Tire Crumb Characterization Study: Field Collection and Laboratory Standard Operating Procedures (SOPs)

Prepared by:
U.S. Environmental Protection Agency
Office of Research and Development

Contributing Authors

National Exposure Research Laboratory	National Risk Management Research Laboratory	Jacobs Technology Inc.	EPA/ORD/NERL SEEs, SSC, ORISE
Christine Alvarez	Xiaoyu Liu	Matt Allen	Lillian Alston, SEE
Nichole Brinkman		Tamira Cousett	Gregory Grissom, SSC
Fu-Lin Chen		Stacy Henkle	Larissa Hassinger, ORISE
Andrea Clements		Denise Popeo	Larry McMillan, SEE
Scott Clifton			Dawn Mills, SSC
Carry Croghan			Sandra Utile-Okechukwu, ORISE
Peter Egeghy			Richard Walker, SEE
Steve Gardner			
Ed Heithmar			
Kathleen Hibbert			
Tammy Jones-Lepp			
Scott Keely			
Kasey Kovalcik			
James McCord			
Myriam Medina-Vera			
Georges-Marie Momplaisir			
Marsha Morgan			
Maliha Nash			
James Noel			
Karen Oliver			
Brian Schumacher			
Brittany Stuart			
Mark Strynar			
Kent Thomas			
Sania Tong-Argao			
Elin Ulrich			
Margie Vazquez			
Don Whitaker			
Alan Williams			
Ron Williams			

Table of Contents

Disclaimer

The research in this document has been funded by the U.S. Environmental Protection Agency (EPA). It has been subjected to the Agency's peer and administrative review process and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The standard operating procedures (SOPs) appended to this report are research methods. They are NOT official EPA methods and have not been validated. They have been reviewed and approved internally by EPA to meet data quality objectives associated with the work herein described. The SOPs are made available as a reference for anyone interested in pursuing additional research, and/or modifying or implementing some of the procedures.

Abstract

This compilation of field collection and laboratory SOPs was assembled for the U.S. Environmental Protection Agency's (EPA) *Tire Crumb Rubber Characterization Study* (TCRS) Pilot Study. This study was developed to address data gaps associated with the safety and use of recycled tire crumb rubber in synthetic turf fields and playgrounds in the United States. The SOPs described in this document contribute to a multi-agency federal research action plan (https://www.epa.gov/chemical-research/federal-research-recycled-tire-crumb-used-playing-fields) to study key environmental human health questions associated with tire crumb rubber on synthetic turf fields and playgrounds. The federal agencies participating in the study include the EPA, the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (CDC/ATSDR), and the U.S. Consumer Product Safety Commission (CPSC).

The federal research action plan has three phases: (1) literature review and data gaps analysis; (2) tire crumb characterization research; and (3) human exposure characterization research. This document focuses on field and laboratory procedures used for the characterization of recycled tire crumb rubber materials and the pilot studies developed to characterize potential human exposures. The tire crumb rubber samples were collected from tire recycling plants and synthetic fields around the United States. The characterization of the tire crumb rubber samples included a wide range of chemical and microbial analyses and physical properties. The chemical laboratory analytes included metals, volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs).

The Executive Summary that follows describes the purpose of this document, background information, the study objectives, and how those objectives were met. The Executive Summary lays the foundation and gives context to the SOPs used for the characterization of the rubber tire crumb material. A summary of the general approach and methods used to collect samples and data is subsequently described and includes: the target analyses relevant to this study and a synopsis of sample collection and laboratory SOPs used in the study (reproduced in full as addendums to Sections 1 and 2).

The SOPs included in the appendices are formatted for field and laboratory use containing details used to collect and analyze the samples from tire recycling facilities and synthetic turf fields around the U.S.

Acronyms and Abbreviations

ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
AQB	Air Quality Branch
CDC	Centers for Disease Control and Prevention
COC	Chain of Custody
CPSC	Consumer Product Safety Commission
ddPCR	droplet digital Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
DNPH	2, 4-dinitrophenylhydrazine
ECB	Environmental Chemistry Branch
EFAB	Environmental Futures Analysis Branch
EHCAB	Ecological & Human Community Analysis Branch
EMMD	Exposure Methods & Measurements Division
EPA	Environmental Protection Agency
EPMA	Electron Probe Micro-analyzer
GC	Gas Chromatography
HCHO	Formaldehyde
HI	Harvard Impactor
HPDE	High Density Polyethylene
HR-ICPMS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IEMB	Integrated Environmental Modeling Branch
LC	Liquid Chromatography
LC/ToF/MS	Liquid Chromatography Time of Flight Mass Spectrometry
mL	Milliliters
MOP	Miscellaneous Operating Procedure
MS	Mass Spectrometry
NERL	National Exposure Research Laboratory
NRMRL	National Risk Management Research Laboratory
NTA	Non-targeted analysis
ORD	Office of Research and Development
PCR	Polymerase chain reaction
PHCB	Public Health Chemistry Branch
PM	Particulate Matter
PSA	Particle Size Analysis
PUF	Polyurethane Foam
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
RH	Relative Humidity
SED	Systems Exposure Division

SEM	Scanning Electron Microscope
SVOCs	Semi-volatile Organic Compounds
SOP	Standard Operating Procedure
TCR	Tire Crumb Rubber
TCRS	Tire Crumb Research Study
ToF MS	Time of Flight Mass Spectrometer
U.S.	United States
VOCs	Volatile Organic Compounds
XRF	X-Ray fluorescence

Acknowledgments
The Tire Crumb Rubber Characterization Study is a collaboration between the U.S. Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. Consumer Product Safety Commission (CPSC). The team acknowledges the contributions of Annette Guiseppi-Elie, Ardra Morgan, Jose Zambrana, Christopher Sibert, Clay Nelson, James Starr, and Sherry Brown. These SOPs would not have been compiled and executed without the support from everyone involved. The authors also want to recognize the invaluable reviews and comments of Dr. Edward Kolodziej (University of Washington) and Dr. Patty Wong (California Environmental Protection Agency's Office of Environmental Health Hazard Assessment). We also acknowledge the invaluable work of Wendy Plessinger as the technical editor.

Executive Summary

Purpose

The purpose of this document is to publish the various methodologies used by EPA for the sample collection and characterization research activities associated with examining recycled tire crumb rubber material used as infill for synthetic turf fields. While the SOPs detailed here are specific to the EPA *Tire Crumb Rubber Characterization Study* and have not been validated, they can easily be modified or applied to studies seeking to collect similar samples and perform multimodal characterization of tire crumbs or similar matrices.

Background

Some in the public have raised concerns about the human safety of recycled tire crumb rubber used in synthetic turf fields and playgrounds in the United States. In response to those concerns, a multi-agency *Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds* (EPA/600/R-16/364) was issued on February 12, 2016. The coordinated effort includes the U.S. Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (CDC/ATSDR), and the Consumer Product Safety Commission (CPSC) as well as key stakeholders (e.g., athletes and parents). The three phases of the strategy include: (1) literature review and data gaps analysis; (2) tire crumb characterization research; and (3) human exposure characterization research. This research effort complements the EPA's limited *Scoping-level Field Monitoring Study of Synthetic Turf Fields and Playgrounds* performed in 2008 (Highsmith, R; Thomas, KW; Williams, RW. (2009); *A Scoping-level Field Monitoring Study of Synthetic Turf and Playgrounds*; EPA/600/R-09/135, National Exposure Research Laboratory, U.S. Environmental Protection Agency). The current effort aims to provide additional information needed to better characterize recycled tire crumb rubber material and understand potential exposures for users of synthetic turf fields.

The EPA, CDC/ATSDR, and CPSC prepared a research protocol that outlines the three phases of the strategy and describes the levels of responsibility for each of the activities. This document focuses on the characterization of the recycled tire crumb rubber including constituents and physical properties. The *Tire Crumb Rubber Characterization Study* can be summarized as the collection of recycled tire crumb material from recycling plants and synthetic turf fields around the United States followed by laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs).

This report compiles the field collection and laboratory SOPs used specifically by EPA on the *Tire Crumb Rubber Characterization Study*. The SOPs include:

- The collection of tire crumb rubber from recycling plants and synthetic turf fields
- Analysis of formaldehyde-DNPH
- Analysis of VOCs
- Analysis of SVOCs by Gas Chromatography/Mass Spectrometry (GC/MS) and sample imaging/topography composition by scanning electron microscope (SEM) and Liquid Chromatography Targeted and Non-targeted approaches

- Analysis of metals by High Resolution Inductive Plasma/Mass Spectrometry (ICP-MS)
- Dust characterization by X-Ray fluorescence (XRF).

A summary of the SOPs is included in Tables 1 and 2.

Table 1. Summary of EPA's Tire Crumb Rubber Field Collection SOPs

Type of Sample Collection	Associated EPA SOPs
Tire crumb rubber from tire recycling plants	D-EMMD-PHCB-038-SOP-01
Tire crumb rubber from synthetic turf fields	D-SED-IEMB-001-SOP-02
Tire crumb rubber from synthetic turf fields for Microbiome Analysis	D-SED-EFAB-009-SOP-01
Questionnaire for administrators of synthetic turf fields on operations, turf history, maintenance, and use	D-SED-EHCAB-002-SOP-01
iButton temperature logging system to assure the integrity of samples collected for microbial analysis	D-SED-EFAB-010-SOP-01

Table 2. Summary of EPA's Tire Crumb Rubber Characterization Study Laboratory SOPs

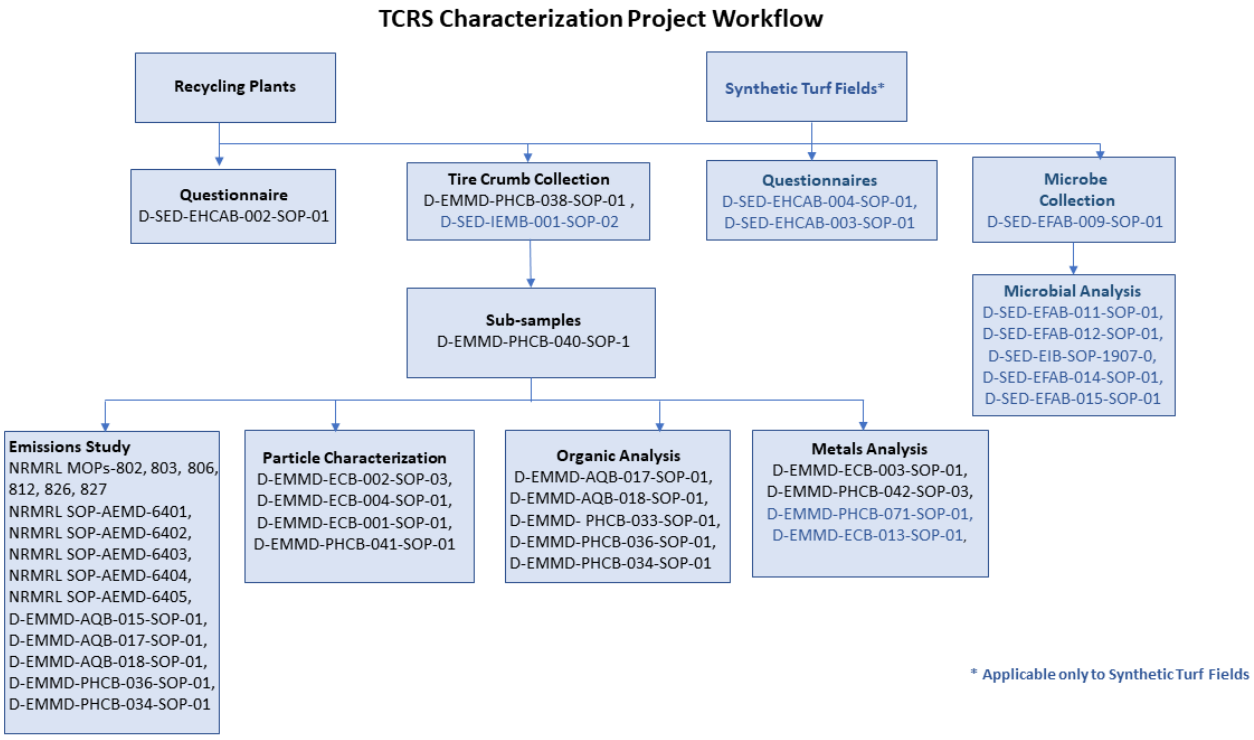
Analytes/Sample or Analysis Type	Type of Analysis	EPA SOP Identification Number
Preparation of Subsamples for Analysis		
SVOCs, metals	Preparation of tire crumb rubber substances for multi-residue characterization	D-EMMD-PHCB-040-SOP-1
Tire Crumb Rubber Particle Characterization		
Particle Size Analysis (PSA)	Sieving procedure for tire crumb rubber samples	D-EMMD-ECB-002-SOP-03
Bulk samples-total element concentration	Innov-X XRF ANALYSIS PROCEDURES: For tire crumb samples	D-EMMD-ECB-004-SOP-01
Particle size & distribution	SEM Analysis of tire crumb particles for sizing and metals	D-EMMD-ECB-001-SOP-01
Particle moisture	Determination of moisture content in tire crumb rubber	D-EMMD-PHCB-041-SOP-01
Tire Crumb Emissions Experiments		
VOCs	Setup and operation of small environmental chambers during testing	NRMRL MOP-802
VOCs	Setup and operation of the Markes Micro-Chamber	NRMRL SOP AEMD-6401
VOCs	Operation of the OPTO Display Software Data Acquisition System in the small chamber laboratory	NRMRL MOP-803

VOCs	Chain of custody procedures for the receipt and transfer of samples	NRMRL SOP AEMD-6402
VOCs	Operation of Clean Air System for the small chamber laboratory	NRMRL MOP-806
VOCs	Sampling and extraction procedures for DNPH-coated silica gel cartridges used to determine air concentrations of formaldehyde and other aldehydes	NRMRL MOP-812
VOCs-Formaldehyde	Operation of the Agilent 1200 HPLC for analysis of DNPH-Carbonyls	NRMRL MOP-826
VOCs-Formaldehyde	High-performance Liquid Chromatography (HPLC) calibration SOP	NRMRL MOP-827
VOCs	Actively loading sorbent tubes with volatile organic compounds	D-EMMD-AQB-015-SOP-01
VOCs	Determination of VOCs desorbed from sorbent tubes using the Markes International Bench ToF-Select ToF GC/MS System	D-EMMD-AQB-017-SOP-01
VOCs	Determination of VOCs desorbed from sorbent tubes using the Markes International Ultra/Unity Thermal Desorption System	D-EMMD-AQB-018-SOP-01 Companion SOP to D-EMMD-AQB-017-SOP-01
VOCs	Collecting air samples from the small environmental testing chambers using Carbopack™ X Sorbent Tubes	NRMRL-SOP-AEMD-6404
SVOCs	Collecting air samples from the Markes Micro-chambers using PUF Plugs	NRMRL-SOP-AEMD-6403-01-0
Chamber clean-up	Glassware and chamber cleaning procedure	NRMRL-SOP-AEMD-6405
SVOCs	SOP for preparation of air samples collected on PUF Plugs for GC/MS Analysis	D-EMMD-PHCB-036-SOP-01
SVOCs	Analytical method for non-targeted and suspect screening in environmental and biological samples using Time of Flight Mass Spectrometry (TOFMS)	D-EMMD-PHCB-034-SOP-01

Organic Analysis		
VOCs	Determination of VOCs desorbed from sorbent tubes using the Markes International Bench ToF-Select ToF GC/MS System	D-EMMD-AQB-017-SOP-01
VOCs	Determination of VOCs desorbed from sorbent tubes using the Markes International Ultra/Unity Thermal Desorption System	D-EMMD-AQB-018-SOP-01 Companion SOP to D-EMMD-AQB-017-SOP-01
Extraction of SVOCs and GC analysis	Extraction and analysis of SVOCs in tire crumb rubber samples	D-EMMD- PHCB-033-SOP-01
SVOCs	SOP for preparation of air samples collected on PUF Plugs for GC/MS Analysis	D-EMMD-PHCB-036-SOP-01
SVOCs LC/ToF/MS analysis	Analytical method for non-targeted and suspect screening in environmental and biological samples	D-EMMD-PHCB-034-SOP-01
Metals Analysis		
Extraction	Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion	D-EMMD-ECB-003-SOP-01
ICP/MS analysis	Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument	D-EMMD-PHCB-042-SOP-03
Microbial Analysis		
Extraction	Extraction of microbes and DNA genomes from samples collected from artificial turf athletic fields	D-SED-EFAB-011-SOP-01
PCR analysis	PCR, Library Preparation and MiSeq Sequencing of Samples for 16S microbiome analysis	D-SED-EFAB-012-SOP-01
Genome analysis	16S rRNA Gene Sequence Analysis	D-SED-EIB-SOP-1907-0
PCR	Droplet digital PCR (ddPCR) analysis of genomic targets	D-SED-EFAB-014-SOP-01
Data analysis	Analysis of data generated from the droplet digital PCR (ddPCR)	D-SED-EFAB-015-SOP-01

Figure 1: Tire Crumb Rubber Study Characterization Project Workflow

A flow diagram shows how the SOPs for the tire characterization were implemented.



Objectives

To communicate and describe EPA's field and laboratory efforts in support of the Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds. To provide technical staff access to research methods, sample collection protocols, and the scientific approach that can be applied or modified for use in matrices similar to tire crumb rubber. In other words, the SOPs included in this document will provide researchers with the tools to perform multimodal characterization of tire crumbs or similar matrices.

Approach

The report provides the SOPs for the *Tire Crumb Rubber Characterization Study*. It outlines the sample collection procedures and the detailed procedures used specifically for each study. Each study section describes the SOPs used for characterizing the tire crumb rubber. In addition, each SOP addendum describes the limitations of the methodology, quality assurance and quality control procedures, and records management.

Tire Crumb Rubber Characterization Study

As part of the Federal Research Action Plan, tire crumb rubber was characterized. Researchers developed procedures for the collection of tire crumb rubber from tire recycling plants and indoor/outdoor synthetic turf fields. Laboratory procedures were developed to measure volatile organic compounds (VOCs) and semi-volatile organic chemicals (SVOCs) emitted and/or present in tire crumb rubber. Metals present in tire crumb rubber were also studied. The microbial populations associated with the tire crumb rubber infill collected from the synthetic turf fields were also characterized. The SOPs developed for the tire crumb rubber characterization are described in the following sections.

Tire Crumb Rubber Field Collection

Tire crumb rubber (TCR) samples were collected from nine tire recycling plants (SOP title: *Collection of Tire Crumb Rubber Samples from Recycling Plants*; SOP# D-EMMD-PHCB-038-SOP-01) and 40 synthetic turf fields across the United States (SOP title: *Collection of Tire Crumb Rubber Samples from Synthetic Turf Fields for Metal, VOC/SVOC, and Particle Analysis*, SOP# D-SED-IEMB-001-SOP-02).

In addition, samples from synthetic turf fields were collected for microbiome analysis (SOP title: *Collection of Samples from Artificial Turf Athletic Fields for Microbiome Analysis*, SOP# D-SED-EFAB-009-SOP-02). A questionnaire was developed to collect data on general facility operations, turf history and maintenance, and public use of synthetic turf fields (SOP title: *Administering the Synthetic Turf Fields Questionnaire*, SOP# D-SED-EHCAB-002-SOP-01).

The samples to be analyzed for VOC/SVOCs were collected using a small handheld stainless-steel comb and placed into a certified, pre-cleaned 250 milliliter (mL) amber glass wide-mouth container. For each field, seven locations were sampled, divided in equal portions, and then composited prior to laboratory analysis. The samples for metal and particle analysis were collected in a similar fashion to those for VOCs/SVOCs with the difference that a small handheld plastic comb was used and samples were placed into certified pre-cleaned 250 mL

high-density polyethylene (HPDE) wide mouth containers. The samples for the microbiome were collected at the same time as the other samples. The individuals collecting the samples wore appropriate personal protective equipment to avoid sample contamination and to assure the samples were aseptically collected for microbiome analysis. Nitrile gloves were used to collect the samples and handle the sampling equipment. A new pair of gloves was used for each of the seven sample collection sites within the field. A sterile polypropylene spatula was used to collect 25 mL of sample into a 50 mL sterile polypropylene container with volumetric lines. The sealed collected samples along with field blanks were placed in a Ziploc bag, stored in a cooler, and delivered to the EPA lab. To assure the integrity of the microbial population, a microchip (iButton) was used to record temperature data to make sure the samples were stored between 2-80 C until laboratory analysis can be performed (SOP title: *iButton Temperature Logging System*, D-SED-EFAB-010-SOP-01).

Sample Preparation

All the tire crumb rubber samples collected (recycling plant and synthetic turf) were sent to the EPA facility in Research Triangle Park where they were logged-in and stored at -20°C until ready for analysis. The tire crumb rubber samples collected from the nine tire recycling plants and 40 synthetic turf fields across the U.S. were removed from the freezer and allowed to warm at room temperature. The composite samples from the synthetic turf fields for organic analysis were prepared by mixing and transferring measured portions of each glass jar sample from a specified location into a single pre-labelled 500 mL amber glass jar (SOP title: *Storage and Preparation of Tire Crumb Rubber Substances for Multi-residue Characterization*, SOP# D-EMMD-PHCB-040-SOP-1). The remaining material was retained for storage and preparing subsets of samples. From each composite sample, subsamples were prepared by weighing specific amounts that were placed in amber glass jars of a specific size depending on the type of analysis to be performed. The weights and jar size used for each subset of composite samples is summarized in Table 3.

Table 3. Preparation of composite subsamples for organic analysis.

Analysis Type	Pre-Cleaned Amber Glass Jar Size	Amount of Composite Sample
SVOC Extraction	60 mL	10 g
Chamber VOC	120 mL	50 g
Chamber SVOC	120 mL	30 g

In addition to composite samples, some samples were collected from individual locations that were not composited. These individual location subsamples were weighted in accordance to the type of analysis to be performed and placed in pre-cleaned 60 mL amber glass jars as summarized in Table 4. The samples were stored at -20ºC until transferred to the appropriate technical lead for analysis.

Table 4. Preparation of subsamples from individual locations collected for organic analysis.

Analysis Type	Amount of Composite Sample
SVOC Extraction	5 g
Chamber VOC	32 g
Chamber SVOC	22 g

Samples (1-L) were also collected from tire recycling plants. Weighted subsamples were taken for each recycling plant for organic analysis and placed in a pre-cleaned 60mL amber glass jar. The amounts of the weights of the subsamples are indicated in the table below (Table 5).

Table 5. Preparation of subsamples from samples collected in tire recycling plants for organic analysis

Analysis Type	Amount of Composite Sample
SVOC Extraction	10 g
Chamber VOC	50 g
Chamber SVOC	30 g

Composite samples for metals analyses were prepared in a similar way as the composite samples prepared for organic analyses. The samples were removed from the freezer and allowed to warm to room temperature. The composite samples from the synthetic turf fields for metal analysis were prepared by mixing and transferring known portions of each HDPE jar sample from a specified location into a single pre-labelled 500 mL HDPE jar. For each type of analysis, measured quantities of the composite sample were placed in a pre-cleaned 120 mL HDPE jar as shown in Table 6.

Table 6. Preparation of composite sub-samples for metal analysis

Analysis Type	Amount of Composite Sample
Metals Digestion	10 g
Metals XRF	10 g

Once the jars for specific analyses have been prepared, capped, and labeled; they were stored at -20ºC or prepared extraction. In addition to the preparation of composite samples, a subset of samples collected from individual locations at a subset of synthetic turf fields was prepared for individual location analysis. Specific weighted amounts were placed in pre-cleaned 120 mL HDPE jars depending on the type of analysis as listed in Table 7.

Table 7. Preparation of subsamples from individual locations collected for metals analysis

Analysis Type	Amount of Individual Location Sample
Metals Digestion	5 g
Metals XRF	5 g

The individual location aliquot samples were stored at -20ºC until transferred to the appropriate technical lead for analysis.

In addition, 1-L HDPE jars from tire recycling plants were collected: two jars for metals analysis and one jar for particle characterization. Weighted subsamples were taken for each recycling plant for metals analysis and placed in a pre-cleaned 120 mL HDPE jar. The amounts of the weights of the subsamples are indicated in Table 8 below.

Table 8. Preparation of sub-samples from samples collected in tire recycling plants for metals analysis.

Analysis Type	Amount of Composite Sample
Metals Digestion	10 g
Metals XRF	10 g

Organic Analysis
The organic analyses were performed for tire crumb rubber samples. The volatile organic compounds emitted from tire crumb rubber samples were collected in controlled chamber experiments. Semi-volatile compounds were extracted with solvents directly from the tire crumb rubber samples. Volatile organic and semi-volatile organic compounds were analyzed by gas chromatography and when applicable, by liquid chromatography.

Volatile Organic Compounds (VOCs)
The following SOPs were used in the emissions chamber studies that were performed for VOCs and SVOCs under different temperature conditions:

- SOP title: Setup and Operation of the Markes Micro-Chamber (SOP# NRMRL SOP AEMD-6401)
- SOP title: Chain of Custody Procedures for the Receipt and Transfer of Samples (SOP# NRMRL SOP AEMD-6402)
- SOP title: Setup and Operation of Small Environmental Chambers During Testing (SOP# MOP802)
- SOP title: Operation of the OPTO Display Software Data Acquisition System in the Small Chamber Laboratory (SOP# MOP803)
- SOP title: Operation of Clean Air System for the Small Chamber Laboratory (SOP# MOP 806)

- SOP title: Collecting Air Samples from the Small Environmental Testing Chambers Using Carbopack™ X Sorbent Tubes (SOP# NRMRL-SOP-AEMD-6404)
- SOP title: Glassware and Chamber Cleaning Procedure (SOP# NRMRL-SOP-AEMD-6405)

Specifically, the emissions were characterized under two different chamber environmental conditions: 25°C and 45% relative humidity (RH); 60°C and 7% RH, respectively, under defined air change rates. The small 53-L chambers were loaded with tire crumb samples and the VOCs emissions were collected using Carbopack™ X sorbent tubes for VOCs and 2, 4-dinitrophenylhydrazine (DNPH) cartridges for formaldehyde (HCHO) (SOP title: *Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes,* SOP# MOP 812). In order to quantitate, VOC standards were loaded into the sorbent tubes prior to sample desorption and analyses (SOP title: *Actively Loading Sorbent Tubes with Volatile Organic Compounds*, SOP# D-EMMD-AQB-015-SOP-01).

The sorbent tubes and cartridges loaded with the sample and standards were desorbed and analyzed by GC for VOCs. A thermal desorption system with a cryo-focusing trap that heats very quickly allowing the analytes to be injected into the GC system was used (SOP title: *Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System,* SOP# D-EMMD-AQB-018-SOP-01). A 60m nonpolar cross-bond dimethyl polysiloxane chromatographic capillary column was used. A time of flight mass spectrometer (ToF MS) set for scanning from 35 to 350 m/z was used as a detector (SOP title: *Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Bench ToF-Select ToF GC/MS System*, SOP# D-EMMD-AQB-017-SOP-01).

Semi-volatile Organic Compounds (SVOCs)
The extraction of tire crumb rubber for SVOCs analysis (SOP title: *Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples*, SOP# D-EMMD-PHCB-033-SOP-01) can be summarized as follows:

1. Weigh 1 g of tire crumb rubber sample and place it into a clean 50 mL polypropylene centrifuge tube.
2. Add the internal standard and 10 mL of 1:1 acetone to hexane.
3. Vortex for 1 minute, let it sit for 2 minutes, vortex for an additional minute.
4. Centrifuge at 4,000 RPM for 5 minutes.
5. Transfer the extract into a 15 mL amber vial with a PTFE-lined cap

For GC/MS/MS analysis, a 1 mL aliquot of the extract was placed into an auto-sampler vial. The analysis was performed using a GC with a capillary injector in split-less mode, an inert 5% phenyl-methyl column (VF-5ms), and a triple quadrupole detector in multiple reaction monitoring (MRM) scan mode. The target analytes for GC analyses are listed in Table 9.

Table 9. Target analytes for GC analyses

Compound	Class	Compound	Class
Cyclohexaneamine	TCR	Dibutyl phthalate	Phthalate
Analine	TCR	2-Mercaptobenzothiazole	TCR
n-Butylbenzene	TCR	Fluoranthene d10	PAH
Naphthalene d8	PAH	Fluoranthene	PAH
Naphthalene	PAH	Pyrene d10	PAH
Benzothiazole	TCR	Pyrene	PAH
Cyclohexylisothiocyanate	TCR	Di-N-hexylphthalate (2)13C2	Phthalate
Resorcinol	TCR	Benzyl butyl phthalate d4	Phthalate
2-Methylnaphthalene	PAH	Benzyl butyl phthalate	Phthalate
1-Methylnaphthalene	PAH	bis(2-Ethylhexyl) adipate	Phthalate
Dicyclohexamine	TCR	Benz(a)anthracene d12	PAH
Dimethyl phthalate	Phthalate	Benz(a)anthracene	PAH
Acenaphthalene d8	PAH	Chrysene d12	PAH
Acenaphthalene	PAH	Chrysene	PAH
Phthalimide	TCR	Bis-2-ethylhexyl phthalate d4	Phthalate
Acenaphthene d10	PAH	Bis-2-ethylhexyl phthalate	Phthalate
Acenaphthene	PAH	1-Hydroxypyrene	PAH
2,6-Di-tert-butyl-p-cresol	TCR	Di-n-octyl phthalate	Phthalate
N,N-Dicyclohexylmethylamine	TCR	Benzo(b)fluoranthene d12	PAH
Diethyl phthalate d4	Phthalate	Benzo(b)fluoranthene	PAH
Diethyl phthalate	Phthalate	Benzo(k)fluoranthene d12	PAH
n-Hexadecane	TCR	Benzo(k)fluoranthene	PAH
Fluorene d10	PAH	Benzo(e)pyrene d12	PAH
Fluorene	PAH	Benzo(e)pyrene	PAH
4-tert-Octylphenol	TCR	Benzo(a)pyrene d12	PAH
2-Bromomethylnaphthalene	PAH	Benzo(a)pyrene	PAH
2-Hydroxybenzothiazole	BT	Perylene d12	PAH
Dibenzothiophene d8	BT	Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	TCR
Dibenzothiophene	BT	Indeno[1,2,3-cd]pyrene d12	PAH
Phenanthrene d10	PAH	Dibenz(a,h)anthracene d14	PAH
Phenanthrene	PAH	Indeno(1,2,3-cd)pyrene	PAH
Anthracene	PAH	Dibenz(a,h)anthracene	PAH
Diisobutyl phthalate	Phthalate	Benzo(g,h,i)perylene d12	PAH

3-Methylphenanthrene	PAH	Benzo(g,h,i)perylene	PAH
2-Methylphenanthrene	PAH	Coronene	PAH
1-Methylphenanthrene	PAH		

The following SOPs were used for the emissions chamber studies that were performed for VOCs and SVOCs under different temperature conditions:

- SOP title: Setup and Operation of Small Environmental Chambers During Testing (SOP# MOP802)
- SOP title: Operation of the OPTO Display Software Data Acquisition System in the small chamber laboratory (SOP# MOP803)
- SOP title: Operation of Clean Air System for the Small Chamber Laboratory (SOP# MOP 806)

For the SVOCs, the chamber emission testing was similar to the procedure used for VOCs with the difference that the chamber air samples were collected on pre-cleaned small polyurethane foam (PUF) plugs (SOP title: *Collecting Air Samples from the Markes Micro-Chambers using PUF plugs*, NRMRL-SOP-AEMD-6403). The VOCs and SVOCs emissions were characterized under two different chamber environmental conditions: 25°C and 45% RH; 60°C and 7% RH, respectively, under defined air change rates.

The PUF samples were extracted using an ultrasonic extraction procedure (SOP title: *Preparation of Air Samples Collected on PUF Plugs for GC/MS analysis*, SOP#: D-EMMD-PHCB-036-SOP-01). The PUF plugs are extracted with 1:1 acetone to hexane and then concentrated to 1 mL under nitrogen. The extracts were transferred to auto-sampler vials for GC analysis.

The extracts from tire crumb rubber samples were also analyzed by liquid chromatography time of flight mass spectrometry (LC/ToF/MS). An aliquot of each extract in 1:1 acetone to hexane was transferred to a vial where the solvent was exchanged to methanol prior to LC/ToF/MS analysis (SOP title: *Analytical Method for Non-targeted and Suspect Screening in Environmental and Biological Samples Using Time of Flight Mass Spectrometry*, SOP# D-EMMD-PHCB-034-SOP-01).

Tire crumb samples from recycling plants and synthetic turf fields as well as the PUF plugs from the emissions experiments were also analyzed using non-targeted analysis (NTA) by GC/MS/MS and LC/MS/MS. The primary difference between targeted and non-targeted analysis is that in the former, specific (targeted) analytes are identified with the use of standards and in the latter the instrument is set up into full scan mode so every peak over a specified threshold can be detected and potentially identified. The first technique provides the traditional quantitative data and the second technique allows for potential discovery and tentative identification of a wide range of compounds in a sample when compared to available databases.

For the GC NTA analysis, a low polarity 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane 60-meter column was used. The detector was set on electron impact mode with a scan range of 50-550 m/z (SOP title: *Extraction and Analysis of SVOCs in Tire Crumb Rubber*

Samples, SOP# D-EMMD-033-SOP-01). For the LC NTA analysis, a C18 column was used with a mobile phase containing methanol and 2 mM formate buffer at a flow rate of 300 µL/min. Electrospray ionization was used and the detection was performed in positive and negative modes (SOP title: *Analytical Method for Non-targeted and Suspect Screening in Environmental and Biological Samples*, SOP# D-EMMD-PHCB-034-SOP-01).

Metals Analysis

The inorganic analyses laboratories received synthetic turf samples collected using 950 mL polyethylene bottles (SOP title: *Storage and Preparation of Tire Crumb Rubber Subsamples for Multi-residue Characterization*, SOP# D-EMMD-PHCB-040-SOP-01) as well as 1-L polyethylene bottles containing recycling plants samples. Particle size analysis was performed to help assess potential exposure risks from inhalable dust and ingestion of small particles. Particle size analysis (PSA) was performed using a sieving procedure that allowed for the rough characterization of particle size for tire crumb samples by using a number of stacked sieves in a shaker (SOP title: *Sieving Procedure for Tire Crumb Rubber Samples*, SOP# D-EMMD-ECB-002-SOP-03). Fractions were collected on each sieve (2.00 mm, 1.00 mm, 0.25 mm, 0.125mm, 0.63mm, and a bottom pan).

X-ray fluorescence (XRF) analysis was done on bulk samples received for metal analysis and on each of the fractions generated by PSA. The XRF procedure was adapted from its use on soil media for use with tire crumb rubber media (SOP title: *Innov-X XRF ANALYSIS PROCEDURES: For Tire Crumb Samples*, D-EMMD-ECB-004-SOP-01). The XRF measured the total element concentration in the original sample without undergoing digestion. In addition, entire PSA fractions retained on the 0.063 mm sieve (nominal sizes 0.063-0.125mm) and the bottom pan (nominal sizes < 0.063 mm) were subsampled and analyzed using a scanning electron microscope (SEM). The SEM method (SOP title: *SEM Analysis of Tire Crumb Particles for Sizing and Metals*, D-EMMD-ECB-001-SOP-01) was developed to be as uniform as possible from sample to sample. It provided measurements of particle size and particle distribution when using the imaging function. Selected particles were analyzed with an electron probe micro-analyzer (EPMA). This technique is similar to the SEM but it has the added capability of chemical analysis.

It is important to note that tire crumb rubber samples collected from synthetic turf fields may contain varying amounts of moisture depending on the environmental conditions. A percent moisture analysis was performed to reduce uncertainty of those analyses that are based on mass of analyte versus mass of rubber (analyte mass/mass of rubber). The analysis was performed for select metals samples at a temperature of 110oC which volatilizes the water as well as some volatile organic compounds (SOP title: *Determination of Moisture Content in Tire Crumb Rubber*, SOP# D-EMMD-PHCB-041-SOP-01).

SOPs were developed and/or modified to characterize metals in tire crumb rubber to inform understanding of potential exposures to toxic contaminants. Metal components were extracted for analysis by using a modified EPA Method 3051A to prepare tire crumb rubber samples for metal analysis by inductively coupled plasma mass spectrometry (ICP-MS) (SOP title: *Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion*, SOP# D-EMMD-

ECB-003-SOP-01). The summarized optimized method for the preparation of bulk tire crumb samples is as follows:

1. Suspend 250mg of tire crumb subsamples in a 3:1 mixture of nitric acid and hydrochloric acid.
2. Seal the sample vessels and warm gently in a microwave system.
3. Let the vessels stand overnight at room temperature.
4. Perform full digestion at 200°C using the parameters in the SOP titled: Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion.

The digested extracts were gravimetrically diluted (nitric acid-2% by volume and hydrochloric acid-1% by volume) to minimize matrix effects. A double-focusing magnetic sector field (high resolution) inductively coupled plasma mass spectrometry (HR-ICPMS) was used for elemental analysis (SOP title: *Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument*, D-EMMD-PHCB-042-SOP-03). The trace elements found in digested tire crumb rubber and analyzed by HR-ICPMS are aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), vanadium (V), and zinc (Zn).

Extraction and Analysis of Microbial Population

Microbes associated with the tire crumb rubber in synthetic fields were studied as part of the tire crumb rubber characterization process. A surfactant solution was added to five grams of the collected tire crumb rubber samples once they arrived into the laboratory. The samples were vortexed vigorously to dislodge any microbes from the sample into the solution (SOP title: *Extraction of Microbes and DNA Genomes from Samples Collected from Artificial Turf Athletic Fields*, SOP# D-SED-EFAB-011-SOP-01). The solution was filtered. The microbes were retained in the nitrocellulose membrane filter which was stored at -20°C until analysis. For each batch of samples, negative and positive TCR extraction controls were prepared. DNA genomes were extracted in a warm water bath at a temperature of 55°C using the PowerWater DNA isolation kit and quantifying DNA yield with a fluorometer. The assay type on the fluorometer was dsDNA High Sensitivity (SOP title: *PCR, Library Preparation and MiSeq Sequencing of Samples for 16S Microbiome Analysis*, SOP# D-SED-EFAB-012-SOP-01 and SOP title: *16S rRNA Gene Sequence Analysis*, SOP# D-SED-EIB-SOP-1907-0). Droplet digital PCR was used to determine the concentrations of targeted microbes (SOP title: *Droplet Digital PCR (ddPCR) Analysis of Genomic Targets*, SOP# D-SED-EFAB-014-SOP-01). The data produced was clustered into positive or negative bins providing the basis to calculate DNA target concentrations (SOP title: *Analysis of Data Generated from the Droplet Digital PCR (ddPCR)*, SOP# D-SED-EFAB-015-SOP-01).

Quality Assurance Summary

The EPA TCR Study was conducted under an approved Quality Assurance Project Plan (QAPP) – Tire Crumb Research Study Sampling and Analysis and Research Protocol – Collections Related to Synthetic Turf Fields with Crumb Rubber Infill

(https://www.epa.gov/sites/production/files/2016-08/documents/tcrs_research_protocol_final_08-05-2016.pdf).

All SOPs were reviewed and approved by the appropriate EPA Branch Chief and Quality Assurance Manager. Prior to approval, each SOP was reviewed and tested (field and lab). The standard operating procedures appended to this report are NOT official EPA methods – instead they are considered research methods. They describe the quality assurance and quality controls, blanks, calibration checks, acceptance ranges, reference materials and procedural checks. All SOPs were reviewed and approved internally by EPA to meet data quality objectives associated with the work herein described. In addition, each part of the project was audited and the data was QA reviewed by a third party not performing the analyses. Details on data analysis and QA/QC are found in the companion report *Synthetic Turf Field Tire Crumb Rubber Research Under the Federal Research Action Plan-Final Report Volume 1-Tire Crumb Characterization Study* [EPA/600/R-18/162], U.S. Environmental Protection Agency, Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry.

Conclusions

There were unique challenges with sampling and analyzing recycled tire crumb rubber materials that had to be addressed in the methods development arena. There is no appropriate surrogate matrix for tire crumb rubber therefore, the methods developed extrapolated the knowledge acquired by working and analyzing other matrices. A fine balance was used to develop methods that would assist in the characterization of the tire crumb rubber without compromising the composition of the material. For example, if an available method for solid matrices existed like EPA SW-846 Method 3051A, then it was modified and applied to the microwave digestion of tire crumbs. Other methods were developed specifically for this effort such as those focusing on the characterization of organic compounds emitted and/or present in recycled tire crumb rubber.

The samples collected from a synthetic turf field had their own particular interferences. They usually had sand and other smaller debris that was difficult to separate from the samples. Minimizing these interferences was particularly challenging when performing particle size analyses and other particle characterization methods. The researchers were also aware that tire crumb rubber can behave as a passive sampler. To better understand the potential contribution of VOCs, the samples collected from tire crumb rubber recycling plants were used to perform controlled emissions experiments. The design of the experiments had challenges such as selecting the temperature and chamber conditions to run the emission experiments that would provide data that would best represent potential field conditions when in use. The data from these experiments supplements the data obtained from the outdoor and indoor synthetic turf fields.

The extraction of tire crumbs, regardless of their origin, required the evaluation of several solvent systems that would be effective without solvating the rubber itself. Specific composite tire crumb rubber samples were prepared and designated as reference samples along with reagent blanks and spikes. Most of the QA/QC samples consisted of duplicate preparations, a reagent spike, reagent blank and the tire crumb rubber sample designated as a reference sample. The GC/MS and LC/MS SOPs were developed to analyze compounds on targeted and non-targeted mode to assist in understanding the complexity of potential analytes present in one sample. None of the analyzed organic analyses methods was optimized for all the compounds resulting in low

detection for some of them. The potential user of these analytical methods is encouraged to use the information provided as a starting point. In addition, microbial analysis, and questionnaire SOPs were developed to provide a better understanding of the characterization and potential exposures beyond the traditional chemical data. All the data produced is reported in the document *Synthetic Turf Field Tire Crumb Rubber Research Under the Federal Research Action Plan – Final Report Volume 1 – Tire Crumb Characterization Study* (EPA/600/R-18/162), U.S. Environmental Protection Agency, Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry.

It is important to keep in mind that the SOPs developed in this compendium are research methods. They are not official EPA methods. They are made available as a reference for anyone interested in pursuing additional research, and/or modifying or implementing some of the procedures.

Section 1: EPA Tire Crumb Rubber Field Collection

Standard Operating Procedures (SOPs)

Type of Sample Collection	Associated EPA SOPs
Tire Crumb Rubber from Tire Recycling Plants	D-EMMD-PHCB-038-SOP-01
Tire Crumb Rubber from Synthetic Turf Fields	D-SED-IEMB-001-SOP-02
Tire Crumb Rubber from Synthetic Turf Fields for Microbiome Analysis	D-SED-EFAB-009-SOP-01
Questionnaire for Administrators of Synthetic Turf Fields on Operations, Turf History, Maintenance, and Use	D-SED-EHCAB-002-SOP-01
iButton Temperature Logging System to Assure the integrity of Samples Collected for Microbial Analysis	D-SED-EFAB-010-SOP-01

This section provides the SOPs related to the tire crumb rubber sample collection. Each individual SOP details the materials used, precautions, shipping procedures, and steps taken to preserve the integrity of the samples.

Collection of Tire Crumb Rubber from Tire Recycling Plants (D-EMMD-PHCB-038-SOP-01)

This SOP summarized the steps taken for sampling tire recycling plants. The field staff arrived at a recycling plant that produces tire crumb rubber and discussed the plant's required safety requirements with plant staff. Following the safety requirements and wearing appropriate safety equipment, the field staff identified the samples to be collected from three separate manufacturing batches. If three batches were not available, the samples were collected from three different storage containers or production lines at the plant. After samples were identified for collection, the field staff filled three 1-L certified pre-cleaned, high-density polyethylene (HDPE) jars (two for metals analysis and one for particle characterization analysis) and two certified pre-cleaned amber glass jars (for organics analysis) completely with tire crumb rubber from each lot, batch, or storage container. The jars were capped and sealed. Jars were labeled with unique sample codes.

Field records included plant project identification number, collection date and time, and initials of the field staff member that collected the sample with required information recorded in the chain of custody (COC) record. Ziplock bags were used to provide secondary containment around the jars. The samples and the completed COC record were shipped using a next day service to the EPA facility in Research Triangle Park (RTP), NC. After receipt, samples were stored in a freezer (-20°C) until subsequent aliquots were prepared for analysis. Samples for particle characterization analysis were shipped to the EPA facility in Las Vegas, NV (now vacant).

Collection of Tire Crumb Rubber from Synthetic Turf Fields for Metal, VOC/SVOC, and Particle Analysis (D-SED-IEMB-001-SOP-02)

Tire crumb rubber samples were collected from synthetic turf fields to support characterization of chemical and microbiological constituents. Field sampling personnel arrived at a facility with synthetic turf fields and discussed the sampling requirements and procedures including any facility-required safety requirements with facility staff.

It is worth noting that substantial variability in tire crumb rubber chemical concentrations have been reported. Therefore, tire crumb rubber samples were collected at seven locations in each synthetic turf field and composited before analysis. In summary, a first set of seven samples was collected from seven locations at each field for volatile organic compounds (VOC) and semi-volatile organic compounds (SVOC) analysis. A small handheld stainless-steel comb was used to pull tire crumb rubber from the field at each location. The collection depth in the field was kept at no more than about 3 centimeters (cm) from the surface. The collected tire crumb rubber was placed into certified pre-cleaned 250-milliliter (mL) amber glass wide-mouth containers with Teflon®-lined lids. Each of the seven containers collected per field was completely filled with tire crumb rubber material. Equal portions of these samples were then composited at the laboratory prior to analysis.

A second set of seven samples was also collected at the same locations for metals analysis. A small handheld plastic comb was used to pull tire crumb rubber from the field at each location.

The collection depth in the field was no more than about 3 cm from the surface. The collected tire crumb rubber was placed into certified pre-cleaned 250 mL high-density polyethylene (HPDE) wide-mouth containers. Each container was completely filled with tire crumb rubber material. Equal portions of these samples were composited at the laboratory prior to analysis.

In addition, a third set of samples was collected at the sample locations for particle analysis. A small handheld plastic comb was used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field was no more than about 3 cm from the surface. The collected tire crumb rubber was placed into certified pre-cleaned 250 mL HPDE wide-mouth containers. Each container at each of the seven field locations was completely filled with tire crumb rubber material. The entire amount of these samples was composited at the laboratory prior to analysis. All samples were shipped the same day as they were collected for next-day delivery service to a central processing laboratory.

Tire Crumb Rubber from Synthetic Turf Fields for Microbiome Analysis (D-SED-EFAB-009-SOP-01)

This SOP describes the steps to aseptically collect samples for microbiome analysis. These tire crumb rubber samples were collected at the same time samples were collected for chemical analysis (section 1.2). Individual 25 mL microbiome samples were collected from each field at all seven locations where samples for metals and VOCs/SVOCs were collected. The individuals collecting the samples wore appropriate personal protective equipment such that they did not contribute their microbiome to the sample. Aseptic techniques were employed while collecting and handling samples or sampling equipment. Nitrile (or appropriate alternative) gloves were worn at all times when handling the sample or sampling equipment. A clean disposable lab coat was worn during sample collection. A freshly opened sterile polypropylene spatula was used at each field location to collect 25 mL samples per sampling location within the field. The sterile spatula was inserted into the athletic field surface at an approximate 30-degree angle to maximum depth of 3 cm from the surface and moved forward to collect tire crumb material. The tire crumb rubber was added to a sterile 50 mL polypropylene container with volumetric lines. The container was filled with tire crumb rubber material to the 25 ml line. The samples were stored on ice immediately after collection and shipped to the laboratory for processing within 24 hours.

Questionnaire for Administrators of Synthetic Turf Fields on Operations, Turf History, Maintenance, and Use (D-SED-EHCAB-002-SOP-01)

Research staff administered the Synthetic Turf Fields Questionnaire to owners or managers using a computer-assisted interview (CAI) method. This questionnaire was used to record specific types of data including type of fields (e.g., indoor or outdoor), age of fields, sports/activities played on fields, frequency and hours of field use, and turf maintenance, cleaning, and replacement practices. Using a laptop computer, the research staff opened the Synthetic Turf Fields questionnaire via the Epi Info software program and asked facility owners or managers each question and recorded their responses. Research staff saved all participant responses recorded in the questionnaires prior to exiting the Epi Info software program. They also made a backup copy of all questionnaire responses using a portable flash drive.

iButton Temperature Logging System to Assure the Integrity of Samples Collected for Microbial Analysis (D-SED-EFAB-010-SOP-01)

Temperature was monitored for samples collected for microbial analysis to assure the integrity of the sample. This SOP describes the steps to program iButtons, use iButtons, and analyze data collected by iButtons. The DS1921G iButton was used to record temperature data within the range of -40°C to 85°C with an accuracy of at least ±1°C and within ±2 minutes. The iButton can store up to 2,048 equally spaced data points at intervals which can be set from 1 to 255 minutes. For this project, the logged data was downloaded with a USB reader probe and One-wire software. The data was viewed and analyzed in Microsoft Excel.

Collection of Tire Crumb Rubber from Tire Recycling Plants (D-EMMD-PHCB-038-SOP-01) – August 6, 2016

U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Standard Operating Procedure for Collection of Tire Crumb Rubber Samples from Recycling Plants

Number: D-EMMD-PHCB-038-SOP-01	Effective Date: 8/17/16

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: M. Scott Clifton

Signature: Date:

Approval

Name: Myriam Medina-Vera
Title: Branch Chief, PHCB

Signature: Date:

Concurrence*

Name: Sania Tong Argao
Title: EMMD QA Manager
Signature: Date:

* Optional Field

STANDARD OPERATING PROCEDURE FOR COLLECTION OF TIRE CRUMB RUBBER SAMPLES FROM RECYCLING PLANTS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

The tire crumb rubber characterization study will involve the collection of crumb rubber material from tire recycling plants and synthetic turf fields around the U.S., with laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and particle/dust characteristics. This standard operating procedure (SOP) describes collection of tire crumb rubber samples from tire recycling plants.

2.0 SUMMARY OF THE METHOD

Field staff will arrive at a recycling plant that produces tire crumb rubber and will discuss plant-required safety requirements with plant staff. Following the safety requirements and while wearing appropriate safety equipment, the field staff will identify samples to be collected from three separate manufacturing batches. If three batches are not available, samples may be collected from three different storage containers or production lines at the plant. After samples have been identified for collection, the field staff will fill three (3) 1-L certified pre-cleaned high-density polyethylene (HDPE) jars (two for metals analysis and one for particle characterization analysis) and two certified pre-cleaned amber glass jars (for organics analysis) completely with tire crumb rubber from each lot, batch, or storage container. The jars will be capped and sealed. Jars will be labeled with unique sample codes.

Field records will include plant project identification number, collection date and time, and initials of the field staff member that collected the sample with required information being recorded in the chain of custody (COC) record. Ziplock bags will be used to provide secondary containment around the jars. Samples and the completed COC record are then shipped using a next-day delivery service to the EPA facility in Research Triangle Park (RTP), NC. After receipt, samples are stored in a freezer (-20°C) until subsequent aliquots are prepared for analysis or samples for particle characterization analysis are shipped to the EPA facility in Las Vegas, NV.

3.0 DEFINITIONS

VOCs – volatile organic compounds
SVOCs – semi-volatile organic compounds
SOP – standard operating procedure
HDPE – high-density polyethylene
COC – chain of custody
RTP – Research Triangle Park
PTFE – Polytetrafluoroethylene
FB – Field Blank

4.0 CAUTIONS

4.1 Field staff will discuss recycling plant safety requirements and procedures with plant staff prior to initiating any sample collection activities.
4.2 Field staff will wear any recycling plant required safety equipment and will follow all plant safety guidelines and procedures.
4.3 Field staff will wear safety glasses and nitrile gloves during sample collection.

5.0 RESPONSIBILITIES

The EPA project staff or contractor will provide the sample collection materials and deliver them to the field staff, which may include preparation of sampling equipment prior to the plant visit. The EPA staff or contractor will also be responsible for receiving tire crumb samples and ensuring proper storage at the EPA facility.

The field staff will be responsible for preparation of sampling equipment prior to the plant visit, collection of tire crumb rubber samples, completing and verifying sample collection records (e.g., sample labels, COC records), and packaging/shipping samples and records to the EPA RTP facility. The field staff is also responsible for following any safety requirements of the recycling plants and wearing appropriate protective equipment including safety glasses and nitrile gloves during sample collection.

6.0 MATERIALS

6.1 1-L HPDE jars, pre-cleaned and certified for trace metals analysis, I-Chem 300 Series or equivalent
6.2 1-L amber glass jars with Teflon-lined lids, pre-cleaned and certified for trace organic chemical analysis, I-Chem 300 Series or equivalent
6.3 Stainless steel collection scoop, medium sized (1/2 cup) or equivalent
6.4 Polycarbonate plastic collection scoop, medium sized (1/2 cup) or equivalent
6.5 Laboratory wipes
6.6 Acetone, HPLC Grade or better
6.7 Hexane, HPLC Grade or better
6.8 Ghost Wipes, Environmental Express P/N 4210
6.9 Deionized water, 18 megohm-cm
6.10 Nitrile gloves
6.11 Safety Glasses
6.12 Ziplock bags, 28” x 30”
6.13 Polytetrafluoroethylene (PTFE) tape
6.14 Sample labels
6.15 Shipping containers and packaging material/tape
6.16 Shipping labels
6.17 Sample collection and chain of custody (COC) record sheet

7.0 PROCEDURES

Samples will be collected for trace metals, trace organics, and particle characterization analysis using slightly different procedures applicable to each type of analyte. The following procedures describe how sampling equipment is prepared prior to the plant visit (section 7.1), how to collect the samples at the plant for trace metals (section 7.2) and trace organics and particle characterization (section 7.3), and how to store and ship the samples (section 7.4).

7.1 *Equipment Preparation*

7.1.1 Clean sample collection scoops and label sample containers in the laboratory prior to transporting to the tire recycling plant.

7.1.2 Wearing nitrile gloves and safety glasses, clean stainless-steel scoops by thoroughly rinsing with tap water followed by wiping dry with a laboratory wipe. After dry, solvent rinse each scoop with acetone followed by hexane using wash bottles filled with the appropriate solvents and rinsing into a waste container. Allow to air dry before storing the cleaned scoops in a Ziplock bag until use.

7.1.3 Clean polycarbonate scoops by thoroughly wiping with a Ghost Wipe. Rinse with deionized water and allow to air dry before storing in a Ziplock bag until use.

7.1.4 Apply pre-printed sample code labels to sample collection containers. For each batch per recycling plant, there will be sample ID codes for two (2) 1-L HDPE jars for metals analysis, one (1) 1-L HDPE jar for particle/dust analysis, and two (2) 1-L amber jars for organics analysis. There will also be sample ID codes for one (1) 1-L HDPE jar and one (1) 1-L amber jar for field blanks per recycling plant. See Table 1 for sample ID codes to be used.

7.1.5 Package cleaned sample collection scoops, labeled sample collection containers, bags, sample collection forms, and COC records for transport to the recycling plant.

7.2 *Trace Metals and Particle Sample Collection by Field Staff at Recycling Plant*

7.2.1 Select the samples to be collected from three different manufacturing batches at the plant. If separate batches cannot be identified, samples may be collected from three different storage containers and/or production lines at each plant.

7.2.2 For the first batch selected, use a pre-cleaned polycarbonate scoop to completely fill three (3) of the pre-cleaned 1-L certified HDPE jars with tire crumb rubber. The same scoop can be used to fill all containers. Two (2) jars will be filled for metals analysis and one (1) jar will be filled for particle analysis.

7.2.3 The sample jars are pre-labeled prior to collection. Field staff must verify that the jar label matches the correct information for the samples being

collected.

7.2.4 After each jar is filled, seal with the lid that was removed from the jar prior to filling. Further seal each lid by wrapping the outside with PTFE tape.

7.2.5 Place each jar into the cardboard box.

7.2.6 Record details of sample collection information on the sample collection form (Figure 1) and COC record (Figure 2) as noted in Section 8.0 Records.

7.2.7 Repeat Steps 7.2.2 – 7.2.6 for the remaining two (2) batches/containers for a total of nine (9) individual samples.

7.3 *Trace Organics Sample Collection by Field Staff at Recycling Plant*

7.3.1 Select the samples to be collected from three (3) different manufacturing batches at the plant. If separate batches cannot be identified, samples may be collected from three (3) different storage containers and/or production lines at each plant.

7.3.2 For the first batch selected, use a pre-cleaned stainless-steel scoop to completely fill two (2) of the pre-cleaned 1-L certified amber jars with tire crumb rubber. The same scoop can be used to fill both sample jars.

7.3.3 The sample jars are pre-labeled prior to collection. Field staff must verify that the jar label matches the correct information for the samples being collected.

7.3.4 After each jar is filled, seal the jar with the PTFE-lined lid that was removed from the jar prior to filling. Further seal each lid by wrapping the outside with PTFE tape.

7.3.5 Place each jar into the cardboard box with sections.

7.3.6 Record details of sample collection information on the sample collection form (Figure 1) and COC record (Figure 2) as noted in Section 8.0 Records.

7.3.7 Repeat Steps 7.3.2 – 7.3.6 for the remaining two (2) batches/containers for a total of six (6) individual samples.

7.4 *Storage and Shipping*

7.4.1 After samples are collected, ensure that all lids are tightly sealed.

7.4.2 Verify that the sample codes on the sample jars match the COC record. Verify that all required information is recorded and complete the COC record and sample collection form.

7.4.3 Place the glass jars into bubble-wrap sleeves. Place the protected glass jars into sections of the cardboard container box and seal the box with packing tape. Place the entire cardboard box into a 28” x 30” Ziplock bag and seal the bag.

7.4.4 Place the HPDE jars into the cardboard container box and seal the box with packing tape. Place the entire cardboard box into a 28” x 30” Ziplock bag and seal the bag.

7.4.5 Package the cardboard container boxes containing sample jars into one or more overpack cardboard boxes with padding material as needed.

7.4.6 Place the COC record(s) and sample collection forms in a Ziplock bag and place it in the overpack cardboard box with the samples.

7.4.7 Place the sample scoops back in their Ziplock bags and place them into the overpack cardboard box for return to the laboratory.

7.4.8 Ship the samples using a next-day delivery service to the EPA/RTP lab using the following address:

Kent Thomas
US EPA/NERL/SED
Chemical Services, Room E-178, Bldg. E Loading Dock
109 T.W. Alexander Dr.
RTP, NC 27709
(919) 541-7939
thomas.kent@epa.gov

7.4.9 Upon receipt at the laboratory, EPA staff or contractors will verify each sample container label against the COC record, initial receipt of the sample, and if needed, record any comments about sample condition upon receipt.

7.4.10 The COC record will be provided to the EPA research manager or his/her designee.

7.4.11 The samples will be placed in a freezer (-20°C) until subsequent aliquots are prepared and analyzed (metals and organics samples) or shipped to the EPA Las Vegas laboratory (particle characterization analysis samples). The storage location will be recorded on the COC record.

8.0 RECORDS

Any information relevant to sample collection should be recorded on the sample collection form (Figure 1) and COC sheet (Figure 2). Required information includes the plant and sample ID code and the process used to generate the tire crumb rubber (typically ambient or cryogenic) recorded by circling the correct category on the sample collection form. The date, time, and initials of the field staff member that collected the sample is recorded on the COC sheet as well. The field staff should also record whether the samples were collected either from different production batches, different storage containers, or from the production line by circling the appropriate category on the sample collection form.

All samples must have a unique sample ID code. See Table 1 for specific codes to be used. Sample codes for tire recycling plants use the following coding scheme:

TCRS-R-VV-W-X-Y-Z

Where:
TCRS = tire crumb rubber research study
R = recycling plant designation
VV = two-digit code unique to each recycling plant, from 80 to 89
W = Recycling plant batch or storage container number, from 1 to 3 (or 9 if Field Blank)
X = Sample jar designation from each batch or storage container; 1 or 2
Y = Sample type identifier M (Metals) or G (organic compounds) or P (particles)
Z = Analysis number code designator – all of these samples are parents with a '0' designator

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Field blank (FB) samples will be collected for each plant. The field blanks will consist of one (1) 1-L HPDE container and one (1) 1-L amber glass container that are taken to the plant site, briefly opened then closed, packaged for shipment just as samples are packaged, and returned to the laboratory with the samples.
9.2 No fortified field controls will be collected because no suitable control materials are available for tire crumb rubber.

10.0 REFERENCE

- *Crumb Rubber Sampling Plan – Manufacturing Site, Office of Health Hazard Assessment,* California Environmental Protection Agency. January 22, 2016.

Table 1. TCRS Sample Codes for Recycling Plant Tire Crumb Rubber Samples

Recycling Plant Tire Crumb Rubber Sample Coding – Metals Analysis
(XX = Plant ID Number)

Sample Code	Description
TCRS-R-XX-1-1-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 1 – Jar 1
TCRS-R-XX-1-2-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 1 – Jar 2
TCRS-R-XX-2-1-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 2 – Jar 1
TCRS-R-XX-2-2-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 2 – Jar 2
TCRS-R-XX-3-1-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 3 – Jar 1
TCRS-R-XX-3-2-M-0	Metals sample – 1-L HPDE Jar – Lot/Batch/Container 3 – Jar 2
TCRS-R-XX-9-1-M-0	Metals sample – 1-L HPDE Jar – Field Blank

Recycling Plant Tire Crumb Rubber Sample Coding – Particle/Dust Analysis
(XX = Plant ID Number)

Sample Code	Description
TCRS-R-XX-1-1-P-0	Particle sample – 1-L HPDE Jar – Lot/Batch/Container 1 – Jar 1
TCRS-R-XX-2-1-P-0	Particle sample – 1-L HPDE Jar – Lot/Batch/Container 2 – Jar 1
TCRS-R-XX-3-1-P-0	Particle sample – 1-L HPDE Jar – Lot/Batch/Container 3 – Jar 1

Recycling Plant Tire Crumb Rubber Sample Coding – Organics Analysis
(XX = Plant ID Number)

Sample Code	Description
TCRS-R-XX-1-1-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 1 – Jar 1
TCRS-R-XX-1-2-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 1 – Jar 2
TCRS-R-XX-2-1-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 2 – Jar 1
TCRS-R-XX-2-2-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 2 – Jar 2
TCRS-R-XX-3-1-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 3 – Jar 1
TCRS-R-XX-3-2-G-0	Organics sample – 1-L Glass Jar – Lot/Batch/Container 3 – Jar 2
TCRS-R-XX-9-1-G-0	Organics sample – 1-L Glass Jar – Field Blank – Jar 1

Recycling Plant Sample ID Code - TCRS-R-VV-W-X-Y-Z
Where:TCRS = tire crumb rubber research study; R = recycling plant designation; VV = two - digit code unique to each recycling plant (from 80 to 89); W = Recycling facility batch or storage container number (from 1 to 3) or 9 if field blank; X = Sample jar designation from each batch or storage container; 1 or 2; Y = sample type identifier M (Metals), G (organic compounds), P (Particle/dust); Z = Assigned analysis number codes for sub-sample aliquots to indicate the type of analysis the sample was prepared for; this is zero for parent samples.

Figure 1. – Sample Collection Form

Form Approved
OMB No. 0923-0054
Exp. Date 01/31/2017

Tire Recycling Plant Sampling Collection Form

Study ID Number _____
Sample Collection Date _____
Collector ID _____

Production Process Type: Ambient Cryogenic (Circle one)

Crumb Rubber Samples Collection – For Metals and Particles (Plastic Containers)

Sample	**Sample Collected**	
Batch/Lot/Storage Bag 1	Yes	No
Batch/Lot/Storage Bag 1	Yes	No
Batch/Lot/Storage Bag 1	Yes	No
Batch/Lot/Storage Bag 2	Yes	No
Batch/Lot/Storage Bag 2	Yes	No
Batch/Lot/Storage Bag 2	Yes	No
Batch/Lot/Storage Bag 3	Yes	No
Batch/Lot/Storage Bag 3	Yes	No
Batch/Lot/Storage Bag 3	Yes	No

Crumb Rubber Samples Collection – For Organics (Glass Containers)

Sample	**Sample Collected**	
Batch/Lot/Storage Bag 1	Yes	No
Batch/Lot/Storage Bag 1	Yes	No
Batch/Lot/Storage Bag 2	Yes	No
Batch/Lot/Storage Bag 2	Yes	No
Batch/Lot/Storage Bag 3	Yes	No
Batch/Lot/Storage Bag 3	Yes	No

Figure 2. Chain of Custody Record

Tire Crumb Research Study				
Site ID Code: Sample Analysis Type:		Site Information if Needed (Do not include facility name or location here):		
Sample ID	Description if Needed	Comments if Needed		
Collected By:	Date Collected:	Collection Start Time:	Collection End Time:	
Shipped By:	Date Shipped:			
Received By:	Date:	Storage:	Relinquished By:	Date:
Received By:	Date:	Storage:	Relinquished By:	Date:
Received By:	Date:	Storage:	Relinquished By:	Date:

Tire Crumb Rubber from Synthetic Turf Fields (D-SED-IEMB-001-SOP-02) – August 12, 2016

U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia; Cincinnati, Ohio; Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Standard Operating Procedure for Collection of Tire Crumb Rubber Samples from Synthetic Turf Fields for Metal, VOC/SVOC, and Particle Analysis

Number: D-SED-IEMB-001-SOP-02	Effective Date: 8/16/16

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: Kent Thomas

Signature: Date:

Approval

Name: Caroline Stevens
Title: Branch Chief, IEMB

Signature: Date:

Concurrence*

Name:
Title:

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

* Optional Field
NERL-SOP.1 (7/2003)

STANDARD OPERATING PROCEDURE FOR COLLECTION OF TIRE CRUMB RUBBER SAMPLES FROM SYNTHETIC TURF FIELDS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

The tire crumb rubber characterization study will involve the collection of tire crumb rubber material from tire recycling plants and synthetic turf fields around the U.S., with laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and particle characteristics. This standard operating procedure (SOP) describes collection of tire crumb rubber samples from synthetic turf fields.

2.0 SUMMARY OF THE METHOD

Tire crumb rubber samples will be collected from synthetic turf fields to support characterization of chemical and microbiological constituents. Field sampling personnel will arrive at a facility with synthetic turf fields and will discuss the sampling requirements and procedures and any facility-required safety requirements with facility staff.

Substantial variability in tire crumb rubber chemical concentrations has been reported. Therefore, tire crumb rubber samples will be collected at seven locations from each synthetic turf field. First, seven samples will be collected from seven locations at each field for VOC and SVOC analysis, the second set of seven samples will be collected at the same locations for metals analysis, and a third set of samples will be collected at the sample location for particle analysis. The seven samples collected for VOC/SVOC analysis and the seven samples collected for metals analysis will be composited in the laboratory prior to analysis.

For VOC/SVOC samples, a small handheld stainless-steel comb will be used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field will be no more than about 3 centimeters (cm) from the surface. Collected tire crumb rubber will be placed into certified pre-cleaned 250-milliliter (mL) amber glass wide-mouth containers with Teflon®-lined lids. Seven containers (one at each of the seven field locations) will be completely filled with tire crumb rubber material. Equal portions of these samples will be composited at the laboratory prior to analysis.

For metals samples, a small handheld plastic comb will be used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field will be no more than about 3 cm from the surface. Collected tire crumb rubber will be placed into certified pre-cleaned 250-mL high density polyethylene (HDPE) wide-mouth containers. Seven containers (one at each of the seven field locations) will be completely filled with tire crumb rubber material. Equal portions of these samples will be composited at the laboratory prior to analysis.

For particle samples, a small handheld plastic comb will be used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field will be no more than about 3 cm from the surface. Collected tire crumb rubber will be placed into

certified pre-cleaned 250-mL HDPE wide-mouth containers. Seven containers (one at each of the seven field locations) will be completely filled with tire crumb rubber material. The entire amount of these samples will be composited at the laboratory prior to analysis.

All samples will be shipped the same day as they are collected with next-day delivery service to a central processing laboratory.

Tire crumb rubber samples will also be collected from synthetic turf fields to support microbiome analysis following the SOP# D-SED-EFAB-009-SOP-01 *Collection of Samples from Artificial Turf Athletic Fields for Microbiome Analysis*. That procedure is briefly described here because tire crumb rubber samples will be collected for chemical and microbiological analyses at the same synthetic turf fields at the same time. Individual 25-mL microbiome samples will be collected from each field at all seven locations where samples for metals and VOCs/SVOCs are collected. Aseptic techniques must be employed while collecting and handling samples or sampling equipment. Nitrile (or appropriate alternative) gloves must be worn at all times when handling the sample or sampling equipment. A clean disposable lab coat will be worn during sample collection. A freshly opened sterile polypropylene spatula will be used at each field location to collect each 25-mL sample. To collect samples, the sterile spatula will be inserted into the athletic field surface at an approximate 30-degree angle to a maximum depth of 3 cm from the surface and moved forward to collect tire crumb material. The tire crumb rubber will be added to a sterile 50-mL polypropylene container with volumetric lines. The container will be filled with tire crumb rubber material to the 25-mL line. Once samples are collected, samples will immediately be placed in a cooler with ice packs. Samples will be shipped the same day as they are collected, in a container with ice packs, with next-day delivery service to the microbiological analysis laboratory. Refer to SOP# D-SED-EFAB-009-SOP-01 for specific procedures.

3.0 DEFINITIONS

SOP – standard operating procedure
COC – chain of custody
QC – quality control
RTP – Research Triangle Park
FB – field blank
HDPE – high-density polyethylene
SVOC – semi-volatile organic compound
VOC – volatile organic compound
CDC – Centers for Disease Control and Prevention
ATSDR – Agency for Toxic Substances and Disease Registry

4.0 CAUTIONS

4.1 Field staff will discuss any synthetic turf field facility safety requirements and

procedures with facility staff prior to initiating any sample collection activities.
4.2 Field staff will wear required safety equipment and will follow all facility safety guidelines and procedures.
4.3 Field staff will wear safety glasses and nitrile gloves during sample collection.
4.4 Field staff will not collect outdoor synthetic turf samples when a thunderstorm is present or in the vicinity.
4.5 Field staff will be aware of potential heat stress during sample collection and will wear appropriate clothing and have drinking water available.

5.0 RESPONSIBILITIES

5.1 The EPA project staff and/or contractors will provide the sample collection materials and deliver them to the field sampling personnel. The EPA staff and/or contractors will also be responsible for receiving tire crumb samples and ensuring proper storage at EPA facilities.
5.2 The field staff is also responsible for following any safety requirements of the facilities and wearing appropriate protective equipment including safety glasses and nitrile gloves during sample collection.

6.0 MATERIALS

6.1 250-mL HDPE jars, pre-cleaned and certified for trace metals analysis I-Chem 300 Series or equivalent
6.2 250-mL amber glass jars with Teflon-lined lids, pre-cleaned and certified for trace organic chemical analysis I-Chem 300 Series or equivalent
6.3 50-mL sterilized Falcon tubes
6.4 Stainless steel tine animal grooming comb (6.3” wide with 6/5” handle, and 1.6” tooth length) modified with an aluminum tire crumb rubber collection tray for pouring samples into containers
6.5 Plastic tine livestock comb (10.3” wide, 7.1” handle, 1.9” high) modified with an acrylic tire crumb rubber collection tray for pouring sample into containers
6.6 9” plastic sterilized spatula
6.7 Laboratory wipes – Kimwipes™ or equivalent
6.8 Acetone – Pesticide Grade or equivalent
6.9 Hexane – Pesticide Grade or equivalent
6.10 Ghost Wipes™ - Environmental Express P/N 4210
6.11 Deionized water
6.12 Nitrile gloves
6.13 Safety Glasses
6.14 Disposable lab coat
6.15 Ziploc storage bags, gallon size
6.16 Ziploc bags, 20” x 18” size
6.17 Sample labels
6.18 Ice packs
6.19 Shipping containers and packaging material

6.20 Shipping labels
6.21 Sample collection form, chain of custody (COC) record sheets, field sketch form
6.22 Construction marker flags for optional field location marking

7.0 PROCEDURES

Samples will be collected for trace metals, trace organics, particle, and microbiological analysis using slightly different procedures applicable to each type of analyte. The following procedures describe how sampling equipment is prepared prior to the synthetic turf field visit and how to collect the samples at the synthetic turf field.

7.1 *Equipment Preparation*

7.1.1 Field staff should don the appropriate laboratory cleaning equipment including nitrile gloves, safety glasses, and lab coat (if needed).
7.1.2 At the laboratory and prior to field use, clean stainless-steel combs by thoroughly rinsing with tap water, being sure to remove all material and dust on the comb tines using a brush if necessary, followed by wiping dry with a laboratory wipe. After drying, solvent rinse each comb with acetone followed by hexane using a wash bottle. Allow to air dry and store the cleaned combs in Ziploc® bags until use.
7.1.3 At the laboratory and prior to field use, clean plastic combs by thoroughly rinsing with tap water, being sure to remove all material and dust on the comb tines using a brush if necessary. Rinse the combs thoroughly with deionized water. Next, wipe the entire comb with one or more Ghost Wipes. Allow to air dry and store the cleaned combs in Ziploc® bags until use.
7.1.4 All waste produced during the cleaning process including wastewater, solvent rinse water, and gloves will be disposed of following the appropriate waste disposal procedure.
7.1.5 All of the materials and supplies needed for synthetic field sample collection will be assembled into ‘kits’ according to the list described in Table 1 (The materials and supplies for the microbiome kit is also included in Table 1 to have all of the information available on one page).

7.2 *General Sampling Requirements and Considerations*

7.2.1 Sample collection at synthetic turf fields must be performed on Monday, Tuesday, Wednesday, or Thursday to ensure that samples shipped overnight to the laboratories can be received and properly processed and stored on a weekday.
7.2.2 Collect tire crumb rubber samples from synthetic turf fields when the fields are not in use.
7.2.3 If possible, collect tire crumb rubber samples from synthetic turf fields under dry weather conditions.
7.2.4 Field staff may mark the seven sample locations at each field using small

marker flags or with other markers prior to sample collection.

7.2.5 One person should collect samples for microbiological analysis at all seven field locations as a continuous effort without performing any other sampling tasks. If only one person is collecting samples, collect all samples for microbiological analysis at all seven locations first before starting the metal, VOC/SVOC, and particle analysis sample collections. Alternatively, a second person could collect samples for microbiological analysis at the same time the other person is collecting the metal, VOC/SVOC, and particulate samples at each location.

7.2.6 Never touch the tire crumb rubber being collected with your hands (whether gloved or not) to avoid potential contamination of the tire crumb rubber samples. For example, do not attempt to wipe tire crumb rubber from sampling combs into containers with your hands.

7.2.7 Following tire crumb rubber sample collection at each location, the disturbed areas and any non-sampled tire crumb rubber brought to the field surface should be returned to its original state by using the sampling comb, foot, or gloved hand to redistribute disturbed material back into the turf.

7.3 *Synthetic Turf Field Sampling Locations*

7.3.1 Collect samples at seven different locations at each synthetic turf field as shown in Figure 1 for soccer/football fields. Follow the same pattern of collection locations for other rectangular fields. Collect samples at softball/baseball fields at seven different locations as shown in Figure 2.

7.3.2 If possible, collect samples from areas at each location that are free of trash or debris. If necessary, remove large trash or debris prior to sample collection, but do not disturb the underlying tire crumb rubber.

7.3.3 Ensure that the sample collection location of each sample container label (Section 8) matches the field location number in Figure 1 or Figure 2 as samples are being collected.

7.3.4 At each synthetic turf field sampling location (see Figures 1 and 2), ensure that the tire crumb rubber sample collection is conducted across an appropriate area that is slightly offset for each type of sample (metals, VOC/SVOC, particles, and microbiological) to avoid removing too much tire crumb rubber from any particular spot, and to avoid collecting tire crumb rubber that is too deep within the field. For example, after collecting 250 mL of tire crumb rubber for metals analysis at a location, move about a meter away to collect 250 mL of tire crumb rubber for VOC/SVOC analysis. Likewise, collect the 250 mL of sample for particle analysis about another meter away.

7.4 *Tire Crumb Rubber Sample Collection for Metals Analysis*

7.4.1 Go to the first field location where samples will be collected. Identify and

open the 250-mL HDPE container labeled for that location.

7.4.2 Using the plastic tine comb, hold the comb with fingers on top of the comb handle and thumb below the comb handle.

7.4.3 Gently insert the comb tines into the tire crumb rubber on the field at about a 30° angle, no more than about 3 cm below the surface. Using a modest side-to-side motion, gently pull the comb forward to collect tire crumb rubber on the comb tines. Use only gentle force to avoid, to the extent feasible, pulling out synthetic grass blades.

7.4.4 When the comb tines are covered with tire crumb rubber, and while the comb is still in contact with the field surface, tip the comb to shift the rubber crumbs onto the comb collection tray. Tilt the tray to add the rubber to the 250-mL HDPE container. Do not attempt to remove any loose synthetic turf blades at this time.

7.4.5 Repeat Steps 7.4.2 and 7.4.3 at immediately adjacent field areas (do not collect multiple times from exactly the same spot) until the 250-mL HDPE container is full. Seal the container tightly with the lid.

7.4.6 A polypropylene spatula that has previously been used to collect a sample for microbiological analysis may be used in alternative collection approach for fields where the comb is not effective. At the first sample collection site of the field, insert the spatula into the field surface at an approximate 30-45 degree angle to a maximum depth of approximately 3 cm from the surface and then push it horizontally through the infill to collect rubber on the spatula. Using this approach, scoop the rubber into the 250-mL HPDE container until it is full. Seal the container tightly with the lid.

7.4.7 Repeat Steps 7.4.1 – 7.4.5 or Step 7.4.6 for each of the remaining six field locations using the same plastic tine comb used at the first location.

7.4.8 Record details of sample collection on the sample collection form (Figure 3) and the COC record (Figure 4).

7.5 *Tire Crumb Rubber Sample Collection for VOC/SVOC Analysis*

7.5.1 Go to the first field location where samples will be collected. Identify and open the 250-mL amber glass container labeled for that location. (Note that samples can be collected in any location order provided that the container label location numbers match the field location number).

7.5.2 Using the stainless-steel tine comb, hold the comb with fingers on top of the comb handle and thumb below the comb handle. (See 7.5.6 for an alternate collection approach if the field blades and/or infill is packed too tightly for the comb to be effective).

7.5.3 Gently insert the comb tines into the tire crumb rubber on the field at about a 30° angle, no more than about 3 cm below the surface. Using a modest side-to-side motion, gently pull the comb forward to collect tire crumb rubber on the comb tines. Use only gentle force to avoid pulling out synthetic grass blades to the extent feasible.

7.5.4 When the comb tines are covered with tire crumb rubber, and while the

comb is still in contact with the field surface, tip the comb to shift the rubber crumbs onto the comb collection tray. Tilt the tray to add the rubber to the 250-mL glass container. Do not attempt to remove any loose synthetic turf blades at this time.

7.5.5 Repeat Steps 7.5.3 and 7.5.4 at immediately adjacent field areas (do not collect multiple times from exactly the same spot) until the 250-mL glass container is full. Seal the container tightly with the lid.

7.5.6 A polypropylene spatula that has previously been used to collect a sample for microbiological analysis may be used in alternative collection approach for fields where the comb is not effective. At the first sample collection site of the field, insert the spatula into the field surface at an approximate 30-45 degree angle to a maximum depth of approximately 3 cm from the surface and then push it horizontally through the infill to collect rubber on the spatula. Using this approach, scoop the rubber into the 250-mL glass container until it is full. Seal the container tightly with the lid.

7.5.7 Repeat Steps 7.5.2 – 7.5.5 or Step 7.4.6 for each of the remaining six field locations using the same stainless-steel tine comb used at the first location.

7.5.8 Record details of sample collection on the sample collection form (Figure 3) and the COC record (Figure 4).

7.6 *Tire Crumb Rubber Sample Collection for Particle Characterization Analysis*

7.6.1 Go to the first field location where samples will be collected. Identify and open the 250-mL HDPE container labeled for that location.

7.6.2 Using the plastic tine comb, hold the comb with fingers on top of the comb handle and thumb below the comb handle. (See 7.6.6 for an alternate collection approach if the field blades and/or infill is packed too tightly for the comb to be effective).

7.6.3 Gently insert the comb tines into the tire crumb rubber on the field at about a 30° angle, no more than about 3 cm below the surface. Using a modest side-to-side motion, gently pull the comb forward to collect tire crumb rubber on the comb tines. Use only gentle force to avoid, to the extent feasible, pulling out synthetic grass blades.

7.6.4 When the comb tines are covered with tire crumb rubber, and while the comb is still in contact with the field surface, tip the comb to shift the rubber crumbs onto the comb collection tray. Tilt the tray to add the rubber to the 250-mL HDPE container. Do not attempt to remove any loose synthetic turf blades at this time.

7.6.5 Repeat Steps 7.6.3 and 7.6.4 at immediately adjacent field areas (do not collect multiple times from exactly the same spot) until the 250-mL HDPE container is full. Seal the container tightly with the lid.

7.6.6 A polypropylene spatula that has previously been used to collect a sample for microbiological analysis may be used in alternative collection approach for fields where the comb is not effective. At the first sample collection site of the field, insert the spatula into the field surface at an

approximate 30-45 degree angle to a maximum depth of approximately 3 cm from the surface and then push it horizontally through the infill to collect rubber on the spatula. Using this approach, scoop the rubber into the 250-mL HPDE container until it is full. Seal the container tightly with the lid.

7.6.7 Repeat Steps 7.6.2 – 7.6.5 or 7.6.6 for each of the remaining six field locations using the same plastic tine comb used at the first location.

7.6.8 Record details of sample collection on the sample collection form (Figure 3) and the COC record (Figure 4).

7.7 *Storage and Shipping*

7.7.1 After each sample is collected, ensure that the lid is tightly sealed.

7.7.2 Verify that the sample codes on the sample container match the COC record. Verify that all required information is recorded and complete the COC record and sample collection form.

7.7.3 Insert the 250-ml amber glass jars for organics analysis into bubble wrap pouches. Place the bubble wrap protected glass jars into sections of the cardboard container box and seal the box. Place the entire cardboard box into an 18” x 20” Ziploc bag and seal the bag.

7.7.4 Place the 250-mL HDPE jars for metals analysis and the 250-mL HDPE jars for particle analysis into two (2) separate cardboard container boxes and seal the boxes. Place each entire cardboard box into an 18” x 20” Ziploc bag and seal the bag.

7.7.5 Package the three (3) cardboard container boxes containing sample jars into the overpack cardboard box.

7.7.6 Place all of the sample collection materials into the fourth cardboard box and place the box into the overpack cardboard box.

7.7.7 Place the completed COC records and sample collection forms in a Ziploc bag and place it in the box with the samples.

7.7.8 Ship the samples using a next-day delivery service to the EPA/RTP lab using the following address:

Kent Thomas (or designated alternate)
US EPA/NERL/SED
Chemical Services, Room E-178, Bldg. E Loading Dock
109 TW Alexander Dr.
RTP, NC 27709
(919) 541-7939
thomas.kent@epa.gov

7.7.9 Contact Kent Thomas or his designee via phone, voice mail, or email on the day that samples are collected and shipped to ensure the laboratories are prepared for receipt.

7.7.10 Upon receipt at the laboratory, EPA, or EPA contract staff will verify each sample container label against the COC record, initial receipt of the

sample, and if needed, record any comments about sample condition upon receipt.

7.7.11 Samples for metals and organics analysis received at the laboratory will either be immediately placed into the subsample preparation process following SOP# D-EMMD-PHCB-40-01, or the samples will be placed in a freezer at -20°C until they are processed.

7.7.12 Samples for particle analysis received at the laboratory will either be immediately placed into shipment to the EPA laboratory in Las Vegas or the samples will be placed in a freezer at -20°C until they are shipped.

7.7.13 The COC record will be provided to the EPA research manager or his/her designee.

8.0 RECORDS

Any information relevant to sample collection should be recorded on the field collection form (Figure 3), and COC record (Figure 4). The required COC record information includes the facility ID, the date and time of sample collection, and initials of the staff member that collected the sample. Field staff should also include, if needed, any information about the sample or sample collection that might be relevant or useful in sample analysis or data interpretation in the comment field of the COC record.

All samples will be labelled with a unique sample ID code using the following scheme:

TCRS-F-VV-W-X-Y-Z

Where:
TCRS = tire crumb rubber research study
F = synthetic turf field designation
VV = two-digit code unique to each synthetic turf field, from 10 to 75
W = location on the field from 1 to 7; except this will be 9 for field blanks
X = sample bottle designation from each location; this will always be 1 for field samples
Y = sample type identifier M (metals) or G (organic compounds) or P (particles)
Z = parent/daughter designator – all of these samples are parents with a ‘0’ designator
The sample codes are defined in Table 2.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Field blank samples will be collected for metals and organic samples at each field. The field blanks will consist of one (1) 250-mL HDPE container and one (1) 250-mL glass container that are taken to the synthetic turf field, briefly opened then closed, and packaged and returned to the laboratory with the samples. No tire crumb rubber is placed into the field blank containers.

9.2 No fortified field controls will be collected because no suitable control materials are

available for tire crumb rubber.

9.3 Field sampling audits will be performed at a subset of the synthetic turf fields by EPA staff and/or contractors or by CDC/ATSDR staff and/or contractors.

10.0 REFERENCES

- Research Protocol. *Collections related to synthetic turf fields with crumb rubber infill*, August 5, 2016. U.S. EPA and CDC/ATSDR.
- Standard Operating Procedure: D-SED-EFAB-009-SOP-01. *Collection of samples from artificial turf athletic fields for microbiome analysis*, August 2016. U.S. EPA.
- Standard Operating Procedure: D-EMMD-PHCB-40-01. *Standard operating procedure for storage and preparation of tire crumb rubber subsamples for multi-residue characterization*, August 2016. U.S. EPA.

11.0 REVISIONS

It was discovered upon initial implementation of this SOP that some field surfaces were too dense for the comb collection method to operate correctly. The SOP was revised to allow optional collection of tire crumb rubber infill using the spatula method that was included as part of SOP D-SED-EFAB-009-SOP-02. This alternative collection option effective date was 08/16/2016. This optional method was communicated immediately to all sample collection staff but was not formalized into a revised SOP until a later date.

Table 1. Synthetic Field Sampling Kit Materials/Supplies Lists

Box 1 (Cooler for microbiological tire crumb samples)	
	Cooler
	8 sterile scoops
	Tyvek lab coat
	1 pair safety glasses
	8 pairs of nitrile gloves (size: large)
	Poly tube rack with 8 pre-labeled Falcon tubes and tube containing an iButton temperature tracking device, all inside a 16" x 16" size Ziploc bag
	10 individual ice packs (packaged as 2 packs in a quart Ziploc bag)
	Layer of bubble wrap
	Ziploc bag containing: • COC record form for microbial samples • Return shipping label

Box 2 (Box for metals, particles, organics tire crumb rubber samples)	
	1 inner box (no dividers) containing seven (7) pre-labeled 250-mL HDPE bottles and box of lab tissues and bubble wrap
	1 inner box (no dividers) containing eight (8) pre-labeled 250-mL HDPE bottles and bubble wrap
	1 inner box w/dividers containing eight (8) pre-labeled 250-mL amber glass bottles with bubble pouches
	1 inner box containing: • 1 pre-cleaned metal comb inside 1 gal Ziploc bag • 1 pre-cleaned plastic comb inside 1 gal Ziploc bag • 8 pairs of size large nitrile gloves in 1 gal Ziploc bag • 1 roll of packaging tape with dispenser • 1 – 12" x 16" Ziploc bag marked "Trash" • 1 pair safety glasses • 7 pink flag markers • 3 – 18" x 20" Ziploc bags for inner box containment for return shipment
	Ziploc bag containing: • COC record forms for metals, organics, and particles • Sampling Collection Form with OMB approval info • Return shipping label

Table 2. TCRS Sample Codes for Synthetic Turf Field Tire Crumb Rubber Samples

Synthetic Field Tire Crumb Rubber Sample Coding – Metals Analysis
(XX = Field ID Number)

Sample Code	Description
TCRS-F-XX-1-1-M-0	Metals sample – 250-mL HDPE Jar – Location 1
TCRS-F-XX-2-1-M-0	Metals sample – 250-mL HDPE Jar – Location 2
TCRS-F-XX-3-1-M-0	Metals sample – 250-mL HDPE Jar – Location 3
TCRS-F-XX-4-1-M-0	Metals sample – 250-mL HDPE Jar – Location 4
TCRS-F-XX-5-1-M-0	Metals sample – 250-mL HDPE Jar – Location 5
TCRS-F-XX-6-1-M-0	Metals sample – 250-mL HDPE Jar – Location 6
TCRS-F-XX-7-1-M-0	Metals sample – 250-mL HDPE Jar – Location 7
TCRS-F-XX-9-1-M-0	Metals sample – 250-mL HDPE Jar – Field Blank

Synthetic Field Tire Crumb Rubber Sample Coding – Particle/Dust Analysis
(XX = Field ID Number)

Sample Code	Description
TCRS-F-XX-1-1-P-0	Particle sample – 250-mL HDPE Jar – Location 1
TCRS-F-XX-2-1-P-0	Particle sample – 250-mL HDPE Jar – Location 2
TCRS-F-XX-3-1-P-0	Particle sample – 250-mL HDPE Jar – Location 3
TCRS-F-XX-4-1-P-0	Particle sample – 250-mL HDPE Jar – Location 4
TCRS-F-XX-5-1-P-0	Particle sample – 250-mL HDPE Jar – Location 5
TCRS-F-XX-6-1-P-0	Particle sample – 250-mL HDPE Jar – Location 6
TCRS-F-XX-7-1-P-0	Particle sample – 250-mL HDPE Jar – Location 7

Synthetic Field Tire Crumb Rubber Sample Coding – Organics Analysis
(XX = Field ID Number)

Sample Code	Description
TCRS-F-XX-1-1-G-0	Organics sample – 250-mL Glass Jar – Location 1
TCRS-F-XX-2-1-G-0	Organics sample – 250-mL Glass Jar – Location 2
TCRS-F-XX-3-1-G-0	Organics sample – 250-mL Glass Jar – Location 3
TCRS-F-XX-4-1-G-0	Organics sample – 250-mL Glass Jar – Location 4
TCRS-F-XX-5-1-G-0	Organics sample – 250-mL Glass Jar – Location 5
TCRS-F-XX-6-1-G-0	Organics sample – 250-mL Glass Jar – Location 6
TCRS-F-XX-7-1-G-0	Organics sample – 250-mL Glass Jar – Location 7
TCRS-F-XX-9-1-G-0	Organics sample – 250-mL Glass Jar – Field Blank

Synthetic Turf Playing Field Sample ID Code - TCRS-F-VV-W-X-Y-Z

Where:TCRS = tire crumb rubber research study; F = playing field designation; VV = two- digit code unique to each playing field (from 10 to 75); W = Playing field sampling location (1-7), Composite Samples (C), Field Blanks (9); X = Sample jar designation, 1 for all samples; Y = Sample type designation, M (Metals), G (Organics), P (Particle/dust), B (Microbial); Z = Assigned analysis number codes for sub-sample aliquots; this is zero for parent samples.

Figure 1. Sample Collection Locations for Synthetic Turf Soccer/Football Fields

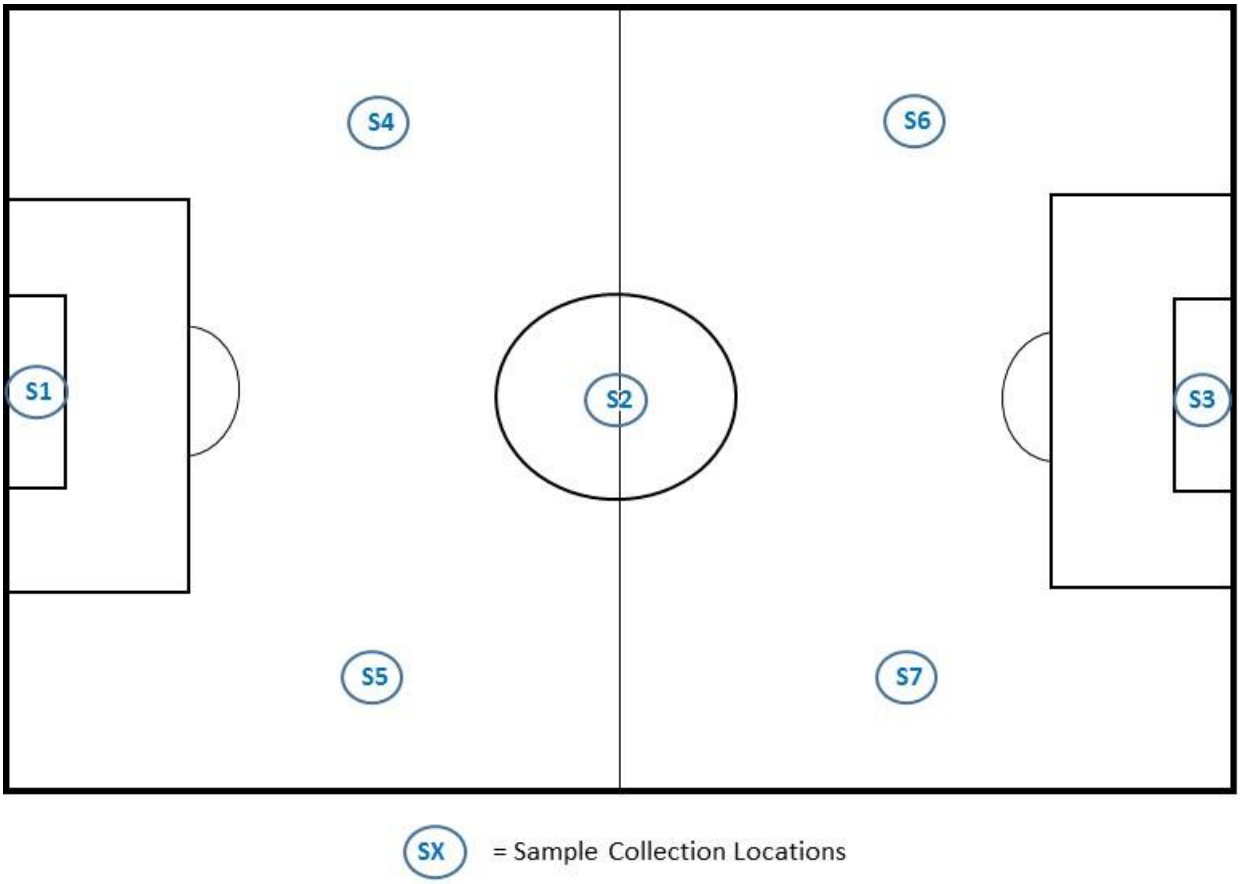


Figure 2. Sample Collection Locations for Synthetic Turf Baseball/Softball Fields

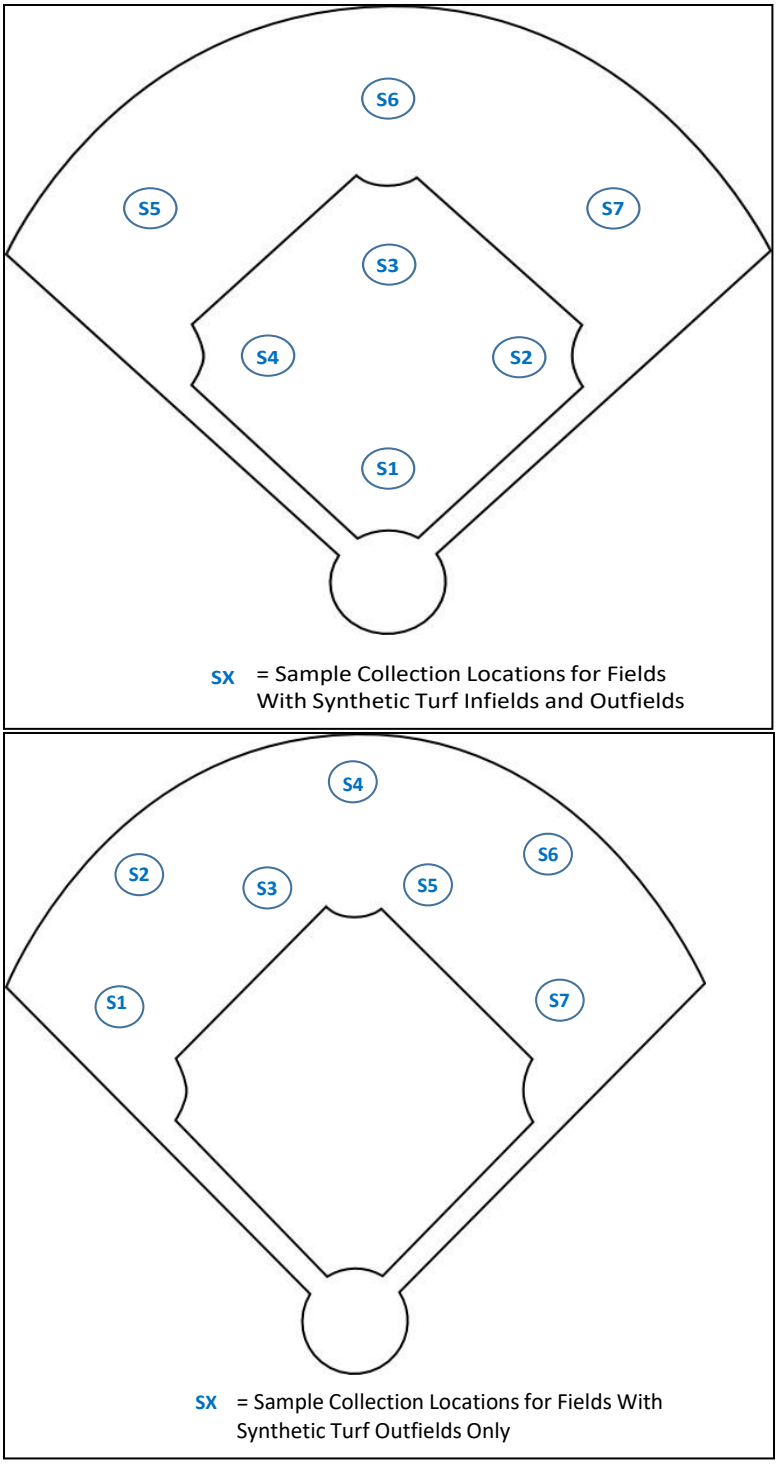


Figure 3. Synthetic Turf Field Sampling Collection Form

Form Approved
OMB No. 0923-0054
Exp. Date 01/31/2017

Study ID Number _____
Sample Collection Date _____
Collector ID _____

Crumb Rubber Samples Collection – For Metals (Plastic Containers)

Field Location	Sample Collected
S1	Yes No
S2	Yes No
S3	Yes No
S4	Yes No
S5	Yes No
S6	Yes No
S7	Yes No

Crumb Rubber Samples Collection – For Organics (Glass Containers)

Field Location	Sample Collected
S1	Yes No
S2	Yes No
S3	Yes No
S4	Yes No
S5	Yes No
S6	Yes No
S7	Yes No

Crumb Rubber Samples Collection – For Microbes (Sterile Plastic Containers)

Field Location	Sample Collected
S1	Yes No
S2	Yes No
S3	Yes No
S4	Yes No
S5	Yes No
S6	Yes No
S7	Yes No

Particle/Dust Sample Collection – For Metals (Plastic Container)

Field Location	Sample Collected
S1	Yes No
S2	Yes No
S3	Yes No
S4	Yes No
S5	Yes No
S6	Yes No
S7	Yes No

Figure 4. Chain of Custody Record

Tire Crumb Research Study Site ID Code: Sample Analysis Type:		Site Information if Needed (Do not include facility name or location here):	
Sample ID	Description if Needed	Comments if Needed	
Collected By: Shipped By:	Date Collected: Date Shipped:	Collection Start Time:	Collection End Time:
Received By:	Date:	Storage:	Relinquished By:
Received By:	Date:	Storage:	Relinquished By:
Received By:	Date:	Storage:	Relinquished By:

Tire Crumb Rubber from Synthetic Turf Fields for Microbiome Analysis (D-SED-EFAB-009-SOP-01) – August 12, 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory Systems
Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Collection of Samples from Artificial Turf Athletic Fields for Microbiome Analysis

Number: D-SED-EFAB-009-SOP-01	Effective Date: June 9, 2016

SOP was developed: ☒In-house ☐ Extramural

Alternative Identification: EFAB-009-01

SOP Steward

Name: Nichole Brinkman, NERL/SED/EFAB

Approval

Name: Valerie Garcia
Title: Chief, NERL/SED/EFAB

Concurrence

Name: Brittany Stuart
Title: SED Quality Assurance Manager

STANDARD OPERATING PROCEDURE FOR COLLECTION OF TIRE CRUMB RUBBER SAMPLES FROM SYNTHETIC TURF FIELDS FOR MICROBIOME ANALYSIS

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of the procedure is to outline the steps necessary to collect tire crumb rubber from artificial fields and soil from natural fields for analysis of the microbiome.

2.0 SCOPE AND APPLICABILITY

The task described in this SOP is limited to the collection of samples on natural and artificial fields. On artificial fields, tire crumb rubber will be collected. Sample collection for microbiome analysis requires aseptic techniques as tools, vessels, and individuals performing the collection can contaminate the sample. This SOP has been developed for the Tire Crumb Microbiome Project.

3.0 SUMMARY OF METHOD

This SOP describes the steps to aseptically collect samples for microbiome analysis. Samples will be collected by individuals wearing appropriate personal protective equipment such that they will not contribute their microbiome to the sample. Additionally, sterile tools and vessels will be used to scoop and store samples. Samples will be stored on ice immediately after collection and shipped to the laboratory for processing within 24 hours.

4.0 DEFINITIONS

BSL – biosafety level
PPE – personal protective equipment
Microbiome – the microorganisms that reside in a particular habitat/niche
PSI – pounds per square inch

5.0 HEALTH AND SAFETY WARNINGS

5.1 Standard laboratory PPE for Biosafety Level (BSL) 1 microorganisms is required. This PPE consists of lab coat, safety glasses, and gloves.

5.2 Known hazards consist of burns from the autoclave to sterilize sampling equipment (stainless steel spatula), accidental puncture using sampling tool when sampling, and exposure to tire crumb rubber and soil samples during sampling from fields.

6.0 CAUTIONS/INTERFERENCES

6.1 Several precautions should be considered to reduce potential contamination and loss/growth of the microbes collected in field samples. Samples should be collected under aseptic conditions so that outside influences (sampler, tools, and storage vessels) do not contaminate the sample. Additionally, microbes present in

samples can begin to degrade or proliferate after collection. To minimize potential microbial loss and growth, samples should be stored at 4°C immediately after collection. Samples should be shipped to the laboratory for arrival at the earliest morning delivery so that samples can be processed within 24 hours of collection.

6.2 A field blank will be used to control for potential introduction of contamination during sampling. The field blank will consist of the sampler opening the vessel and placing a sterile sampling tool into the vessel, but omitting a sample. To evaluate temperature after sample collection and during shipping, iButtons (see SOP# D-SED-EFAB-010-SOP-01 for the *iButton Temperature Logging System*) will be added to coolers to monitor temperature during shipping.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The only qualification of the sampler required to perform this task is that he/she wears appropriate PPE and handles sampling tools and storage containers while wearing gloves.

8.0 EQUIPMENT AND SUPPLIES

8.1 To collect and ship samples, the following materials are needed:

- 8.1.1 Lab coat
- 8.1.2 Safety goggles
- 8.1.3 Laboratory gloves (nitrile or latex) Sterile disposable polypropylene spatula
- 8.1.4 Sterile 50-ml polypropylene conical tube with graduations Cooler (16 quarts should be more than sufficient)
- 8.1.5 Cold blocks or ice packs in Ziploc bag
- 8.1.6 Packing material
- 8.1.7 iButton and USB Temperature Data Logger
- 8.1.8 The USB Temperature Data Logger is needed to evaluate temperature recorded with the iButton system.

9.0 PROCEDURE

9.1 Put on lab coat, safety goggles, and gloves. A new, clean pair of gloves should be donned for each sample collection location (each of the seven sites on the field).

9.2 Obtain materials listed above for collection and storage of samples (all is needed except the USB Temperature Data Logger).

9.3 At each sample collection site of the field, insert the sterile spatula into the athletic field or natural soil surface at an approximate 30-45-degree angle to a maximum depth of 1 inch (2.54 cm) from the surface.

9.4 Scoop the crumb rubber or soil into a sterile 50-ml polypropylene conical tube and fill to the 25-ml line.

9.5 Immediately seal the conical tube and label with date, time, field identification (as specified in the Field Sampling QAPP), sample collection location, and analyst's initials.

9.6 Place samples in a Ziploc bag and store in a cooler filled with ice packs or cold

blocks.

9.7 To collect a field blank, at field level of sample collection site S2 (see Figure 1), open the sterile 50-ml conical tube, insert an empty sterile spatula, remove spatula then close the tube. Label tube with date, time, field location, "Field Blank", and analyst's initials. Place conical tube in Ziploc bag with samples and store in cooler filled with ice packs or cold blocks.

9.8 Place an iButton in a separate 50-ml conical tube and place in a Ziploc bag and store with the samples in the cooler filled with ice packs or cold blocks. Do not place iButton near or directly on top of ice packs or cold blocks. Record iButton serial number on the Field Sample Collection Form (Figure 2).

9.9 Record data in the Field Sample Collection Form (Figure 2) and Chain of Custody record, sign forms for custody control and place in Ziploc bag and add to cooler.

9.10 Pack the cooler with packing material and ship to:

Nichole Brinkman
26 West Martin Luther King Drive
MS 587
Cincinnati, OH 45268

9.11 Upon receipt, the analyst receiving the samples will verify temperature of samples with the iButton and USB Temperature Data Logger, as described in SOP# D-SED-EFAB- 010-SOP-01.

10.0 DATA AND RECORDS MANAGEMENT

The Field Sample Collection Form (Figure 2) and Chain of Custody record will be incorporated into lab notebooks of the analyst receiving the samples. The analyst will assess the temperature conditions of samples during shipment using SOP# D-SED-EFAB-010-SOP- 01, and produce a temperature plot and alarm log, which will also be incorporated into the lab notebook.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 The quality control procedures required to demonstrate successful performance of sample collection and shipment are field blanks and the use of the iButton to monitor sample temperature after collection and during shipment to the analytical lab.

11.2 Field blanks will consist of a negative control to monitor for potential sampler contributions during collection of field samples. All equipment and procedures will be used as outlined above.

11.3 One field blank will be collected at every field sampled and will be used to assess the quality of the seven samples collected at each field. One iButton will be used with the seven (7) samples collected at each field.

11.4 If the iButton results show that the sample temperatures exceeded the 2 - 8°C acceptable temperature range, this information will be recorded and used in the interpretation of sample data. The field blank will be processed in subsequent

SOPs to verify sterility in sample collection. If samples show the presence of microbes, this information will be recorded and used in the interpretation of sample data.

11.5 The results of the quality control procedures will be documented in the analyst's notebook. All quality control failures will be reported to the Technical Lead. The quality control results will be described in a report at the completion of the project.

11.6 Additional quality control samples may be incorporated into individual field sampling quality assurance project plans.

12.0 REFERENCES

- SOP# D-SED-EFAB-009-SOP-01: *iButton temperature logging system*
- SOP# D-SED-IEMB-007-QAPP-01: *Field sampling of tire crumb rubber to characterize chemical and microbial constituents*

13.0 DOCUMENT HISTORY

Date of Revision	Revision #	Description of Changes
6/09/2016	0	First version

Figure 1. Sample locations of athletic fields

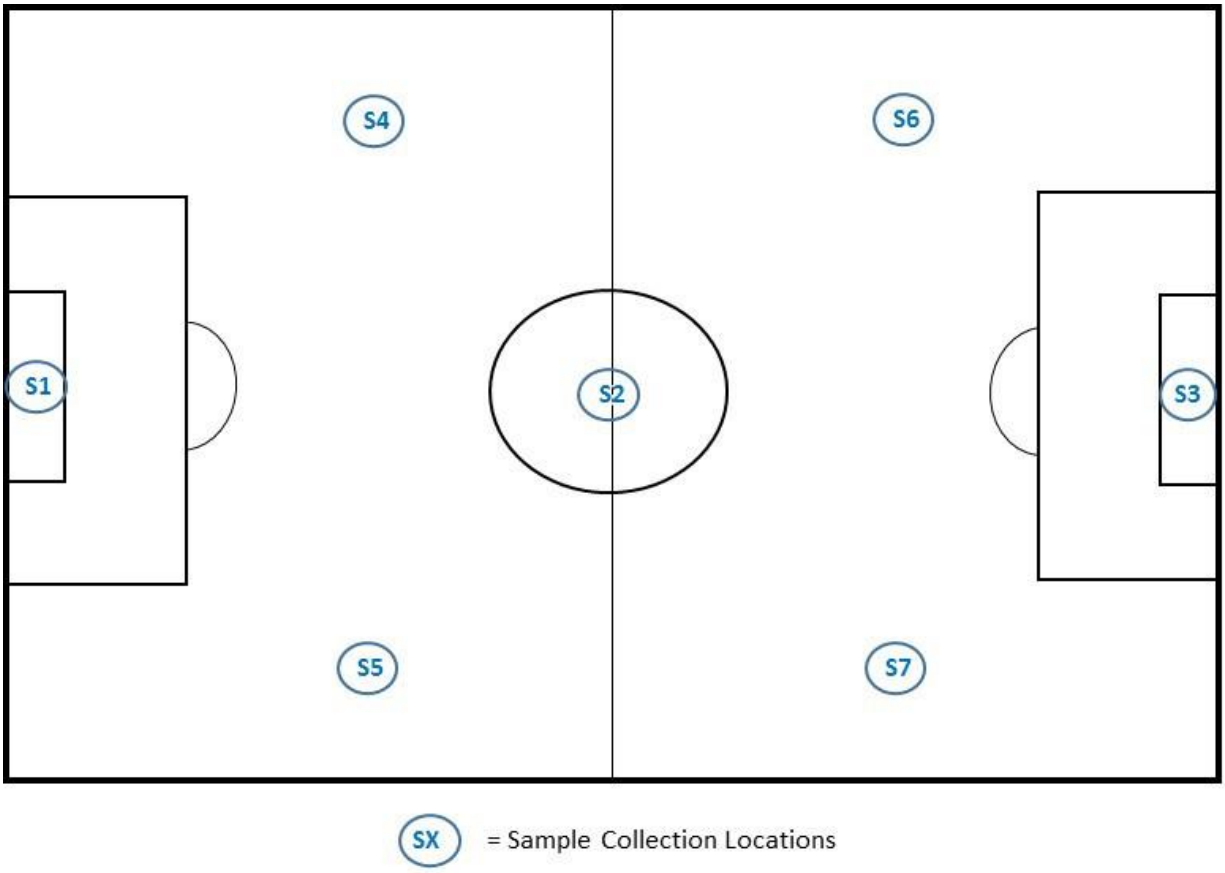


Figure 2. Tire Crumb Rubber Sample Collection Form

Tire Crumb Rubber Sample Collection Form	
Sample Collection Information	
Date and time	
Analyst	
Field Location/ID	
GPS Coordinates	
iButton Serial Number (one per field site)	

Environmental Variables	
Air Temperature (°C)	
Field Temperature (°C)	
Weather description (circle)	Sunny, partly cloudy, overcast, raining
Relative humidity (%)	
Are humans present? (circle)	Yes No If No, when was the last report of human activity?

Questionnaire for Administrators of Synthetic Turf Fields on Operations, Turf History, Maintenance, and Use (D-SED-EHCAB-002-SOP-01) – TBD

U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Standard Operating Procedure for Administering the Synthetic Turf Fields Questionnaire (Owner/Manager)

Number: D-SED-EHCAB-002-SOP-01	Effective Date: TBD

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EHCAB-002-01

SOP Steward

Name: Marsha K. Morgan

Signature: Date:

Approval

Name: Kent W. Thomas
Title: Tire Crumb Leader

Signature: Date:

Concurrence*

Name: Brittany Stuart
Title: QA Manager, Systems Exposure Division

Signature: Date:

STANDARD OPERATING PROCEDURE FOR QUESTIONNAIRE FOR ADMINISTRATORS OF SYNTHETIC TURF FIELDS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

The Synthetic Turf Fields Questionnaire is used to collect data from managers or owners of athletic facilities that have artificial turf fields with tire crumb infill. The purpose of the questionnaire is to collect information on general facility operations, turf history and maintenance, and public use at the facilities.

2.0 SUMMARY OF THE METHOD

Research staff administer the Synthetic Turf Fields Questionnaire to owners or managers using a computer-assisted interview (CAI) method. This questionnaire is used to record specific types of data including type of fields (e.g., indoor or outdoor), age of fields, sports/activities played on fields, frequency and hours of field use, and turf maintenance, cleaning, and replacement practices. Using a laptop computer, research staff open the Synthetic Turf Fields questionnaire via the Epi Info software program and ask facility owners or managers each question and record their responses. Research staff save all participant responses recorded in the questionnaires prior to exiting the Epi Info software program. They also make a backup copy of all questionnaire responses using a portable flash drive.

3.0 DEFINITIONS

SOP – standard operating procedure
CAI – computer-assisted interview
COC – chain of custody

4.0 CAUTIONS

Research staff must keep all completed participant questionnaires on the laptop computer and portable flash drive in a secure location at all times.

5.0 RESPONSIBILITIES

5.1 The EPA Database Manager will be responsible for providing the questionnaire (Epi Info and PDF versions) to the EPA Tire Crumb Team Leader.
5.2 The EPA Tire Crumb Team Leader will be responsible for providing the questionnaire to the appropriate research staff members responsible for questionnaire administration.
5.3 The research staff member(s) designated to administer the questionnaire will be responsible for completing the participant questionnaires. They are also be responsible for returning the completed questionnaires (via portable flash drives), including Chain of Custody (COC) record, to the EPA Tire Crumb Team Leader.

6.0 MATERIALS

6.1 Laptop computer
6.2 Epi Info software, version 7.1.5
6.3 Adobe Acrobat XI Pro
6.4 Encrypted, portable USB thumb drives (see Reference section)
6.5 COC records

7.0 PROCEDURES

7.1 *Administration of the Questionnaire (See Appendix A)*

7.1.1 Turn on the designated laptop computer (password protected) assigned to this project.
7.1.2 Open the Epi Info software program located on the desktop of the laptop computer. On the main screen of this software program, click on the "Enter Data" button. At the top of the screen, click on "Open Form" and then click on the button (with three dots) to open a current project folder. Next, find the Tire Crumb Questionnaire folder located under Epi Info 7 Projects Folder. In this folder, select the Facility Info.prj file, click the "Open" button, and then the "OK" button.
7.1.3 At the top of the screen, click on the "New Record" button. This questionnaire has a total of 30 questions. Record the Site ID Number (obtained via the EPA Tire Crumb Team Leader), Interviewer Date, and Interviewer ID at the top of the questionnaire form.
7.1.4 Begin the questionnaire. Ask the facility owner or manager the first question and record his/her response to the question. Repeat this procedure for each question.
7.1.5 After the questionnaire is completed, go to the top of the screen, select "File", click on the "Save" button and save the file as FacilitySiteXX (example: FacilitySite01).
7.1.6 For the next participant, repeat steps 7.1.3 – 7.1.5.
7.1.7 Exit the Epi Info software program by selecting "File" at the top of the screen and then click on "Exit."
7.1.8 Make a backup copy of the Tire Crumb Questionnaire folder using only the study-designated portable flash drive. Label the flash drive with a unique identifier (example: TCRSFacility Questionnaire). *All files will be uploaded to this one flash drive.

8.0 RECORDS

COC records (Appendix B) will be used to document the transfer of the participant questionnaire data.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

Proper COC records shall be kept documenting the transfer and receipt of all questionnaire data by EPA's Tire Crumb Team Leader at the EPA laboratory in Research Triangle Park, NC.

10.0 REFERENCES

- *Quality Assurance Project Plan, Activity Characterization for the Tire Crumb Research Study*, National Exposure Research Laboratory, Research Triangle Park, N.C., 2016.
- Epi Info 7 User Guide. 2016. https://www.cdc.gov/epiinfo/user-guide/
- How to securely encrypt a USB flash drive. http://www.online-tech-tips.com/computer-tips/encrypt-usb-flash-drive/ (accessed on June 24, 2016).

Appendix A: Synthetic Turf Fields Questionnaire

Form Approved
OMB No. 0923-XXXX
Exp. Date xx/xx/20xx

Owner/Manager Synthetic Turf Fields Questionnaire

Site ID Number	Interview Date	Interviewer ID

Interviewer: In this interview, I would like to ask you some general questions about your role and responsibilities at this facility and about the operation, maintenance, and use of the synthetic turf fields with crumb rubber infill at your facility.

A1. Who owns the facility?

A1.a What type of organization owns the facility?
☐Private
☐School
☐City
☐County
☐State
☐Military/Federal
☐(enter other if necessary)

A2. What is your profession and relationship to this facility?
(owner or manager)

A3. How long have you operated this facility?

Months	**Years**

A4. May I have your phone number and E-mail address for future contact?
Phone

E-mail

Facility User Information

A5. Are the synthetic fields at this facility open to the public?

☐Yes
☐No
☐Don't Know
☐Refused

A6. Is there an open or free-play schedule at this facility?

☐Yes
☐No
☐Don't Know
☐Refused

A7. Is field use at this facility limited to organization membership or school use only?

☐Yes
☐No
☐Don't Know
☐Refused

If yes, what organization(s) use the synthetic fields?

A8. How many days per week are the synthetic fields open at this facility during each season?

Days per week (spring)

Days per week (summer)

Days per week (fall)

Days per week (winter)

A9. What is the average number of hours per day that people use the synthetic fields at this facility during the four seasons?

Hours per day (spring)	
Hours per day (summer)	
Hours per day (fall)	
Hours per day (winter)	

A10a. On average, how many people per day use the synthetic fields at this facility during spring?

A10b. On average, how many people per day use the synthetic fields at this facility during summer?

A10c. On average, how many people per day use the synthetic fields at this facility during fall?

A10d. On average, how many people per day use the synthetic fields at this facility during winter?

A11. For each of the different age groups, what sports or other activities are played on the synthetic turf fields at this facility during which seasons (*check all that apply*)?

			Spring	**Summer**	**Fall**	**Winter**
☐ < 6	☐	Soccer	☐	☐	☐	☐
	☐	Football	☐	☐	☐	☐
	☐	Field Hockey	☐	☐	☐	☐
	☐	Baseball	☐	☐	☐	☐
	☐	Softball	☐	☐	☐	☐
	☐	Rugby	☐	☐	☐	☐
	☐	Ultimate Frisbee	☐	☐	☐	☐
	☐	Physical Training (PT)	☐	☐	☐	☐
	☐	Physical Education (PE)	☐	☐	☐	☐
	☐	Other:	☐	☐	☐	☐
	☐	Other:	☐	☐	☐	☐
☐ 6 – 11	☐	Soccer	☐	☐	☐	☐
	☐	Football	☐	☐	☐	☐
	☐	Field Hockey	☐	☐	☐	☐
	☐	Baseball	☐	☐	☐	☐
	☐	Softball	☐	☐	☐	☐
	☐	Rugby	☐	☐	☐	☐
	☐	Ultimate Frisbee	☐	☐	☐	☐
	☐	Physical Training (PT)	☐	☐	☐	☐
	☐	Physical Education (PE)	☐	☐	☐	☐
	☐	Other:	☐	☐	☐	☐
	☐	Other:	☐	☐	☐	☐

A11 ***(continued)***. For each of the different age groups, what sports or other activities are played on the synthetic turf fields at this facility during which seasons (*check all that apply*)?

				Spring	Summer	Fall	Winter
☐	12 – 18	☐	Soccer	☐	☐	☐	☐
		☐	Football	☐	☐	☐	☐
		☐	Field Hockey	☐	☐	☐	☐
		☐	Baseball	☐	☐	☐	☐
		☐	Softball	☐	☐	☐	☐
		☐	Rugby	☐	☐	☐	☐
		☐	Ultimate Frisbee	☐	☐	☐	☐
		☐	Physical Training (PT)	☐	☐	☐	☐
		☐	Physical Education (PE)	☐	☐	☐	☐
		☐	Other:	☐	☐	☐	☐
		☐	Other:	☐	☐	☐	☐
☐	18+	☐	Soccer	☐	☐	☐	☐
		☐	Football	☐	☐	☐	☐
		☐	Field Hockey	☐	☐	☐	☐
		☐	Baseball	☐	☐	☐	☐
		☐	Softball	☐	☐	☐	☐
		☐	Rugby	☐	☐	☐	☐
		☐	Ultimate Frisbee	☐	☐	☐	☐
		☐	Physical Training (PT)	☐	☐	☐	☐
		☐	Physical Education (PE)	☐	☐	☐	☐
		☐	Other:	☐	☐	☐	☐
		☐	Other:	☐	☐	☐	☐

Facility Information

A12. Do you have any standard practices in place to reduce tire crumb exposure to people using the synthetic fields?

If so, please describe:

Outdoor Field Only

A13. Are there outdoor fields at this facility?

A14. When was each outdoor synthetic field installed at this facility?

Field	Month	Year

A15. Which company or companies installed these fields?

A16. Do you ever replace all of the tire crumb infill on the outdoor synthetic turf field(s) at your facility?

- ☐Yes
- ☐No
- ☐Don't Know
- ☐Refused

If yes, how often do you replace all of the tire crumb infill on the synthetic turf fields?

- ☐Never/Rarely
- ☐Every 6 months
- ☐Yearly
- ☐Every 2-3 years
- ☐Every 3-5 years
- ☐Every 5-7 years
- ☐More than 7 years
- ☐Don't Know
- ☐Refused

A17. Do you ever refresh or add tire crumb infill to your outdoor synthetic turf field(s) at your facility?

☐Yes
☐No
☐Don't Know
☐Refused

If yes, how often do you refresh or add tire crumb infill to your synthetic turf fields?

☐Never/Rarely
☐Every 6 months
☐Yearly
☐Every 2-3 years
☐Every 3-5 years
☐Every 5-7 years
☐More than 7 years
☐Don't Know
☐Refused

A18. What was the date of the most recent replacement/refreshment?

A19. Which company or companies provide(s) crumb rubber infill material for replacement/refreshment?

A20. Are the following routine field maintenance activities performed on the outdoor synthetic field(s) at this facility?

	Activity	**Times**	**Per**
☐	Sweeping		Day / week / month / year
☐	Brushing		Day / week / month / year
☐	Redistribution/leveling		Day / week / month / year
☐	Aerating		Day / week / month / year
☐	Magnet sweep		Day / week / month / year
☐	Rejuvenation		Day / week / month / year

☐ Deep Cleaning ____ Day / week / month / year

A21. Has the outdoor synthetic field(s) ever been treated with biocides, herbicides, insecticides, fungicides, or other agents?

☐Yes
☐No
☐Don't Know
☐Refused

A22. Have any of the following chemicals been used on the field and how often (*check all that apply*)?

	Chemical	Times	Per
☐	Algae Died B		Day / week / month / year
☐	Qualgex		Day / week / month / year
☐	Steri-maX		Day / week / month / year
☐	Other (specify)		Day / week / month / year
☐	Unknown Biocide		Daily/weekly/ monthly/annually

<u>Indoor Fields Only</u>

A23. Are there indoor fields at this facility?

A24. When was each indoor synthetic field installed at this facility?

Field	Month	Year

A25. Which company or companies installed these fields?

A26. Do you ever replace all of the tire crumb infill on the indoor synthetic turf field(s) at your facility?

☐Yes
☐No
☐Don't Know
☐Refused

If yes, how often do you replace all of the tire crumb infill on the indoor synthetic turf fields?

☐Never/Rarely
☐Every 6 months
☐Yearly
☐Every 2-3 years
☐Every 3-5 years
☐Every 5-7 years
☐More than 7 years
☐Don't Know
☐Refused

A27. Do you ever refresh or add tire crumb infill to your indoor synthetic turf field(s) at your facility?

☐Yes
☐No
☐Don't Know
☐Refused

If yes, how often do you refresh or add tire crumb infill to your indoor synthetic turf fields?

☐Never/Rarely
☐Every 6 months
☐Yearly
☐Every 2-3 years
☐Every 3-5 years
☐Every 5-7 years
☐More than 7 years
☐Don't Know
☐Refused

A28. What was the date of the most recent replacement/refreshment?

A29. What company or companies provide(s) crumb rubber infill material for replacement/refreshment?

A30. Are the following routine field maintenance activities performed on the indoor synthetic field(s) at this facility?

	Activity	Times	Per
☐	Sweeping		Day / week / month / year
☐	Brushing		Day / week / month / year
☐	Redistribution/leveling		Day / week / month / year
☐	Aerating		Day / week / month / year
☐	Magnet sweep		Day / week / month / year
☐	Rejuvenation		Day / week / month / year
☐	Deep Cleaning		Day / week / month / year

A31. Has the outdoor synthetic field(s) ever been treated with biocides, herbicides, insecticides, fungicides, or other agents?

☐Yes
☐No
☐Don't Know
☐Refused

A32. Have any of the following chemicals been used on the field? (check all that apply) and how often?

	Chemical	Times	Per
☐	Algae Died B		Day / week / month / year
☐	Qualgex		Day / week / month / year
☐	Steri-maX		Day / week / month / year
☐	Other (specify)		Day / week / month / year
☐	Unknown Biocide		Daily/weekly/ monthly/annually

A33. Do you know the outdoor air fraction ventilation rates for this facility during each season?

If yes (please specify):

Spring		(cfm)
Summer		(cfm)
Fall		(cfm)
Winter		(cfm)

If you do not know, can you identify a person, including their phone number, who can provide us with your facility ventilation rates?

Full name

Phone number

Thank you so much for your time. I know that your time is valuable. If you have any questions or concerns, please refer to the contact sheet for information on who to contact.

Appendix B: Questionnaire Chain of Custody format

FD ID[b]	**Site ID**	**PID**	**Received**		**Comments**
			Date	Initials	

[a] For hardcopy versions, place n/a in the flash drive ID column
[b] Flash drive ID

iButton Temperature Logging System to Assure the Integrity of Samples Collected for Microbial Analysis (D-SED-EFAB-010-SOP-01) – July 2017

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory Systems
Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: iButton Temperature Logging System

Number: D-SED-EFAB-010-SOP-01	Effective Date: June 9, 2016

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EFAB-010-01

SOP Steward

Name: Nichole Brinkman, NERL/SED/EFAB

Approval

Name: Valerie Garcia
Title: Chief, NERL/SED/EFAB

Concurrence

Name: Brittany Stuart
Title: SED Quality Assurance Manager

STANDARD OPERATING PROCEDURE FOR iBUTTON TEMPERATURE LOGGING SYSTEM TO ASSURE THE INTEGRITY OF SAMPLES

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of the procedure is to collect and assess iButton data to determine whether samples were maintained within the optimal temperature range after collection and during shipment.

2.0 SCOPE AND APPLICABILITY

The task described in this SOP is limited to the collection of samples that will be analyzed for microbial analysis. To maintain the integrity of the microbial population collected in field samples, they should be stored between 2-8°C after collection and until they can be processed in the laboratory. iButtons are temperature logging systems that can be used to monitor temperature in containers where samples are stored and shipped. This SOP describes the use and analysis of the iButtons for monitoring temperature of collected field samples.

3.0 SUMMARY OF METHOD

This SOP describes the steps to program iButtons, use iButtons, and analyze data collected by iButtons. The DS1921G iButton records temperature data within the range of -40°C-85°C and is accurate to at least ±1°C and within ±2 minutes. The iButton can store up to 2,048 equally spaced data points at intervals which can be set from 1 to 255 minutes. The logged data is downloaded with a USB reader probe and One-wire software. Data can be viewed and analyzed in Microsoft Excel.

4.0 DEFINITIONS

PPE – personal protective equipment

5.0 HEALTH AND SAFETY WARNINGS

5.1 Standard laboratory PPE for Biosafety Level (BSL) 1 microorganisms is required. This PPE consists of a lab coat, safety glasses, and gloves.

5.2 Known hazards consist of exposure to samples shipped in containers along with iButtons. PPE should be worn when retrieving iButtons from shipping containers.

6.0 CAUTIONS/INTERFERENCES

Experience has shown that if the iButton is not near the sample, then temperature logs will not accurately reflect sample temperature after collection and during shipment. For example, if the iButton is taped to the side of the shipping container, away from the sample and cold source, it may record temperatures above the targeted range even though samples may have been in the targeted range. To reduce this potential misrepresentation, it is recommended that iButtons be intermixed with samples to ensure the temperature of samples is recorded.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The only qualification of the person required to perform this task is that he/she has the iButton reader and One-wire software.

8.0 EQUIPMENT AND SUPPLIES

The following materials are needed to use the iButtons to monitor temperature:

8.1 Lab coat
8.2 Safety goggles
8.3 Laboratory gloves (nitrile or latex)
8.4 USB adapter
8.5 iButton (DS1921G or DS1922L) iButton Reader
8.6 One-wire Viewer software

The iButton Reader and One-wire software is needed to evaluate temperatures recorded with the iButtons.

9.0 PROCEDURE

These procedures were adapted from the manufacturer's manual (see REFERENCE below):

9.1 *Program iButton to begin logging data: Begin Mission*

9.1.1 Open One-wire viewer software and ensure reader is attached via USB connection.
9.1.2 Insert an iButton into the reader.
9.1.3 Select iButton on device list, it will not be the first line. Ensure you have the correct iButton if there are more than one iButtons in the reader. Device will disappear if removed from reader.
9.1.4 Select "Thermochron" tab (Figure 1) for DS1921G, or "Mission" tab for DS1922L.
9.1.5 Select "Status" sub-tab.
9.1.6 Ensure that "Is Mission Active?" reads "False"; if not, press "Disable Mission" and wait until it reads "False".
9.1.7 Ensure Celsius is checked (only for DS1921G).
9.1.8 Select "Start New Mission", this opens "Initialize New Mission" window (Figure 2).
9.1.9 Check "Synchronize Real-time Clock".
9.1.10 If you wish rollover to occur check "Enable Rollover". Note: If rollover is checked, after rollover only the maximal number of readings will be retained.
9.1.11 Enter "Sampling Rate" (see Table 1 and 2 for rates and associated maximal mission periods).
9.1.12 Enter "Mission Start Delay" in minutes, if a delay is desired.

9.1.13 Select “OK” and wait for “Is Mission Active?” to change to “True” on Status sub-tab.
9.1.14 Remove iButton from reader and use for temperature logging. NOTE: Alarm programming not included as data cannot be downloaded.
9.1.15 Programmed iButton is then taken to the field to be installed with collected samples.

9.2 *Viewing or Ending Mission and Saving Results*
9.2.1 Open One-wire viewer software and ensure reader is attached via USB connection.
9.2.2 Insert an iButton into the reader.
9.2.3 Select iButton on device list.
9.2.4 Select “Thermochron” or “Mission” tab (Figure 1)
9.2.5 Select “Status” sub-tab.
9.2.6 If you wish to only view partial results then skip Step 7, go to Step 8.
9.2.7 Press “Disable Mission”, skip Step 8, go to Step 9.
9.2.8 Press “Refresh Mission Results”.
9.2.9 Results can be viewed in two formats.
9.2.10 Select “Histogram” sub-tab. This is view only (Figure 3) and only for DS1921G.
9.2.11 Select “Temperatures” sub-tab (Figure 4).
9.2.12 To rescale graph, right click and select “Rescale Graph”.
9.2.13 To import into Excel, right click and select “Save data to .csv File”.
9.2.14 Remove iButton from reader, return to sample or store.

9.3 *Opening and Graphing Results in Excel*
9.3.1 Open Excel, select new workbook.
9.3.2 Select open file.
9.3.3 Select files of type: text.
9.3.4 Open .csv file and save as “iButton Serial Number_sample collection date.xlsx” to create a new document for analysis.
9.3.5 Delete 2nd column (only delete units).
9.3.6 Delete any rows from periods not of interest in file.
9.3.7 Select “Date/Time” and “Value data” (Note: value is the temperature).
9.3.8 Select “Insert scatter chart with data points and lines”.
9.3.9 Select “Chart Layout Type 1”.
9.3.10 Label y-axis Temperature (°C), x-axis Date and Time, and Title.
9.3.11 Delete legend.
9.3.12 Select “Home” menu, select x-axis, then alignment angle text.
9.3.13 Save file.

10.0 DATA AND RECORDS MANAGEMENT

The temperature data logged from the iButtons for each shipment of samples will be recorded and placed in the analyst’s lab notebook. Any alarms – temperatures recorded outside of the targeted range of 2-8°C – will be noted.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

If the iButton results show that the sample temperatures exceeded the 2-8°C acceptable temperature range, this information will be recorded and used in the interpretation of sample data. The results of the quality control procedures will be documented in the analysts' notebook. All quality control failures will be reported to the Technical Lead. The quality control results will be described in a report at the completion of the project.

12.0 REFERENCE

- *Determining the Mission Parameters for Temperature Logger iButton® Devices.* http://www.embeddeddatasystems.com/assets/images/supportFiles/tutorials/AN5335.pdf. Accessed June 9, 2016.

13.0 DOCUMENT HISTORY

Date of Revision	Revision #	Description of Changes
6/9/2016	0	First version

14.0 TABLES

Table 1: Sampling rate and mission time for DS1921G

Sampling Rate (min)	Maximal Mission Period (day)
1	1.4
10	14
30	43
255	362

Table 2: Sampling rate and mission time for DS1922L

Sampling Rate (sec)	Maximal Mission Period (hours)
1	2.28
2	4.55
3	6.83
4	9.10
5	11.37
11	25.03
60	5.68 days

15.0 FIGURES

Figure 1. Thermochron menu (DS1922L will say Mission)

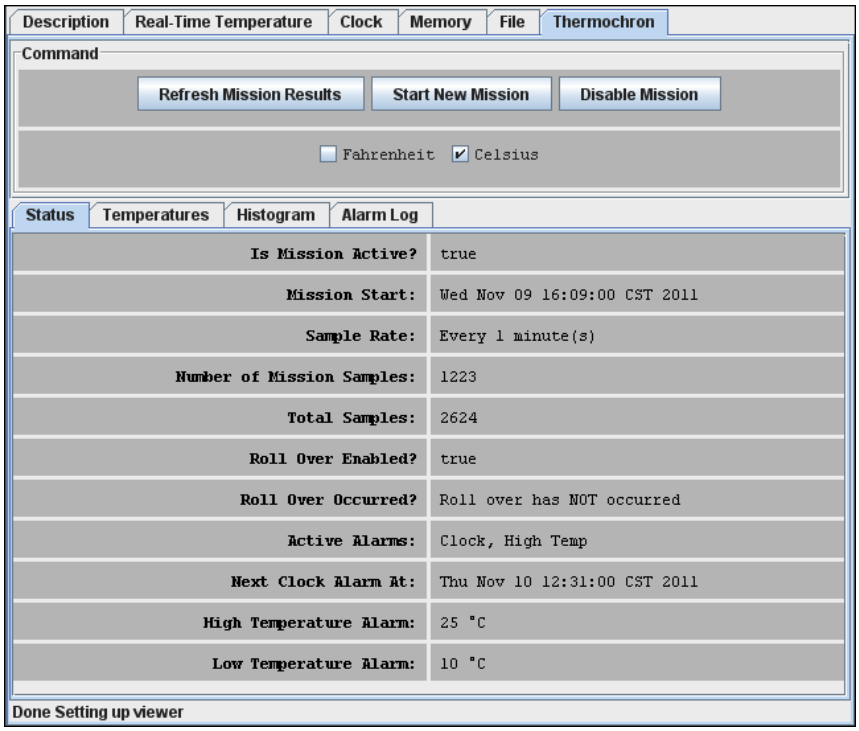


Figure 2. Initialize new mission window

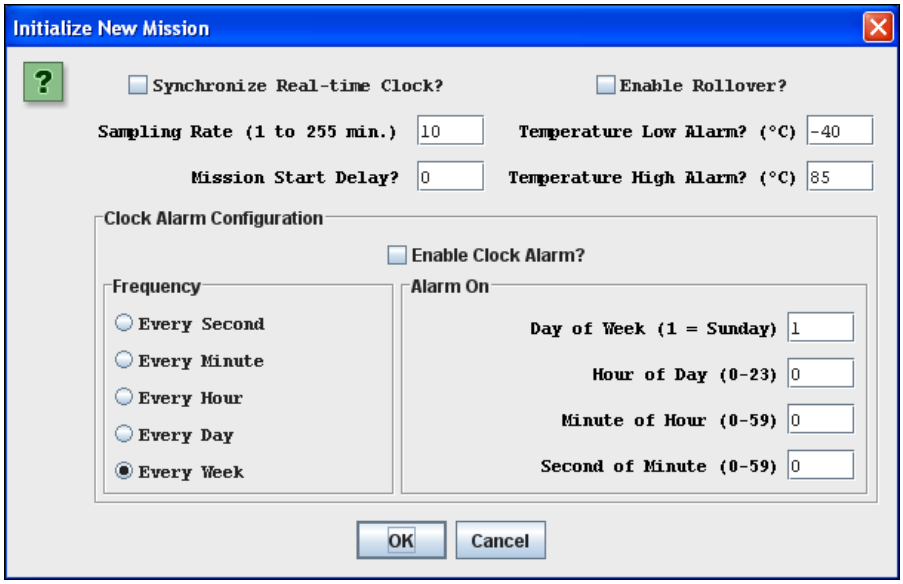


Figure 3. Histogram tab

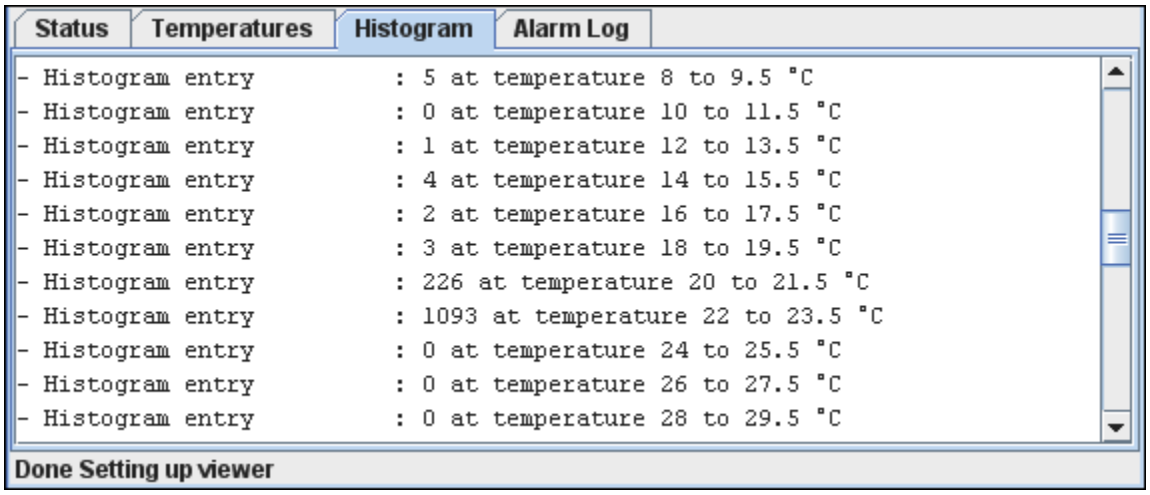
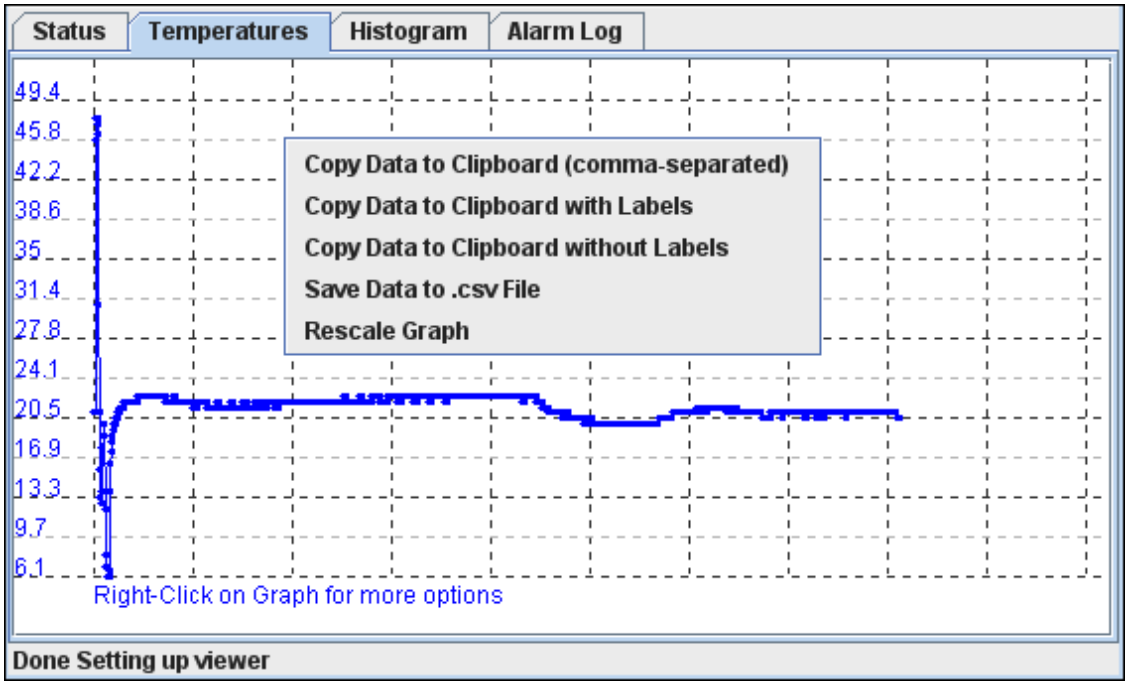


Figure 4. Temperature plot



Section 2: EPA Tire Crumb Rubber Sample Preparation and Analysis

Standard Operating Procedures (SOPs) and Miscellaneous Operating Procedures (MOPs)

Type of Sampling and Analysis	Associated EPA SOPs/MOPs
Storage and Preparation of Tire Crumb Rubber Sub-samples for Analyses	D-EMMD-PHCB-040-SOP-01
Desorbing VOCs with TD 50:50 and Unity 2	D-EMMD-AQB-SOP-3465-0
SEM Tire Crumb Characterization	D-EMMD-ECB-011-SOP-01
Sieving Procedure for Tire Crumb Rubber Samples	D-EMMD-ECB-002-SOP-03
Total Nitric Acid Extractable Metals from Solid Samples	D-EMMD-ECB-003-SOP-01
Innov-X XRF Analysis Procedures for Tire Crumb Samples	D-EMMD-ECB-004-SOP-01
Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples	D-EMMD-PHCB-033-SOP-01
Air Samples Collected on PUF Plugs for GC/MS Analysis	D-EMMD-PHCB-036-SOP-01
Determination of Moisture Content in Tire Crumb Rubber	D-EMMD-PCHB-041-SOP-01
Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument	D-EMMD-PHCB-042-SOP-03
Extraction of Microbes and DNA Genomes from Samples Collected from Artificial Turf Athletic Fields	D-SED-EFAB-011-SOP-01
PCR, Library Preparation and MiSeq Sequencing of Samples for 16S Microbiome Analysis	D-SED-EFAB-012-SOP-01
Droplet Digital PCR (ddPCR) Analysis of Genomic Targets	D-SED-EFAB-014-SOP-01
Analysis of Data Generated from Droplet Digital PCR	D-SED-EFAB-015-SOP-01
16S rRNA Gene Sequence Analysis	D-SED-EIB-SOP-1907-0
Setup and Operation of Small Environmental Emissions Chambers During Testing	MOP 802
Operation of the Opto Display Software Data Acquisition System (DAS) in the Small Chamber Laboratory	MOP 803
Operation of the Clean Air System for the Small Chamber Laboratory	MOP 806
Sampling and Extraction Procedures for DNPH-coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes	MOP 812
Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls	MOP 826
High-Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure	MOP 827
Setup and Operation of the Markes Micro-Chamber Thermal Extractor and Humidifier Accessory	SOP 6401
Chain of Custody Procedures for the Receipt and Transfer of Samples	SOP 6402
Collecting Air Samples from the Markes Micro-Chambers Using PUF Plugs	SOP 6403
Collecting Air Samples from the Small Environmental Testing Chambers Using Carbopack™ X Sorbent Tubes	SOP 6404
Glassware and Chamber Cleaning Procedure	SOP 6405

Section 2. Summary of EPA's Tire Crumb Rubber Characterization Study Laboratory SOPs/MOPs

This section provides the SOPs/MOPs used for the sample preparation and characterization of the recycled tire crumb rubber samples. The samples collected were stored at -20oC temperature which was considered adequate since the sampled material used at the fields was opened to ambient conditions for weeks and years, and the material collected from recycling plants was processed in the open and stored before used for a few days. No formal storage stability studies were performed due to the many unknowns and variables that made difficult the establishment of a blanket timeline on stability. One of goals of the tire crumb rubber characterization study was to understand the characteristics of the samples collected and provide the basis for potential future research. In the end, the samples were composited where applicable and distributed to each lab where they were analyzed as soon as possible after receipt.

This section also includes the SOPs for the emissions experiments. The samples were stored in the RTP freezers from up to a couple of days, probably up to three weeks or so before experiments. Note that the samples collected for microbial analysis were sent directly to the EPA facility in Cincinnati where they were processed on the day of receipt. For metals, samples were stored in RTP freezers at first, but then sent to LV for digestion, on the order of days to weeks. For particles, samples were received in RTP and stored in freezers, then prepared and shipped to LV for particle size analyses.

Preparation of Sub-samples for Analysis

Preparation of Tire Crumb Rubber Substances for Multi-Residue Characterization- D-EMMD-PHCB-040-SOP-1

The Tire Crumb Rubber (TCR) samples collected at either a recycling plant or synthetic turf playing field were received in the EPA facility in Research Triangle Park (RTP), NC, logged-in and stored at -20º C until the appropriate aliquots were prepared for each type of sample analysis. To prepare the individual aliquots for those analyses, the bulk samples were removed from the freezer storage location and allowed to warm to room temperature.
For samples collected from synthetic turf fields, composite samples were prepared by mixing and transferring a known portion of each sample collected at a specified location into a single jar. The composite material was mixed and weighed into a pre-labelled sample container for each type of analysis.
For samples collected from recycling plants, the bulk TCR material collected is mixed and weighed into a pre-labelled sample container for each type of analysis. Remaining material from both the synthetic turf fields and recycling facilities were retained and returned to the freezer storage location.
Sample aliquots were transferred to the appropriate technical lead for analysis. The aliquots remained stored at -20 ºC until analysis. The sample aliquots were sent in a cooler with ice packs using a next day delivery service in cases where shipping from RTP to the technical lead was required.

Bulk tire crumb rubber was also collected from recycling plants and synthetic turf fields for particle characterization analysis. Those samples were shipped without further processing to the NERL Las Vegas analysis laboratory.

The vortex extraction method combined with ceramic homogenizers was aggressive and efficient in terms of throughput, which was very important to meet tight timeline for completing the laboratory work. The results achieved were comparable to results found in the literature from previous studies. The use of multiple sequential extractions using the technique was evaluated. A determination was made that most of the extractable organics were removed in the first extraction cycle. This along with little loss due to no heating, evaporation, or extensive sample handling made the method ideal for the project objectives. This method was also evaluated for linearity across tire crumb mass as well as precision of replicates and was found to perform well across the range of semi-volatile organics we were measuring. Note that this is not a total extraction method.

Tire Crumb Rubber Particle Characterization

Sieving Procedure for Tire Crumb Rubber Samples- D-EMMD-ECB-002-SOP-03

This procedure allowed for a rough characterization of particle size for tire crumb samples and collection fines (particles < 150 µm in size) for further analysis. The procedure may be suitable for use on other types of solid matrices.
In summary, the tire crumb rubber samples were shaken through a number of stacked sieves. The fraction left in each sieve, along with the fines that filter through the entire stack, was weighed and reported.

Innov-X XRF ANALYSIS PROCEDURES: For Tire Crumb Samples- D-EMMD-ECB-004-SOP-01

An Innov-X Field Portable XRF was used in this procedure. The instrument is a field screening tool that returns values for some metals with parts per million sensitivity. This analyzer has a mode for soil media that which was adapted for use with tire crumb rubber media.

Air dried tire crumb samples were placed analyzed for 300 seconds in standard mode for heavy metals, and 300 seconds for light element analysis. The analyzer combined the data from the two modes and gave concentration data for a range of elements.

SEM Analysis of Tire Crumb Particles for Sizing and Metals- D-EMMD-ECB-001-SOP-01

This SOP describes the scanning electron microscope (SEM) analysis method used to determine particle size range and overall size distribution of the particles. Also, it performed qualitative elemental composition determination of individual particles of interest.
The samples were imaged with the SEM to determine the particle size and size distribution. Spectra was obtained from an energy dispersive system (EDS) for qualitative metals analysis. Each sample was prepared by placing a carbon adhesive tab on a metal stub and firmly placing

the stub in the sample. All loose particles were removed by blowing compressed air tangential to the sample. At least 5 separate positions on an SEM sample holder were analyzed. Different detectors highlight different features of the sample. Information on picking a detector and the expected resolution can be found in the user manual.

Determination of Moisture Content in Tire Crumb Rubber- D-EMMD-PHCB-041-SOP-01

Tire crumb rubber samples collected from playing fields may contain varying amounts of water based on environmental conditions at the sampling location. Since many of the analyses of tire crumb rubber were based on mass of analyte/mass of rubber, the percent moisture was determined to accurately quantify the levels of analyte in the rubber sample. Moisture analysis was comprised of not only the amount of water in a sample, but the amount of other materials that are volatile at 110º C. Since the mass of other materials was likely to be below the weight sensitivity of the moisture analyzer, weight loss was assumed to be mostly water. This SOP describes the procedure used to determine the amount of moisture in tire crumb rubber samples using the Mettler-Toledo HE53 Moisture Analyzer based on industry standard procedures prescribed in ASTM Method D1509-15.

To determine the moisture content, the sample was allowed to reach room temperature while the moisture analyzer is set-up. Prior to measurement, the balance calibration was verified using certified check weights. When the sample had equilibrated to room temperature, the moisture analysis process begun. A disposable sample pan was placed onto the moisture analyzer and tared. Tire crumb sample (2g) was spread in a thin even layer across the total surface of the pan and the weight was recorded on a Moisture Analysis Form (Appendix A). The moisture analysis was then started and continued until mass loss was less than 1 mg/30 s. The HE53 Moisture Analyzer displayed the % moisture content which should was recorded on a form.

Tire Crumb Emissions Experiments

Note that for the tire crumb VOC emissions characterization, experiments in the small chamber were performed at both 25°C and 60°C. These conditions mimic the mild and the hot summer field conditions. The tests conformed to ASTM D5116, ASTM D7706 and ISO 16000-25.

Setup and operation of small environmental chambers during testing- NRMRL MOP-802

This SOP describes the setup and operation of small environmental emissions chambers used for the Tire Crumb Rubber Characterization Study. The small environmental chambers were utilized to evaluate emissions from sources (like tire crumb rubber) in a controlled temperature, relative humidity (RH) and air exchange rate atmosphere over extended time periods. The chambers conformed to ASTM Standard Guide D5116-11 — Standard Guide for Small-Scale Environmental Chamber Determinations of Organic Emissions from Indoor Materials/Products.

Setup and Operation of the Markes Micro-Chamber Thermal Extractor and Humidifier Accessory - NRMRL SOP AEMD-6401

This SOP provides a written, repeatable procedure for the operation and use of the Markes120 and 250 series Micro-Chamber / Thermal Extractor and Humidity Accessory.

Operation of the OPTO Display Software Data Acquisition System in the small chamber laboratory- NRMRL MOP-803

This SOP describes the setup of the Opto Display acquisition system. The Opto Display Runtime (OPTO) data acquisition system controls, monitors, collects, and records environmental conditions within the 53-L small chamber testing system through the management of a series of mass flow controllers. During chamber setup, the OPTO is used in conjunction with the clean supply air system and incubators to establish environmental conditions (air exchange and humidity) within test chambers as specified by project test plans. Chamber airflows and humidity levels are controlled by the OPTO system, while the chamber temperatures are controlled using the incubator temperature setpoints. During testing, the OPTO is used to monitor, maintain, and record environmental conditions within test chambers.

Chain of Custody Procedures for the Receipt and Transfer of Samples- NRMRL SOP AEMD-6402

This SOP provides a written procedure for the receipt or transfer of samples using a chain of custody (COC) form.

Operation of Clean Air System for the Small Chamber Laboratory- NRMRL MOP-806

The clean air system is a critical part of the small chamber laboratory, because the research performed requires an endless supply of clean conditioned air to the chambers. This supply of air must be free of most volatile organic compounds (VOC). This SOP describes how to set up the supply air in a way that meets the critical criteria such as pressure of at least 70 psig and an oil-free source.

Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes -NRMRL MOP-812

This procedure describes the sampling and extraction procedures for determining air concentrations of formaldehyde and other aldehydes from environmental small chamber effluent and ambient samples using 2, 4-dinitrophenylhydrazine (DNPH)-silica gel cartridges. In summary, a stream of air sample exiting an environmental small chamber or at an ambient location is directed through a DNPH-silica gel cartridge using a vacuum pump. Aldehydes in the air are absorbed and derivatized by DNPH. The DNPH cartridge is then extracted with acetonitrile and the aldehyde derivative is analyzed via high performance liquid chromatography (HPLC) to determine the concentration of the aldehyde present in the air sample.

Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls- NRMRL MOP-826

This SOP provides instructions to the laboratory analyst in powering on the HPLC system, preparing the HPLC for analysis, creating analytical run sequence tables, creating and/or

modifying an analytical method (if necessary), running an analytical sequence, and analyzing data acquired from the HPLC.

High Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure- NRMRL MOP-827

The SOP explains the procedure for the preparation of carbonyl-dinitrophenylhydrazine (DNPH) standards for calibrating the Agilent 1200 HPLC. Two stock standards were used Cerilliant ERA-020 and Supelco CRM4M7285. Both are multicomponent aldehyde/ketone-DNPH stock standards. The concentration of each analyte in the solution was 15 µg/mL. After standard preparation and analysis, the calibration of the instrument ranged from 0.03 to 15.00 µg/mL.

Actively loading sorbent tubes with volatile organic compounds- D-EMMD-AQB-015-SOP-01

A standards dilution system capable of accurately diluting certified gaseous volatile organic compound (VOC) standards has been constructed for purposes of preparing multipoint calibration standards on PerkinElmer-type sorbent tubes for instrument calibration and QC samples. All gas flows are controlled by mass flow controllers (MFCs) and the humidity is controlled by an adjustable water bubbler. A nominal 1 ppmv TO-14A VOC gaseous standards mixture is diluted with ultrazero humidified air to generate suitable low ppbv (or ng/L) gaseous standard concentrations. A Markes Easy-VOC grab sampler is used to pull a measured portion of the gaseous standards mixture directly through the sorbent tube to load the tube with nanogram masses of VOCs.

Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Bench ToF-Select ToF GC/MS System - D-EMMD-AQB-017-SOP-01

This standard operating procedure (SOP) is applicable to the determination of volatile organic compounds (VOCs) using the Markes International Bench TOF-Select time-offlight (TOF) mass selective detector (MSD) system after desorption from sorbent tubes using the Markes International Ultra/Unity 2 thermal desorption (TD) system. Method parameters are provided for the analysis of VOCs from Carbopack X sorbent tubes used in the Tire Crumb rubber characterization study.

Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System- D-EMMD-AQB-018-SOP-01 Companion SOP to D-EMMD-AQB-017-SOP-01

This standard operating procedure (SOP) is applicable to the determination of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and non-targeted compounds of interest using the Markes International gas chromatograph–mass spectrometer (GC-MS) time-of-flight (TOF) system following desorption from sorbent tubes using the Markes International thermal desorber 50:50 (TD 50:50) and the Unity 2. The TD 50:50 serves as an auxiliary thermal desorption unit that is capable of desorbing up to 100 sorbent tubes in one analytical sequence. Primary tube desorption occurs in the TD 50:50, and collected analytes are transferred to the

Unity 2, focused on the cold trap, desorbed off the trap, and transferred to the GC for further separation before final quantitation in the TOF MS.

Collecting Air Samples from the Small Environmental Testing Chambers Using CarbopackTM X Sorbent Tubes- NRMRL-SOP-6404

This SOP describes a reproducible method for collecting VOCs from air samples with volumes greater than 50 mL on CarbopackTM X sorbent tubes. Common Carbopack™ X VOC target analytes, their boiling points, and their breakthrough volumes are shown in the procedure.

Collecting Air Samples from the Markes Micro-Chambers Using PUF Plugs- NRMRL-SOP-AEMD-6403-01-0

This SOP describes the collection of air samples on polyurethane foam (PUF) plug samplers from the Markes micro-chamber/thermal extractor system. The air samples were collected on PUF plugs during the emission experiments performed with the Markes micro-chamber/thermal extractor emissions chambers.

Glassware and Chamber Cleaning Procedure- NRMRL-SOP-AEMD-6405

This SOP describes the cleaning of glassware and testing chambers in the Indoor Air Quality Small Chamber Laboratory.

Standard Operating Procedure for Preparation of Air Samples Collected on PUF Plugs for GC/MS Analysis- D-EMMD-PHCB-036-SOP-01

This SOP details the extraction and work-up procedures for air samples collected on precleaned polyurethane foam (PUF) plugs. This method is applicable for extraction of both indoor and outdoor field samples as well as laboratory generated samples, including collection from emissions experiments. This SOP is written to encompass a wide range of analytes and to be applicable across many studies. Analytical performance will need to be assessed for specific analytes prior to use.

There are two acceptable methods for extraction and processing of PUF plugs that will be detailed in this SOP that involve either Soxhlet or ultrasonic extraction. The method chosen will depend on availability of materials and equipment, resources, and analytical performance. For both methods, samples are allowed to equilibrate to room temperature after removing from freezer storage.

With the Soxhlet extraction method, samples are transferred to clean 150 mL Soxhlet extractors. Internal standard solution is spiked onto the surface of the samples. Boiling flasks are filled with 300 mL of 1:1 acetone:hexane along with several boiling chips. The extractors are assembled on a heating mantle with condensers and heat is applied so the extraction rate is ~20 cycles per hour. The samples are extracted for 16 hours (overnight is convenient). The extracts in the boiling flasks are allowed to cool and are then concentrated to 2-5 mL on a rotary evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL

hexane rinses of the boiling flask. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.

For ultrasonic extraction, samples are transferred to clean 60 mL amber jars. Internal Standard solution is added to the PUF. Each jar is filled with 50 mL of 1:1 acetone:hexane and is then sealed with a PTFE-lined cap. The jars are placed in an ultrasonic cleaner with water level well below the level of the jar cap. The ultrasonic cleaner is then turned on for 15 minutes. Sample jars are removed from the cleaner and the extracts are transferred through funnels into 250 mL narrow mouth bottles. The funnels are rinsed with hexane from a wash bottle after the extracts are added. The solvent addition, extraction, and transfer is repeated two more times. The extracts in the bottles are then evaporated to 2-5 mL using a parallel evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL hexane rinses of the bottle. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.

Analytical method for non-targeted and suspect screening in environmental and biological samples using Time of Flight Mass Spectrometry (TOFMS)- D-EMMD-PHCB-034-SOP-01

This standard operating procedure (SOP) describes a method for the non-targeted analysis (NTA) and suspect screening of chemicals in environmental and biological media using Time of Flight Mass Spectrometry (TOFMS). This method is used to discover xenobiotic chemicals in various environmental and biological media screening against various data bases. Specifically, the SOP describes the use of the high-performance liquid chromatography time-of-flight mass spectrometry for the analysis of samples using NTA and suspect screening of chemicals in various media.

Organic Analysis

This section describes the standard operating procedures (SOPs) used for the organic analysis of the samples. It is worth noting that many of the SOPs in this section are also included in previous sections as the SOPs often describe the parameters used for analysis.

Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Bench ToF-Select ToF GC/MS System- D-EMMD-AQB-017-SOP-01

This standard operating procedure (SOP) is applicable to the determination of volatile organic compounds (VOCs) using the Markes International BenchTOF-Select time-offlight (TOF) mass selective detector (MSD) system after desorption from sorbent tubes using the Markes International Ultra/Unity 2 thermal desorption (TD) system. Method parameters for analysis of VOCs are provided in multiple appendices. Appendix C describes the specific analytical conditions used for the tire crumb rubber characterization study.
This SOP is a companion to D-EMMD-AQB-018-SOP-01, “Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International TD 50:50/Unity 2 Thermal Desorption System” and refers to it often.

Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System- D-EMMD-AQB-SOP-3465-0; Alternate ID: D-EMMD-AQB-018-SOP-01

This standard operating procedure (SOP) is applicable to the determination of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and non-targeted compounds of interest using the Markes International gas chromatograph–mass spectrometer (GC-MS) time-of-flight (TOF) system following desorption from sorbent tubes using the Markes International thermal desorber 50:50 (TD 50:50) and the Unity 2. The TD 50:50 serves as an auxiliary thermal desorption unit that is capable of desorbing up to 100 sorbent tubes in one analytical sequence. Primary tube desorption occurs in the TD 50:50, and collected analytes are transferred to the Unity 2, focused on the cold trap, desorbed off the trap, and transferred to the GC for further separation before final quantitation in the TOF MS. The parameters of the analytical method are provided in the Appendix A of the SOP.

This SOP is written as a companion to D-EMMD-AQB-017-SOP-01, "Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International BenchTOF-Select GC-MS TOF System."

Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples-D-EMMD- PHCB-033-SOP-01

Tire crumb rubber (TCR) is a material made from recycled tires that is used as infill for synthetic turf athletic fields. In order to characterize its components, it is essential to measure the chemicals from the rubber itself. This method describes the extraction and analysis of semi-volatile organic compounds (SVOCs) from the TCR.

Samples of TCR were stored in a freezer at -20º C after receipt at the EPA lab. The samples were allowed to warm to room temperature, then each sample was homogenized inside of the collection jar by shaking in a manner to cycle the content from the bottom to the top of the jar. Two separate 1g aliquots were removed from each sample, shaking between each aliquot, with each being transferred to a clean 50 mL polypropylene centrifuge tube. An Internal Standard solution was added to each tube along with a ceramic homogenizer. 10 mL of 1:1 acetone:hexane was then added to each sample tube. The tubes were capped and were vortex mixed for 1 minute, allowed to sit for 2 minutes, then vortex mixed for an additional minute. The tubes were then centrifuged at 4000 RPM for 5 minutes. The solvent was removed and transferred to a 15 mL vial. A 1 mL aliquot of the extract was transferred to an autosampler vial for GC/MS/MS analysis. Another aliquot was transferred to a vial where it was solvent exchanged to methanol for LC/TOF analysis. Since there is no appropriate surrogate matrix for TCR, QAQC samples consisted of duplicate preparations, a reagent spike, reagent blank and a TCR sample prepared from a TCR designated as a reference sample.

Standard Operating Procedure for Preparation of Air Samples Collected on PUF Plugs for GC/MS Analysis- D-EMMD-PHCB-036-SOP-01

This SOP details the extraction and work-up procedures for air samples collected on precleaned polyurethane foam (PUF) plugs. This method is applicable for extraction of both indoor and

outdoor field samples as well as laboratory generated samples, including collection from emissions experiments. This SOP is written to encompass a wide range of analytes and to be applicable across many studies. Analytical performance will need to be assessed for specific analytes prior to use.

There are two acceptable methods for extraction and processing of PUF plugs that will be detailed in this SOP that involve either Soxhlet or ultrasonic extraction. The method chosen will depend on availability of materials and equipment, resources, and analytical performance. For both methods, samples are allowed to equilibrate to room temperature after removing from freezer storage.

With the Soxhlet extraction method, samples are transferred to clean 150 mL Soxhlet extractors. Internal standard solution is spiked onto the surface of the samples. Boiling flasks are filled with 300 mL of 1:1 acetone:hexane along with several boiling chips. The extractors are assembled on a heating mantle with condensers and heat is applied so the extraction rate is ~20 cycles per hour. The samples are extracted for 16 hours (overnight is convenient). The extracts in the boiling flasks are allowed to cool and are then concentrated to 2-5 mL on a rotary evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL hexane rinses of the boiling flask. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.
For ultrasonic extraction, samples are transferred to clean 60 mL amber jars. Internal Standard solution is added to the PUF. Each jar is filled with 50 mL of 1:1 acetone:hexane and is then sealed with a PTFE-lined cap. The jars are placed in an ultrasonic cleaner with water level well below the level of the jar cap. The ultrasonic cleaner is then turned on for 15 minutes. Sample jars are removed from the cleaner and the extracts are transferred through funnels into 250 mL narrow mouth bottles. The funnels are rinsed with hexane from a wash bottle after the extracts are added. The solvent addition, extraction, and transfer is repeated two more times. The extracts in the bottles are then evaporated to 2-5 mL using a parallel evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL hexane rinses of the bottle. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.

Analytical method for non-targeted and suspect screening in environmental and biological samples- D-EMMD-PHCB-034-SOP-01

This standard operating procedure (SOP) describes a method for the non-targeted analysis (NTA) and suspect screening of chemicals in environmental and biological media using Time of Flight Mass Spectrometry (TOFMS). This method is used to discover xenobiotic chemicals in various environmental and biological media screening against various in-house and purchased data bases. Traditional methods use target analysis of a small number of chemicals of interest in select media. However, newer high-resolution mass spectrometry (HR-MS) techniques (such as TOFMS) allow for the rapid screening of analytes of interest based on accurate mass measurements and spectral isotope matching. As the technique is kept consistent through the screening of various media, the result will be the ability to detect chemicals in different media, as well as co-occurrence of chemicals in specific media. These data will help to prioritize existing high-throughput toxicology assays and modeling efforts for chemicals of interest to the agency.

Metals Analysis

As part of the Federal Research Action Plan, tire crumb rubber was characterized. Researchers developed procedures for the collection of tire crumb rubber from tire recycling plants and indoor/outdoor synthetic turf fields. Metals present in tire crumb rubber were studied and the standard operating procedures uses are described below.

Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion- D-EMMD-ECB-003-SOP-01

This document describes a procedure for the preparation of solid matrices such as tire crumb rubber, soils, sediments or sludge and can be adapted for biological matrices and wipes, for analysis by inductively coupled plasma mass spectrometry (ICP-MS). It uses a mixture of nitric acid and hydrochloric acid to improve the extractability of metal analytes.

Organic material in the sample matrix is destroyed and metals that are extractable with a mixture of nitric and hydrochloric acid are solubilized by microwave digestion in a sealed, pressurized Teflon vessel. The sample is first allowed to pre-digest at room temperature, and then subjected to a microwave heating program that increased the temperature of the mixture slowly to 200 ◦C and kept it at this temperature for another 30 minutes. The MARS-5 microwave unit (CEM Corporation, Matthews, NC) used is fitted with a fiber optic temperature sensor to monitor the temperature of the reference vessel. The instrument has the ability to regulate the temperature of the sample by adjusting the amount of applied power.
After cooling, the samples are diluted with deionized water and transferred to an acid cleaned polyethylene or Teflon container that can be centrifuged if needed to separate solid particles.

Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument- D-EMMD-PHCB-042-SOP-03

Inductively coupled plasma mass spectrometry (ICPMS) is a widely accepted analytical tool for trace and ultra-trace elemental analysis. It has been the technique of choice for accurate and precise measurements needed for today's demanding applications.

In ICPMS, an inductively coupled plasma (a gas consisting of ions, electrons, and neutral particles) is formed from argon gas under an intense electromagnetic field. The plasma is used to atomize and ionize the sample matrix. The resulting ions are then passed through a series of apertures into the high-vacuum mass analyzer. Isotopes of the elements are identified by their mass-to-charge ratio (m/z), and the intensity of a specific peak in the mass spectrum is proportional to the amount of that isotope (element) in the original sample.
Because of the enormous number of possible interferences, the ability to isolate analytes from interfering species is critical to any analytical ICPMS instrument. Double-focusing magnetic sector field ICPMS, often called high resolution ICPMS (HR-ICPMS), provides a straightforward solution to most of the polyatomic and isobaric interferences by separating the analyte of interest from its interfering species. The HR ICPMS was used in the analysis of tire

crumb rubber samples transferred to a Class 100 clean lab. The SOP provides the following information:

- Descriptions of various components of E2 and its control software (v3.0)
- The basic operational settings of the E2
- Autosampler control procedures
- Procedures to prepare reagents and calibration standards
- Procedures for verifying performance measures of the E2 on a daily basis
- Instructions for preparing various logs
- Instructions for setting up a sequence for automated use of the instrument with QC checks
- Need-based instrument conditioning and maintenance procedures such as changing recirculating fluid, establishing mass calibration, and changing the entrance slit assembly

Microbial Analysis

Extraction of microbes and DNA genomes from samples collected from artificial turf athletic fields- D-SED-EFAB-011-SOP-01

This standard operating procedure (SOP) describes the extraction of microbes from tire crumb rubber collected from artificial turf athletic field and the procedure for extracting the genomic material from the extracted microbes. It covers laboratory processing steps to extract microbes from particles in the sample, and extract and quantify genomic material, such as deoxyribose nucleic acid (DNA).

Extraction of microbes from sample

The purpose of this method is to concentrate the sample and extract the genomic DNA for downstream genetic analysis such as polymerase chain reaction (PCR) and metagenomic sequencing. Once samples arrive in the lab, a solution containing surfactants is added to the crumb rubber sample and then the sample is vortexed vigorously to dislodge any microbes from the sample into solution. The solution is then filtered through a 0.45um filter to capture and retain microbes on the filter. The sides of the filter apparatus are washed twice with sterile phosphate buffered saline (PBS) to remove potential inhibitors and to help remove any microbes attached to the sides of the filter apparatus. The filter is then stored -20˚C.

Extraction of DNA genomes and determination of yield

Nucleic acids are extracted from the microbes captured on filters using the PowerWater DNA Isolation Kit (MoBio Laboratories) and the DNA yield is quantified using the Qubit Fluorometer 2.0 (Invitrogen).
The method reporting limit is dictated by the Qubit Fluorimeter which is 0.5ng/mL of DNA.

PCR, Library Preparation and MiSeq Sequencing of Samples for 16S microbiome analysis- D-SED-EFAB-012-SOP-01

The purpose of this SOP is to outline the steps necessary to perform 16S PCR, then prepare and sequence 16S libraries generated from samples for analysis of the microbiome.

The tasks described in this SOP are applicable to a variety of matrices, including, but not limited to, water, soil, mouse gut tissue, mouse feces, zebrafish tissue and cat feces. Implementation of this SOP assumes that high quality DNA genomes have been prepared from samples.

Specifically, the SOP describes the steps to amplify and barcode specific variable regions of the 16S rRNA genes extracted from samples followed by purification of amplicons and a procedure to normalize amplicon concentration across samples. Normalized amplicon libraries are sequenced to assess the composition of the 16S microbiome. To amplify and barcode 16S genes in samples, a thermal cycler is needed. Amplification is verified by gel electrophoresis, which requires gel boxes and power supply. Once libraries are purified and normalized, they are quantified with the Qubit Fluorimeter or through qPCR via a real-time PCR instrument (i.e., Life Technologies StepOne Plus) or ddPCR via QX200 ddPCR system. Additionally, the average fragment size of amplicon libraries are assessed with an Agilent Bioanalyzer 2100. Finally, libraries are sequenced using an Illumina MiSeq.

Note: The SOP is an adaptation from a published method from researchers at the University of Michigan (Kozich et al. 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology 79(17):5112 DOI:10.1128/AEM.01043-13).

16S rRNA Gene Sequence Analysis- D-SED-EIB-SOP-1907-01

The purpose of this procedure is to describe the steps to generate 16SrRNA gene sequences using Illumina's MiSeq platform using paired end reads. The SOP uses indexed reads to multiplex a large number of samples (i.e. 384) on a single MiSeq experiment. Mothur software is used to filter the sequences and determine their taxonomic content.

Droplet digital PCR (ddPCR) analysis of genomic targets- D-SED-EFAB-014-SOP-01

Droplet digital PCR (ddPCR) can be used to quantify RNA and DNA targets of prokaryotes, eukaryotes, archaea and viruses in a variety of sample matrices, including, but not limited to, water, air and rubber surfaces. This standard operating procedure (SOP) describes the amplification and quantitation of genomic targets using ddPCR. The use of this procedure is contingent upon sample preparation that releases RNA and/or DNA from cells or viral capsids.

Quantitative PCR (qPCR) to determine the concentrations of targeted microbes in environmental samples has become a standard tool used in microbiology because it is sensitive and specific for the intended microbial targets. The quantitative nature of qPCR relies on the linear relationship that exists between log10 target sequences in a sample and the number of thermal cycles needed to reach a defined threshold (often referred to as the cycle threshold) where fluorescence is detectable above background but is still increasing exponentially due to PCR amplification. Fluorescence can be produced either by the binding of intercalating dyes or by the cleavage of target sequence specific probes that both occur stoichiometrically with the generation of amplified target sequence fragments. Based on this relationship, standard curves of log10 target sequences vs. cycle threshold measurements can be generated from well characterized source

material (standards) using a linear regression model and target sequences in unknown samples can be quantified by interpolation of their cycle threshold measurements on the standard curves. In this way, qPCR provides quantitation of unknown samples relative to known standards.

Analysis of data generated from the droplet digital PCR (ddPCR)- D-SED-EFAB-015-SOP-01

This SOP describes the procedure to analyze data generated from the BioRad QX200 Droplet Digital PCR System. The data produced by the QX200 Droplet Digital PCR system is composed of fluorescence amplitude data from thousands of droplets produced in a ddPCR reaction. Based on the amplitude value, each droplet will be clustered into positive or negative bins. The number of droplets in each bin is counted and the number of total and positive droplets is used to determine the number of target DNA molecules present from a Poisson distribution. The QuantaSoft Software is used to cluster droplets and determine DNA target concentration (1). On occasion, the software is not able to cluster the droplets and, in this case, an alternative method is used to assess the fluorescence amplitude threshold to cluster droplets. Here, the mean and standard deviation of the fluorescence amplitude data from the droplets of the no template control reactions are used to determine a negative threshold value. The value is applied manually in the QuantaSoft software and DNA target concentrations are calculated. Finally, the total number of DNA target molecules per unit volume of sample are calculated by accounting for sample processing steps.

Storage and Preparation of Tire Crumb Rubber Subsamples for Analyses (D-EMMD-PHCB-040-SOP-01) – August 16, 2016

U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Standard Operating Procedure for Storage and Preparation of Tire Crumb Rubber Subsamples for Multi-residue Characterization

Number: D-EMMD-PHCB-040-SOP-01	Effective Date: 8/16/2016

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: M. Scott Clifton

Signature: Date:

Approval

Name: Myriam Medina-Vera
Title: Branch Chief, PHCB

Signature: Date:

Concurrence*

Name: Sania W. Tong Argao
Title: EMMD QA Manager

Signature: Date:

* Optional Field

STANDARD OPERATING PROCEDURE FOR STORAGE AND PREPARATION OF TIRE CRUMB RUBBER SUBSAMPLES FOR ANALYSES

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

The tire crumb rubber (TCR) characterization study will involve the collection of crumb rubber material from tire recycling plants and synthetic turf fields around the U.S., with laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and particle characteristics. This standard operating procedure (SOP) describes storage and preparation of tire crumb rubber aliquots for each type of multi-residue characterization analyses to be performed.

2.0 SUMMARY OF THE METHOD

After TCR samples collected at either a recycling plant or synthetic turf playing field are received at the EPA facility in Research Triangle Park (RTP), NC, they are logged-in and stored at -20ºC until the appropriate aliquots can be prepared for each type of sample analysis. To prepare the individual aliquots for those analyses, the bulk samples will first be removed from the freezer storage location and allowed to warm to room temperature.

For samples collected from synthetic turf fields, composite samples will be prepared by mixing and transferring a known portion of each sample collected at a specified location into a single jar. The composite material is then mixed and weighed into a pre-labelled sample container for each type of analysis. For a subset of fields, samples from individual locations on the field, selected by the research coordinator, will also be mixed and weighed into a pre-labelled sample container for each type of analysis.

For samples collected from recycling plants, the bulk TCR material collected is mixed and weighed into a pre-labelled sample container for each type of analysis. Remaining material from both the synthetic turf fields and recycling facilities will be retained and returned to the freezer storage location.

Sample aliquots will be transferred to the appropriate technical lead for analysis. The aliquots will remain stored at -20ºC until analysis. In cases where aliquots require shipping from RTP to the technical lead, the samples will be sent in a cooler with ice packs using a next-day delivery service.

Bulk tire crumb rubber is also collected from recycling plants and synthetic turf fields for particle characterization analysis. Those samples will be shipped without further processing to the NERL Las Vegas analysis laboratory.

3.0 DEFINITIONS

3.1 TCR – tire crumb rubber
3.2 VOCs – volatile organic compounds
3.3 SVOCs – semi-volatile organic compounds
3.4 SOP – standard operating procedure
3.5 COC – chain of custody
3.6 QC – quality control

3.7 DCM – Dichloromethane
3.8 RTP – Research Triangle Park
3.9 HDPE – high-density polyethylene

4.0 CAUTIONS

Staff handling the tire crumb material must wear appropriate personal protective equipment. At a minimum, safety glasses and nitrile gloves should be worn.

5.0 RESPONSIBILITIES

The EPA laboratory staff or contractor assigned to prepare aliquots will prepare composite samples and aliquots as directed by this SOP. They will also verify that the appropriate transfer and storage materials are used. In addition, they will complete any Chain of Custody (COC) documentation that accompanies the field collection samples and generate subsequent custody documents to follow sample aliquots. Those staff will also be responsible for ensuring that the balances used are calibrated and verified prior to each use and that freezers used for storage are monitored and maintained at ≤ -20ºC. Laboratory staff or contractors will also prepare and ship samples to technical leads when necessary.

The EPA project staff or contractors are responsible for providing sample transfer and storage materials and appropriate storage locations for samples and sample aliquots. The project staff or contractors are also responsible for transferring custody documents to the laboratory staff and addressing any technical issues that may arise.

6.0 MATERIALS

6.1 Freezer, capable of storage at ≤ -20ºC
6.2 Sample Aliquot Preparation Forms
6.3 Labels for jars
6.4 Plastic spoons, 1 tsp sterile, Fisher 14-375-256 or equivalent
6.5 Stainless steel spoons or spatulas
6.6 Certified, pre-cleaned amber glass jars:
  6.6.1 60 mL, I-Chem P/N 341-0060 or equivalent
  6.6.2 120 mL, I-Chem P/N 341-0120 or equivalent
  6.6.3 250 mL, I-Chem P/N 341-0250 or equivalent
  6.6.4 500 mL, I-Chem P/N 341-0500 or equivalent
6.7 Certified, pre-cleaned high-density polyethylene (HDPE) jars:
  6.7.1 120 mL, C&G Containers P/N 30621-236 or equivalent
  6.7.2 250 mL, C&G Containers P/N 30621-222 or equivalent
  6.7.3 500 mL, C&G Containers P/N 30621-226 or equivalent
  6.7.4 950 mL, C&G Containers P/N 30621-214 or equivalent
6.8 Nitrile gloves
6.9 Safety glasses
6.10 Ziploc bags, gallon size

6.11 Ziploc bags, quart size
6.12 Bubble wrap bags, various sizes
6.13 Ice packs (chilled)
6.14 Coolers for shipping
6.15 Laboratory Notebook
6.16 Sample collection and COC record sheet
6.17 Dichloromethane (DCM) – HPLC Grade or better

7.0 PROCEDURES

Important Note on Balance Calibration Verification: Prior to the weighing of any samples for aliquots, verify the balance calibration according to Section 9.1, and only use a verified balance for the following procedures.

7.1 *Preparation of Samples from Synthetic Turf Fields*
Samples collected from each synthetic turf playing field consist of 7 individual location samples collected in both glass (organics) and HDPE (metals) 250 mL jars. In addition to those 14 samples, there are seven (7) 250 mL HDPE jars filled with samples for particle characterization analysis that will not have subsample preparation. Jars for composite and subsamples may be pre-labeled prior to sample preparation or they may be labeled at the time of preparation.

7.1.1 Composite Sample Preparation – Organics

7.1.1.1 Identify the field-collected samples to be prepared and remove from freezer storage. Allow to warm to room temperature before weighing.

7.1.1.2 Place a certified, pre-cleaned amber glass 500 mL jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 300 g.

7.1.1.3 Mix the content of the 250 mL glass jar containing the TCR material collected from location #1 for organics by shaking and stirring from the bottom to the top using a DCM-rinsed, stainless-steel spoon or spatula.

7.1.1.4 Tare the balance and weigh 35 g ± 1 g of TCR material collected at Location # 1 into the 500 mL jar. Replace the cap on the sample from Location # 1. Record the actual weight of material on the Sample Aliquot Preparation Form (Appendix A).

7.1.1.5 Repeat steps 7.1.1.3 and 7.1.1.4 for the samples collected from the remaining six locations while making sure to cap the 500 mL jar and shake and roll the container to mix the material after the addition of each aliquot. Record the sum weight on the Sample Aliquot Preparation Form by adding each individual location's weight together.

7.1.1.6 Label the jar with Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.

7.1.1.7 Retain the remaining sample material from the field-collected

samples for storage or preparation of a subset of individual aliquots as noted in the next section. Complete required COC record (Appendix F).

7.1.2 Composite Subsample Preparation – Organics
Use the following chart and follow steps 7.1.2.1 – 7.1.2.4 below to measure out subsample aliquots for each composite field sample for organics analysis.

Analysis Type	**Pre-Cleaned Amber Glass Jar Size**	**Amount of Composite Sample**
SVOC Extraction	60 mL	10 g
Chamber VOC	120 mL	50 g
Chamber SVOC	120 mL	30 g
SVOC Bioaccessibility - Saliva	60 mL	20 g
SVOC Bioaccessibility - Gastric	60 mL	20 g
SVOC Bioaccessibility - Sweat	60 mL	20 g

7.1.2.1 For each analysis type, place the appropriately sized certified, pre-cleaned amber glass jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g.
7.1.2.2 Thoroughly mix the content of the glass jar containing the composite TCR organics sample (prepared in section 7.1.1) by shaking and stirring from the bottom to the top using a DCM-rinsed stainless-steel spoon or spatula.
7.1.2.3 Tare the balance and weigh the appropriate amount (in grams) of the composite sample into the correct sized amber jar for the analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix A).
7.1.2.4 Cap and label the amber jar with Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.
7.1.2.5 Repeat steps 7.1.2.1 – 7.1.2.4 for each analysis type for each composite sample.
7.1.2.6 Cap the jar containing the composite sample and store at -20ºC for future use.
7.1.2.7 Complete the Sample Aliquot Preparation Form for these samples (Appendix A). Store composite subsamples at -20ºC until transferred to the appropriate technical lead for analysis. Complete the required COC record (Appendix F).

7.1.3 Individual Location Sample Preparation – Organics

7.1.3.1 The EPA research coordinator will identify a subset of samples collected from individual locations at a subset of synthetic turf fields for individual location analysis. It is likely that samples will only be prepared for the first three locations from five fields, but the number and locations is subject to change.

7.1.3.2 Identify the samples and remove from freezer storage. Allow to warm to room temperature before weighing, then use the following chart and follow steps 7.1.3.3 – 7.1.3.6 below to weigh out the individual location samples to be analyzed for organics.

Analysis Type	**Pre-Cleaned Amber Glass Jar Size**	**Amount of Individual Location Sample**
SVOC Extraction	60 mL	5 g
Chamber VOC	60 mL	32 g
Chamber SVOC	60 mL	22 g
SVOC Bioaccessibility - Saliva	60 mL	10 g
SVOC Bioaccessibility - Gastric	60 mL	10 g
SVOC Bioaccessibility - Sweat	60 mL	10 g

7.1.3.3 For each analysis type, place a certified, pre-cleaned amber glass 60 mL jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g.

7.1.3.4 Mix the content of the 250 mL glass jar containing the TCR material collected from the first individual location sample to be aliquoted for organics by shaking and stirring from the bottom to the top using a DCM-rinsed stainless-steel spoon or spatula.

7.1.3.5 Tare the balance and weigh the appropriate amount (in grams) of the individual location sample into the 60 mL jar for the analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix A).

7.1.3.6 Cap and label the amber jar with Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.

7.1.3.7 Repeat steps 7.1.3.3 – 7.1.3.6 for each analysis type and for each selected individual location sample.

7.1.3.8 Cap each individual location sample, complete the COC record, and return the remaining individual location samples to freezer storage at -20ºC.

7.1.3.9 Complete the Sample Aliquot Preparation Form for these samples (Appendix A). Store individual location aliquot samples at -20ºC until transferred to the appropriate technical lead for

analysis.

7.1.4 Composite Sample Preparation – Metals

7.1.4.1 Identify the samples and remove from freezer storage. Allow to warm to room temperature before weighing.

7.1.4.2 Place a certified, pre-cleaned 500 mL HDPE jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 300g. Verify the balance calibration prior to use.

7.1.4.3 Thoroughly mix the content of the 250 mL HDPE jar containing the TCR material collected from field location #1 for metals by shaking and stirring from the bottom to the top using a clean plastic spoon.

7.1.4.4 Tare the balance and weigh 35g ± 1 g of TCR material collected at Location # 1 into the 500 mL jar. Replace the cap on the sample from Location # 1. Record the actual weight of material on the Sample Aliquot Preparation Form (Appendix B).

7.1.4.5 Repeat step 7.1.4.3 and 7.1.4.4 for the samples collected from the remaining six locations while making sure to cap the 500 mL container and shake and roll the container to mix the material after the addition of each aliquot. Record the sum weight on the Sample Aliquot Preparation Form (Appendix B) by adding each individual location's weight together.

7.1.4.6 Label the jar with the Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.

7.1.4.7 Retain the remaining sample material from the field-collected samples for storage or preparation of a subset of individual aliquots as noted in the next section. Complete required COC record (Appendix F).

7.1.5 Composite Sub-Sample Preparation – Metals

Use the following chart and follow steps 7.1.5.1 – 7.1.5.4 below to measure out sub-sample aliquots for each composite field sample for metals analysis.

Analysis Type	**Pre-Cleaned HDPE Jar Size**	**Amount of Composite Sample**
Metals Digestion	120mL	10 g
Metals XRF	120mL	10 g
Metals Bioaccessibility – Saliva	120mL	20 g
Metals Bioaccessibility – Gastric	120mL	20 g
Metals Bioaccessibility - Sweat	120 mL	20 g

7.1.5.1 For each analysis type, place a certified, pre-cleaned 120 mL HDPE jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g.

7.1.5.2 Mix the content of the glass jar containing the composite TCR metals sample (prepared in section 7.1.4) by shaking and stirring from the bottom to the top using a clean plastic spoon.

7.1.5.3 Tare the balance and weigh the appropriate amount (in grams) of the composite sample into the 120 mL jar for the analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix B).

7.1.5.4 Cap and label the jar with Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.

7.1.5.5 Repeat steps 7.1.5.1 – 7.1.5.4 for each analysis type for each composite sample.

7.1.5.6 Cap the jar containing the composite sample and store at -20ºC for future use.

7.1.5.7 Complete the Sample Aliquot Preparation Form for these samples (Appendix B). Store composite field sub-samples at -20ºC until transferred to the appropriate technical lead for analysis. Complete required COC record (Appendix F).

7.1.6 Individual Location Sample Preparation – Metals

7.1.6.1 The EPA research coordinator will identify a subset of samples collected from individual locations at a subset of synthetic turf fields for individual location analysis. It is likely that samples will only be prepared for the first three locations from five fields, but the number and locations is subject to change.

7.1.6.2 Identify the samples and remove from freezer storage. Allow to warm to room temperature before weighing, then use the following chart and follow steps 7.1.6.3 – 7.1.6.6 below to weigh out the individual location samples to be analyzed for metals.

Analysis Type	**Pre-Cleaned HDPE Jar Size**	**Amount of Individual Location Sample**
Metals Digestion	120 mL	5 g
Metals XRF	120 mL	5 g
Metals Bioaccessibility – Saliva	120 mL	10 g
Metals Bioaccessibility – Gastric	120 mL	10 g
Metals Bioaccessibility - Sweat	120 mL	10 g

7.1.6.3 For each analysis type, place a certified, pre-cleaned 120 mL HDPE jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g. Verify the balance calibration prior to use.

7.1.6.4 Mix the content of the 250 mL HDPE jar containing the TCR material collected from the first individual location sample for metals by shaking and stirring from the bottom to the top using a clean plastic spoon.

7.1.6.5 Tare the balance and weigh the appropriate amount (in grams) of the individual location sample into the 120 mL jar for the analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix B).

7.1.6.6 Cap and label the jar with Sample ID as indicated in Section 8 for field samples and the Sample Aliquot Preparation Form.

7.1.6.7 Repeat steps 7.1.6.3 – 7.1.6.6 for each analysis type for each selected individual location sample.

7.1.6.8 Cap each individual location sample, complete the COC record, and return the remaining individual location samples to freezer storage at -20ºC.

7.1.6.9 Complete the Sample Aliquot Preparation Form for these samples (Appendix B). Store individual location aliquot samples at -20ºC until transferred to the appropriate technical lead for analysis.

7.2 *Preparation of Samples from Recycling Plants*

Samples collected from tire recycling plants will consist of two (2) 950 mL amber glass jars (organics) and three (3) 950 mL HDPE jars (two for metals analysis and one for particle characterization) collected across three manufacturing batches, containers, or production lines. Only one glass and one HDPE jar will need to be removed per batch/container/production line in order to prepare subsamples. Jar #1 will always be used for preparation of the subsamples and jar #2 will remain in storage. Subsamples will not be prepared for particle characterization samples. Jars for subsamples may be pre-labeled prior to sample preparation or they may be labeled at the time of preparation.

7.2.1 <u>Sub-Sample Preparation for Organics</u>

7.2.1.1 Identify the samples and remove from freezer storage. Allow to warm to room temperature before weighing, then use the following chart and follow steps 7.2.1.2 – 7.2.1.5 below to measure out subsample aliquots for each recycling plant subsample for organics analysis.

Analysis Type	**Pre-Cleaned Amber Glass Jar Size**	**Amount of Composite Sample**
SVOC Extraction	60mL	10 g
Chamber VOC	60 mL	50 g
Chamber SVOC	60 mL	30 g
SVOC Bioaccessibility - Saliva	60 mL	20 g
SVOC Bioaccessibility - Gastric	60 mL	20 g
SVOC Bioaccessibility - Sweat	60 mL	20 g

7.2.1.2 For each analysis type, place a certified, pre-cleaned amber glass 60 mL jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g.

7.2.1.3 Mix the content of the 950 mL glass jar containing the TCR material collected from one batch for organics by shaking and stirring from the bottom to the top using a DCM-rinsed, stainless-steel spoon or spatula.

7.2.1.4 Tare the balance and weigh the appropriate amount (in grams) of the recycling plant organics sample into the 60 mL jar for the analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix C).

7.2.1.5 Cap and label the jar with Sample ID as indicated in Section 8 for recycling plant samples and the Sample Aliquot Preparation Form.

7.2.1.6 Repeat steps 7.2.1.2 – 7.2.1.5 for each analysis type for each batch of recycling plant samples for a total of 18 subsamples (six (6) analysis types from three (3) lot/batch/storage samples).

7.2.1.7 Cap the jar containing the remainder of the recycling plant sample batch, record the amount removed and other information as needed in the COC record (Appendix F), and return to freezer storage at -20ºC.

7.2.1.8 Complete the Sample Aliquot Preparation Form for these samples (Appendix C). Store recycling plant subsamples at -20ºC until transferred to the appropriate technical lead for analysis.

7.2.2 Sub-Sample Preparation for Metals

7.2.2.1 Identify the samples and remove from freezer storage. Allow to warm to room temperature before weighing, then use the following chart and follow steps 7.2.2.2 – 7.2.2.5 below to measure out subsample aliquots for each recycling plant subsample for metals analysis.

Analysis Type	**Pre-Cleaned HDPE Jar Size**	**Amount of Composite Sample**
Metals Digestion	120 mL	10 g
Metals XRF	120 mL	10 g
Metals Bioaccessibility – Saliva	120 mL	20 g
Metals Bioaccessibility – Gastric	120 mL	20 g
Metals Bioaccessibility - Sweat	120 mL	20 g

7.2.2.2 For each analysis type, place a 120 mL certified, pre-HDPE jar onto a verified (see Section 9.1) laboratory balance capable of weighing the jar plus 100 g.

7.2.2.3 Mix the content of the 950 mL HDPE jar containing the TCR material collected from one batch for metals by shaking and stirring from the bottom to the top using a clean plastic spoon.

7.2.2.4 Tare the balance and weigh the appropriate amount (in grams) of the recycling plant metals sample into the 120 mL jar for the

analysis type as listed in the chart above. Record the weight of material on the Sample Aliquot Preparation Form (Appendix D).

7.2.2.5 Cap and label the jar with Sample ID as indicated in Section 8 for recycling plant samples and the Sample Aliquot Preparation Form.

7.2.2.6 Repeat steps 7.2.2.2 – 7.2.2.5 for each analysis type for each batch of recycling plant samples for a total of 18 sub-samples (six (6) analysis types from three (3) lot/batch/storage samples).

7.2.2.7 Cap the jar containing the remainder of the recycling plant sample batch, record the amount removed and other information as needed in the COC record (Appendix F), and return to freezer storage at -20ºC.

7.2.2.8 Complete the Sample Aliquot Preparation Form for these samples (Appendix C). Store recycling plant subsamples at -20ºC until transferred to the appropriate technical lead for analysis.

8.0 RECORDS

Any information relevant to sample aliquot preparation should be recorded in a laboratory notebook (e.g., visual observations, deviations, etc.). Sample preparation will be recorded in the appropriate "Sample Aliquot Preparation Form" for the type of sample being prepared (Appendix A-D). All samples should have a unique sample ID code that is relevant to where the sample was collected and what type of subsample they represent. A detailed list of sample codes is included in Appendix E. Samples collected from recycling plants will be coded as follows.

Recycling Plant Sample ID Code:

TCRS-R-VV-W-X-Y-Z

Where:
TCRS = tire crumb rubber research study
R = recycling plant designation
VV = two-digit code unique to each recycling plant, from 80 to 89
W = recycling facility batch or storage container number, from 1 to 3; field blanks are 9
X = sample jar designation from each batch or storage container; 1 or 2
Y = sample type identifier M (metals), G (organic compounds), P (particle characterization)
Z = assigned analysis number codes for subsample aliquots to indicate the type of analysis the sample was prepared for. The analysis type numbers are assigned as follows in Table 1:

Table 1. Assigned Analysis Numbers for Sample Codes

Analysis Type	Number Assigned
Composite/Parent Sample – No direct analysis	0
SVOC Extraction	1
Chamber VOC	2
Chamber SVOC	3
SVOC or Metals Bioaccessibility -Saliva	4
SVOC or Metals Bioaccessibility - Gastric	5
SVOC or Metals Bioaccessibility - Sweat	6
Metals Digestion	7
Metals XRF	8

Examples for tire crumb rubber research study recycling plant samples:

TCRS-R-81-1-1-M-4	Plant 81, Batch 1, Sample Jar 1, Metals, SVOC or Metals Bioaccessibility - Saliva aliquot
TCRS-R-85-3-1-G-3	Plant 85, Batch 3, Sample Jar 1, Organic Compounds, Chamber SVOC aliquot

Samples collected from synthetic turf playing fields will follow a very similar scheme with some modifications to reflect the different types of samples being collected. The coding for fields is as follows:

Synthetic Turf Playing Field Sample ID Code:

TCRS-F-VV-W-X-Y-Z

Where:
TCRS = tire crumb rubber research study
F = playing field designation
VV = two-digit code unique to each playing field, from 10 to 75
W = playing field sampling location; 1-7; Composite samples will have the designation “C” replacing the playing field location; field blanks are 9
X = sample jar designation; will always be 1 for fields or 1-2 for particle samples
Y = sample type designation, M (metals), G (organic compounds), P (particle characterization)
Z = assigned analysis number codes for sub-sample aliquots from Table 1

Examples for the synthetic field composite sub-samples:

TCRS-F-10-C-1-M-4	Field 10, Composite Sample, Sample Jar 1, Metals, SVOC or Metals Bioaccessibility – Saliva sample
TCRS-F-30-C-1-G-3	Field 30, Composite Sample, Sample Jar 1, Organic Compounds, Chamber SVOC sample

The individual location subsamples will use the sample code from field collection with the analysis number from Table 1 above.

Examples for the synthetic field individual location sub-samples:

TCRS-F-10-1-1-M-4	Field 10, Field Sampling Location 1, Sample Jar 1, Metals, SVOC or Metals Bioaccessibility – Saliva sample
TCRS-F-30-3-1-G-3	Field 30, Field Sampling Location 3, Sample Jar 1, Organic Compounds sample, Chamber SVOC sample

The COC record from sample collection will be completed to describe the transfer and handling of those samples in preparation of the subsamples. The mass removed from each sample will be recorded along with the details of sample handling transfer and storage. A different COC record will be used to document the storage and transfer of subsample aliquots to the respective analytical laboratories. This COC record may include different sample types transferred at the same time, but should only contain samples sent to a single laboratory. Field collected quality control samples may also be transferred using this form providing that the original COC appropriately indicates the transfer of the sample. The form to be used can be found as Appendix F to this SOP.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

Balance calibration will be checked with two different check weights prior to use that encompass the range in which samples will be weighed. This check will be documented on the sample aliquot preparation forms. The acceptance range for the balance is +/-1% of the weight being measured. Only annually verified weights will be used to check the balance. If the balance calibration does not meet the acceptance criteria, the calibration will be re-checked using different weights from the same set that encompass the range of use. If the balance is still out of calibration, then another balance will be used and checked in the same manner prior to use.

APPENDIX A – Sample Aliquot Preparation Playing Fields – Organics

Sample Aliquot Preparation Form – Field Samples – Organics

SOP#: D-EMMD-PHCB-040-SOP-01 (Sections 7.1.1 - 7.1.3 – Synthetic Field Samples – Organics)

Prepared By: _____ Balance: _____

Date: _____ Check Wt. 1: _____

Pass: ____ Check Wt. 2: _____
Pass: ____

Composite Preparation – Organics (Section 7.1.1)

Sample ID	Weight (g)	Sum Weight (g)
Location 1 ID: TCRS - F - _ _ -1-1-G-0		
Location 2 ID: TCRS - F - _ _ -2-1-G-0		
Location 3 ID: TCRS - F - _ _ -3-1-G-0		
Location 4 ID: TCRS - F - _ _ -4-1-G-0		
Location 5 ID: TCRS - F - _ _ -5-1-G-0		
Location 6 ID: TCRS - F - _ _ -6-1-G-0		
Location 7 ID: TCRS - F - _ _ -7-1-G-0		
Composite ID: TCRS - F - _ _ -C-1-G-0	NA	

Subsample Preparation – Organics (Section 7.1.2)

Sample Type	Sample ID[1]	Weight (g)
SVOC Extraction	TCRS - F - _ _ - C-1-G-1	
Chamber VOC	TCRS - F - _ _ - C-1-G-2	
Chamber SVOC	TCRS - F - _ _ - C-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - F - _ _ - C-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - F - _ _ - C-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - F - _ _ - C-1-G-6	

Individual Location Aliquots – Organics (Section 7.1.3)

Location 1

Sample Type	Sample ID	Weight (g)
SVOC Extraction	TCRS - F - _ _ -1-1-G-1	
Chamber VOC	TCRS - F - _ _ -1-1-G-2	
Chamber SVOC	TCRS - F - _ _ -1-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - F - _ _ -1-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - F - _ _ -1-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - F - _ _ -1-1-G-6	

Location 2

Sample Type	Sample ID	Weight (g)
SVOC Extraction	TCRS - F - _ _ -2-1-G-1	
Chamber VOC	TCRS - F - _ _ -2-1-G-2	
Chamber SVOC	TCRS - F - _ _ -2-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - F - _ _ -2-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - F - _ _ -2-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - F - _ _ -2-1-G-6	

Location 3

Sample Type	Sample ID	Weight (g)
SVOC Extraction	TCRS - F - _ _ -3-1-G-1	
Chamber VOC	TCRS - F - _ _ -3-1-G-2	
Chamber SVOC	TCRS - F - _ _ -3-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - F - _ _ -3-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - F - _ _ -3-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - F - _ _ -3-1-G-6	

[1] Sample ID Code: TCRS-F-VV-W-X-Y-Z; Where: TCRS = tire crumb rubber research study; F = playing field designation; VV = two-digit code unique to each playing field from 10 to 75; W = Playing field sampling location from 1 to 7 or C for Composite Samples and 9 for field blanks; X = Sample jar designation, will always be 1 for fields; Y = Sample type designation, M (Metals), G (Organic Compounds), P (Particle characterization); Z = Assigned analysis number codes for sub-sample aliquots from Table 1 of SOP #D-EMMD-PHCB-040-SOP-01.

APPENDIX B – Sample Aliquot Preparation Playing Fields – Metals

Sample Aliquot Preparation Form – Field Samples – Metals

SOP#: D-EMMD-PHCB-040-SOP-01 (Sections 7.1.4 - 7.1.6 – Playing Field Samples – Metals)

Prepared By: _____ Balance: _____

Date: _____ Check Wt. 1: _____

Pass: ____ Check Wt. 2: _____
Pass: ____

Composite Preparation – Metals (Section 7.1.4)

Sample ID	Weight (g)	Sum Weight (g)
Location 1 ID: TCRS - F - _ _ -1-1-M-0		
Location 2 ID: TCRS - F - _ _ -2-1-M-0		
Location 3 ID: TCRS - F - _ _ -3-1-M-0		
Location 4 ID: TCRS - F - _ _ -4-1-M-0		
Location 5 ID: TCRS - F - _ _ -5-1-M-0		
Location 6 ID: TCRS - F - _ _ -6-1-M-0		
Location 7 ID: TCRS - F - _ _ -7-1-M-0		
Composite ID: TCRS - F - _ _ -C-1-M-0	NA	

Subsample Preparation – Metals (Section 7.1.5)

Sample Type	Sample ID[2]	Weight (g)
Metals Digestion	TCRS - F - _ _ - C-1-M-7	
Metals XRF	TCRS - F - _ _ - C-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - F - _ _ - C-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - F - _ _ - C-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - F - _ _ - C-1-M-6	

Individual Location Aliquots – Metals (Section 7.1.6)

Location 1

Sample Type	Sample ID	Weight (g)
Metals Digestion	TCRS - F - _ _ -1-1-M-7	
Metals XRF	TCRS - F - _ _ -1-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - F - _ _ -1-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - F - _ _ -1-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - F - _ _ -1-1-M-6	

.

Location 2

Sample Type	Sample ID	Weight (g)
Metals Digestion	TCRS - F - _ _ -2-1-M-7	
Metals XRF	TCRS - F - _ _ -2-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - F - _ _ -2-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - F - _ _ -2-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - F - _ _ -2-1-M-6	

Location 3

Sample Type	Sample ID	Weight (g)
Metals Digestion	TCRS - F - _ _ -3-1-M-7	
Metals XRF	TCRS - F - _ _ -3-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - F - _ _ -3-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - F - _ _ -3-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - F - _ _ -3-1-M-6	

[1] Sample ID Code: TCRS-F-VV-W-X-Y-Z; Where: TCRS = tire crumb rubber research study; F = playing field designation; VV = two-digit code unique to each playing field from 10 to 75; W = Playing field sampling location from 1 to 7 or C for Composite Samples and 9 for field blanks; X = Sample jar designation, will always be 1 for fields; Y = Sample type designation, M (Metals), G (Organic Compounds), P (Particle characterization); Z = Assigned analysis number codes for sub-sample aliquots from Table 1 of SOP #D-EMMD-PHCB-040-SOP-01.

APPENDIX C – Sample Aliquot Preparation Recycling Plants – Organics

Sample Aliquot Preparation Form – Recycling Plant Samples – Organics

SOP#: D-EMMD-PHCB-040-SOP-01 (Section 7.2.1 – Recycling Plant Samples – Organics)

Prepared By: _____ Balance: _____

Date: _____ Check Wt. 1: _____

Pass: ____ Check Wt. 2: _____
Pass: ____

Sample Storage Location: _____

Comments: _____

Lot/Batch/Container 1 (Sample ID: TCRS - R - _ _ -1-1-G-_)

Sample Type	**Sample ID**[3]	**Weight (g)**
SVOC Extraction	TCRS - R - _ _ -1-1-G-1	
Chamber VOC	TCRS - R - _ _ -1-1-G-2	
Chamber SVOC	TCRS - R - _ _ -1-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - R - _ _ -1-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - R - _ _ -1-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - R - _ _ -1-1-G-6	

Lot/Batch/Container 2 (Sample ID: TCRS - R -_ _ -2-1-G- _)

Sample Type	**Sample ID**	**Weight (g)**
SVOC Extraction	TCRS - R - _ _ -2-1-G-1	
Chamber VOC	TCRS - R - _ _ -2-1-G-2	
Chamber SVOC	TCRS - R - _ _ -2-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - R - _ _ -2-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - R - _ _ -2-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - R - _ _ -2-1-G-6	

Lot/Batch/Container 3 (Sample ID: TCRS - R -_ _ -3-1-G- _)

[1] Sample ID Code: TCRS-F-VV-W-X-Y-Z; Where: TCRS = tire crumb rubber research study; F = playing field designation; VV = two-digit code unique to each playing field from 10 to 75; W = Playing field sampling location from 1 to 7 or C for Composite Samples and 9 for field blanks; X = Sample jar designation, will always be 1 for fields; Y = Sample type designation, M (Metals), G (Organic Compounds), P (Particle characterization); Z = Assigned analysis number codes for sub-sample aliquots from Table 1 of SOP #D-EMMD-PHCB-040-SOP-01.

.

Sample Type	**Sample ID**	**Weight (g)**
SVOC Extraction	TCRS - R - _ _ -3-1-G-1	
Chamber VOC	TCRS - R - _ _ -3-1-G-2	
Chamber SVOC	TCRS - R - _ _ -3-1-G-3	
SVOC Bioaccessibility - Saliva	TCRS - R - _ _ -3-1-G-4	
SVOC Bioaccessibility - Gastric	TCRS - R - _ _ -3-1-G-5	
SVOC Bioaccessibility - Sweat	TCRS - R - _ _ -3-1-G-6	

APPENDIX D – Sample Aliquot Preparation Recycling Plants – Metals

Sample Aliquot Preparation Form – Recycling Plant Samples – Metals

SOP#: D-EMMD-PHCB-040-SOP-01 (Section 7.2.2 – Recycling Plant Samples – Metals)

Prepared By: _____ Balance: _____

Date: _____ Check Wt. 1: _____

Pass: ____ Check Wt. 2: _____
Pass: ____

Sample Storage Location: _____

Comments: _____

Batch 1 (Sample ID: TCRS - R - _ _ -1-1-M- _)

Sample Type	**Sample ID**[4]	**Weight (g)**
Metals Digestion	TCRS - R - _ _ -1-1-M-7	
Metals XRF	TCRS - R - _ _ -1-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - R - _ _ -1-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - R - _ _ -1-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - R - _ _ -1-1-M-6	

Batch 2 (Sample ID: TCRS - R - _ _ -2-1-M- _)

Sample Type	**Sample ID**	**Weight (g)**
Metals Digestion	TCRS - R - _ _ -2-1-M-7	
Metals XRF	TCRS - R - _ _ -2-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - R - _ _ -2-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - R - _ _ -2-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - R - _ _ -2-1-M-6	

Batch 3 (Sample ID: TCRS - R - _ _ -3-1-M- _)

Sample Type	**Sample ID**	**Weight (g)**
Metals Digestion	TCRS - R - _ _ - 3-1-M-7	
Metals XRF	TCRS - R - _ _ - 3-1-M-8	
Metals Bioaccessibility - Saliva	TCRS - R - _ _ - 3-1-M-4	
Metals Bioaccessibility - Gastric	TCRS - R - _ _ - 3-1-M-5	
Metals Bioaccessibility - Sweat	TCRS - R - _ _ -3-1-M-6	

.

[1]Sample ID Code: TCRS-F-VV-W-X-Y-Z; Where: TCRS = tire crumb rubber research study; F = playing field designation; VV = two-digit code unique to each playing field from 10 to 75; W = Playing field sampling location from 1 to 7 or C for Composite Samples and 9 for field blanks; X = Sample jar designation, will always be 1 for fields; Y = Sample type designation, M (Metals), G (Organic Compounds), P (Particle characterization); Z = Assigned analysis number codes for sub-sample aliquots from Table 1 of SOP #D-EMMD-PHCB-040-SOP-01.

APPENDIX E – Sample Codes for Tire Crumb Subsamples

Synthetic Field and Recycling Plant ID Number Information
(A unique 2-digit code will be used for each synthetic field or recycling plant)

TCRS Sample Codes for Recycling Plant Tire Crumb Rubber Subsamples
Recycling Plant Tire Crumb Rubber Subsample Coding – Metals Analysis
(XX = Plant ID Number) *

Sample Code	Description	TCR Mass
TCRS-R-XX-1-1-M-7	Batch 1 Metals Digestion – 120 mL HDPE jar	10 g
TCRS-R-XX-2-1-M-7	Batch 2 Metals Digestion – 120 mL HDPE jar	10 g
TCRS-R-XX-3-1-M-7	Batch 3 Metals Digestion – 120 mL HDPE jar	10 g
TCRS-R-XX-1-1-M-8	Batch 1 Metals XRF – 120 mL HDPE jar	10 g
TCRS-R-XX-2-1-M-8	Batch 2 Metals XRF – 120 mL HDPE jar	10 g
TCRS-R-XX-3-1-M-8	Batch 3 Metals XRF – 120 mL HDPE jar	10 g
TCRS-R-XX-1-1-M-4	Batch 1 Metals Bioaccessibility - Saliva – 120 mL HDPE jar	20 g
TCRS-R-XX-2-1-M-4	Batch 2 Metals Bioaccessibility - Saliva – 120 mL HDPE jar	20 g
TCRS-R-XX-3-1-M-4	Batch 3 Metals Bioaccessibility - Saliva – 120 mL HDPE jar	20 g
TCRS-R-XX-1-1-M-5	Batch 1 Metals Bioaccessibility - Gastric – 120 mL HDPE jar	20 g
TCRS-R-XX-2-1-M-5	Batch 2 Metals Bioaccessibility - Gastric – 120 mL HDPE jar	20 g
TCRS-R-XX-3-1-M-5	Batch 3 Metals Bioaccessibility - Gastric – 120 mL HDPE jar	20 g
TCRS-R-XX-1-1-M-6	Batch 1 Metals Bioaccessibility - Sweat – 120 mL HDPE jar	20 g
TCRS-R-XX-2-1-M-6	Batch 2 Metals Bioaccessibility - Sweat – 120 mL HDPE jar	20 g
TCRS-R-XX-3-1-M-6	Batch 3 Metals Bioaccessibility - Sweat – 120 mL HDPE jar	20 g

*Jar 1 from each batch will be used for all aliquots.

TCRS Sample Codes for Recycling Plant Tire Crumb Rubber Subsamples (*continued*)
Recycling Plant Tire Crumb Rubber Subsample Coding – Organics Analysis
(XX = Plant ID Number) *

Sample Code	Description	TCR Mass
TCRS-R-XX-1-1-G-1	Batch 1 Organics SVOC Extraction – 60 mL Glass Jar	10 g
TCRS-R-XX-2-1-G-1	Batch 2 Organics SVOC Extraction – 60 mL Glass Jar	10 g
TCRS-R-XX-3-1-G-1	Batch 3 Organics SVOC Extraction – 60 mL Glass Jar	10 g
TCRS-R-XX-1-1-G-2	Batch 1 Organics Chamber VOC – 60 mL Glass Jar	50 g
TCRS-R-XX-2-1-G-2	Batch 2 Organics Chamber VOC – 60 mL Glass Jar	50 g
TCRS-R-XX-3-1-G-2	Batch 3 Organics Chamber VOC – 60 mL Glass Jar	50 g
TCRS-R-XX-1-1-G-3	Batch 1 Organics Chamber SVOC – 60 mL Glass Jar	30 g
TCRS-R-XX-2-1-G-3	Batch 2 Organics Chamber SVOC – 60 mL Glass Jar	30 g
TCRS-R-XX-3-1-G-3	Batch 3 Organics Chamber SVOC – 60 mL Glass Jar	30 g
TCRS-R-XX-1-1-G-4	Batch 1 Organics SVOC Bioaccessibility - Saliva – 60 mL Glass Jar	20 g
TCRS-R-XX-2-1-G-4	Batch 2 Organics SVOC Bioaccessibility - Saliva – 60 mL Glass Jar	20 g
TCRS-R-XX-3-1-G-4	Batch 3 Organics SVOC Bioaccessibility - Saliva – 60 mL Glass Jar	20 g
TCRS-R-XX-1-1-G-5	Batch 1 Organics SVOC Bioaccessibility - Gastric – 60 mL Glass Jar	20 g
TCRS-R-XX-2-1-G-5	Batch 2 Organics SVOC Bioaccessibility - Gastric – 60 mL Glass Jar	20 g
TCRS-R-XX-3-1-G-5	Batch 3 Organics SVOC Bioaccessibility - Gastric – 60 mL Glass Jar	20 g
TCRS-R-XX-1-1-G-6	Batch 1 Organics SVOC Bioaccessibility - Sweat – 60 mL Glass Jar	20 g
TCRS-R-XX-2-1-G-6	Batch 2 Organics SVOC Bioaccessibility - Sweat – 60 mL Glass Jar	20 g
TCRS-R-XX-3-1-G-6	Batch 3 Organics SVOC Bioaccessibility - Sweat – 60 mL Glass Jar	20 g

*Jar 1 from each batch will be used for all aliquots.

TCRS Sample Codes for Synthetic Turf Field Tire Crumb Rubber Subsamples
Synthetic Field Tire Crumb Rubber Subsample Coding – Metals Analysis
(XX = Field ID Number)[5]

Sample Code	Description	TCR Mass
TCRS-F-XX-C-1-M-0	Composite Metals sample – 500 mL HPDE Jar	245 g
TCRS-F-XX-C-1-M-7	Composite Metals sample - Digestion – 120 mL HDPE Jar	10 g
TCRS-F-XX-C-1-M-8	Composite Metals sample - XRF – 120 mL HDPE Jar	10 g
TCRS-F-XX-C-1-M-4	Composite Metals sample - Bioaccessibility - Saliva – 120 mL HDPE Jar	20 g
TCRS-F-XX-C-1-M-5	Composite Metals sample - Bioaccessibility - Gastric – 120 mL HDPE Jar	20 g
TCRS-F-XX-C-1-M-6	Composite Metals sample - Bioaccessibility - Sweat – 120 mL HDPE Jar	20 g
TCRS-F-XX-1-1-M-7	Location 1 Metals - Digestion – 120 mL HDPE Jar	5 g
TCRS-F-XX-2-1-M-7	Location 2 Metals - Digestion – 120 mL HDPE Jar	5 g
TCRS-F-XX-3-1-M-7	Location 3 Metals - Digestion – 120 mL HDPE Jar	5 g
TCRS-F-XX-1-1-M-8	Location 1 Metals sample - XRF – 120 mL HDPE Jar	5 g
TCRS-F-XX-2-1-M-8	Location 2 Metals sample - XRF – 120 mL HDPE Jar	5 g
TCRS-F-XX-3-1-M-8	Location 3 Metals sample - XRF – 120 mL HDPE Jar	5 g
TCRS-F-XX-1-1-M-4	Location 1 Metals - Bioaccessibility - Saliva – 120 mL HDPE Jar	10 g
TCRS-F-XX-2-1-M-4	Location 2 Metals - Bioaccessibility - Saliva – 120 mL HDPE Jar	10 g
TCRS-F-XX-3-1-M-4	Location 3 Metals - Bioaccessibility - Saliva – 120 mL HDPE Jar	10 g
TCRS-F-XX-1-1-M-5	Location 1 Metals - Bioaccessibility - Gastric – 120 mL HDPE Jar	10 g
TCRS-F-XX-2-1-M-5	Location 2 Metals - Bioaccessibility - Gastric – 120 mL HDPE Jar	10 g
TCRS-F-XX-3-1-M-5	Location 3 Metals - Bioaccessibility - Gastric – 120 mL HDPE Jar	10 g
TCRS-F-XX-1-1-M-6	Location 1 Metals - Bioaccessibility - Sweat – 120 mL HDPE Jar	10 g
TCRS-F-XX-2-1-M-6	Location 2 Metals - Bioaccessibility - Sweat – 120 mL HDPE Jar	10 g
TCRS-F-XX-3-1-M-6	Location 3 Metals - Bioaccessibility - Sweat – 120 mL HDPE Jar	10 g

[a]Subsamples will be prepared from individual field location samples for only a subset of fields to be selected by the research coordinator (approximately 5 out of 40 fields). It is anticipated that the individual field locations will be 1, 2, and 3. However, the research coordinator may provide guidance to designate the use of other field locations if necessary.

TCRS Sample Codes for Synthetic Turf Field Tire Crumb Rubber Subsamples (*continued*)
Synthetic Field Tire Crumb Rubber Subsample Coding – Organics Analysis
(XX = Field ID Number)

Sample Code	Description	TCR Mass
TCRS-F-XX-C-1-G-0	Composite Organics sample – 500 mL Glass Jar	245 g
TCRS-F-XX-C-1-G-1	Composite Organics sample - SVOC Extraction – 60 mL Glass Jar	10 g
TCRS-F-XX-C-1-G-2	Composite Organics sample - Chamber VOC – 120 mL Glass Jar	50 g
TCRS-F-XX-C-1-G-3	Composite Organics sample - Chamber SVOC – 120 mL Glass Jar	30 g
TCRS-F-XX-C-1-G-4	Composite Organics sample - Bioaccessibility - Saliva – 60 mL Glass Jar	20 g
TCRS-F-XX-C-1-G-5	Composite Organics sample - Bioaccessibility - Gastric – 60 mL Glass Jar	20 g
TCRS-F-XX-C-1-G-6	Composite Organics sample - Bioaccessibility - Sweat – 60 mL Glass Jar	20 g
TCRS-F-XX-1-1-G-1	Location 1 Organics - SVOC Extraction – 60 mL Glass Jar	5 g
TCRS-F-XX-2-1-G-1	Location 2 Organics - SVOC Extraction – 60 mL Glass Jar	5 g
TCRS-F-XX-3-1-G-1	Location 3 Organics - SVOC Extraction – 60 mL Glass Jar	5 g
TCRS-F-XX-1-1-G-2	Location 1 Organics - Chamber VOC – 60 mL Glass Jar	20 g
TCRS-F-XX-2-1-G-2	Location 2 Organics - Chamber VOC – 60 mL Glass Jar	20 g
TCRS-F-XX-3-1-G-2	Location 3 Organics - Chamber VOC – 60 mL Glass Jar	20 g
TCRS-F-XX-1-1-G-3	Location 1 Organics - Chamber SVOC – 60 mL Glass Jar	20 g
TCRS-F-XX-2-1-G-3	Location 2 Organics - Chamber SVOC – 60 mL Glass Jar	20 g
TCRS-F-XX-3-1-G-3	Location 3 Organics - Chamber SVOC – 60 mL Glass Jar	20 g
TCRS-F-XX-1-1-G-4	Location 1 Organics - Bioaccessibility - Saliva – 60 mL Glass Jar	10 g
TCRS-F-XX-2-1-G-4	Location 2 Organics - Bioaccessibility - Saliva – 60 mL Glass Jar	10 g
TCRS-F-XX-3-1-G-4	Location 3 Organics - Bioaccessibility - Saliva – 60 mL Glass Jar	10 g
TCRS-F-XX-1-1-G-5	Location 1 Organics - Bioaccessibility - Gastric – 60 mL Glass Jar	10 g
TCRS-F-XX-2-1-G-5	Location 2 Organics - Bioaccessibility - Gastric – 60 mL Glass Jar	10 g
TCRS-F-XX-3-1-G-5	Location 3 Organics - Bioaccessibility - Gastric – 60 mL Glass Jar	10 g
TCRS-F-XX-1-1-G-6	Location 1 Organics - Bioaccessibility - Sweat – 60 mL Glass Jar	10 g
TCRS-F-XX-2-1-G-6	Location 2 Organics - Bioaccessibility - Sweat – 60 mL Glass Jar	10 g

TCRS-F-XX-3-1-G-6	Location 3 Organics - Bioaccessibility - Sweat – 60 mL Glass Jar	10 g

[a]Subsamples will be prepared from individual field location samples for only a subset of fields to be selected by the research coordinator (approximately 5 out of 40 fields). It is anticipated that the individual field locations will be 1, 2, and 3. However, the research coordinator may provide guidance to designate the use of other field locations if necessary.

APPENDIX F – Chain of Custody for Subsample Aliquots

Chain of Custody Record

Project Name: **Tire Crumb Rubber Research Study**				
Organization:				
Address:				
City/State/Zip:				
Phone:				
Sample ID	Description if Needed	Date Prepared	Date Analyzed	Comments if Needed
Prepared By:		Date:	Storage:	Relinquished By:
Received By:		Date:	Storage:	Relinquished By:
Received By:		Date:	Storage:	Relinquished By:
Received By:		Date:	Storage:	Relinquished By:

Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System (D-EMMD-AQB-SOP-3465-0; Alternate ID: D-EMMD-AQB-018-SOP-01; Companion SOP to D-EMMD-AQB-017-SOP-01) – October 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurements Division
Air Quality Branch

STANDARD OPERATING PROCEDURE

SOP Title: Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System for the Tire Crumb Research Study

SOP ID: D-EMMD-AQB-SOP-3465-0	Effective Date: October 1, 2016

SOP was developed: ☐ In-house ☒ Extramural: Jacobs WA 3-111

SOP Discipline*: Organic Chemistry

Alternative Identification: #D-EMMD-AQB-018-SOP-01

SOP Contact Signature

Name: Karen Oliver
Signature/Date:

Management Signature

Name: Surender Kaushik (Tad Kleindienst signing on behalf of Surender Kaushik)
Title: Branch Chief
Signature/Date:

QA Signature

Name: Sania Tong Argao
Title: Quality Assurance Manager
Signature/Date:

*See discipline descriptions on the NERL Scientific and Technical SOP intranet site.

Revision History

Revision No.	Name	Date of Revision	Description of Change(s)
1	Karen Oliver	11/21/2018	This SOP was revised to reflect current Division and Branch. Previous EMMD ID #D-EMMD-AQB-018-SOP-01.

Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System for the Tire Crumb Research Study – TBD

Contributors

Karen Oliver
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Tamira Cousett
Jacobs Technology Inc.
Research Triangle Park, NC

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

1.1 This standard operating procedure (SOP) is applicable to the determination of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and non-targeted compounds of interest using the Markes International gas chromatograph–mass spectrometer (GC-MS) time-of-flight (TOF) system following desorption from sorbent tubes using the Markes International thermal desorber 50:50 (TD 50:50) and the Unity 2. The TD 50:50 serves as an auxiliary thermal desorption unit that is capable of desorbing up to 100 sorbent tubes in one analytical sequence. Primary tube desorption occurs in the TD 50:50, and collected analytes are transferred to the Unity 2, focused on the cold trap, desorbed off the trap, and transferred to the GC for further separation before final quantitation in the TOF MS. The analysis method for VOCs, Tire Crumb Compounds, and non-targeted compounds is provided in Appendix A.

1.2 This SOP is written as a companion to SOP#: D-EMMD-AQB-017-SOP-01, *Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International BenchTOF-Select GC-MS TOF System for the Tire Crumb Research Study.*

1.3 The following VOCs are the compounds of interest from the EPA Compendium Method TO-14A target list (U.S. EPA, 1999a), PAHs of interest from the EPA Compendium Method TO-13A target list (U.S. EPA, 1999b), and target Tire Crumb Research Study (TCRS) compounds of interest from in-house pilot studies.

VOCs		**PAHs**	**Tire Crumb**
1,2-Dichloro-1,1,2,2-tetrafluoroethane	Toluene	Naphthalene	*tert*-butylamine
1,3-Butadiene	Tetrachloroethene	Acenaphthalene	*trans*-2-butene
Trichlorofluoromethane	Chlorobenzene	Acenaphthene	*cis*-2-butene
1,1-Dichloroethene	Ethylbenzene	Fluorene	Methyl isobutyl ketone
1,1,2-Trichloro-1,2,2-trifluoroethane	*m,p*-Xylene	Phenanthrene	Benzothiazole
1,1-Dichloroethane	Styrene	Anthracene	
cis-1,2-Dichloroethene	*o*-Xylene	Fluoranthene	
1,2-Dichloroethane	4-Ethyltoluene	Pyrene	
1,1,1-Trichloroethane	1,3,5-Trimethylbenzene		
Benzene	*m*-Dichlorobenzene		
Carbon tetrachloride	*p*-Dichlorobenzene		
1,2-Dichloropropane	*o*-Dichlorobenzene		
Trichloroethene			

2.0 SUMMARY OF METHOD

VOCs are desorbed from sorbent tubes that have been previously exposed using SOPs D-EMMD-AQB-016-SOP-01, D-EMMD-AQB-015-SOP-01, or D-EMMD-AQB-007-SOP-01 and conditioned using SOP#: D-EMMD-AQB-008-SOP-01 (Markes TC-20 tube conditioner). The tubes are fitted with a stainless-steel DiffLok cap on the rear end (outlet) of the tube while an inert coated stainless-steel cap is placed on the grooved end (inlet) of the tube. Tubes are loaded horizontally into the sampling trays with the fritted (grooved) end of the tube (inlet) pointing toward the right-hand side. An analytical sequence is created and initiated using the Ultra-TD software to select the appropriate desorption method and run the sequence. The TD 50:50/Unity 2

method for determining VOCs, TCRS compounds, and non-targeted compounds is summarized in Appendix A.

An additional method has been optimized on the system to desorb VOCs and PAHs from Carbograph 2 TD and Carbograph 1 TD dual-bed sorbent tubes. This method is referred to throughout the SOP as the VOC_PAH method and is not in the scope of daily laboratory analytical practices; the operating parameters and compound target lists are specified separately in Appendix B (VOC_PAH method).

3.0 DEFINITIONS

D1	duplicate
DQO	data quality objective
ECC	electronic carrier control
GC	gas chromatograph
FB	field blank
FS	field spike
ID	identification
in.	inch
MDL	method detection limit
MFC	mass flow controller
min	minute
mL	milliliter
MS	mass spectrometer
PAH	polycyclic aromatic hydrocarbon
ppbv	parts per billion by volume
pptv	parts per trillion by volume
psig	pounds per square inch gauge
PTFE	polytetrafluoroethylene
SA	sample
SB	shipping blank
SOP	standard operating procedure
TCRS	tire crumb research study
TD	thermal desorber
TOF	time of flight
VOC	volatile organic compound

4.0 HEALTH AND SAFETY

4.1 Gases in high-pressure cylinders are in use in this laboratory. Operators must exercise extreme care in working with high-pressure gas cylinders.

4.2 Certain areas of the Markes International TD 50:50/Unity 2 are extremely hot, so caution should be used when attempting to retrieve a hot or jammed tube. Also, the power to the TD 50:50 should be switched off when attempting to retrieve a jammed tube and when troubleshooting and performing routine maintenance.

5.0 INTERFERENCES

5.1 Prior to sampling, sorbent tubes should be conditioned for one (1) hour using the tube conditioning procedure described in SOP#: D-EMMD-AQB-008-SOP-01 (Markes TC-20) to remove any VOC contaminants. This process should remove any contaminants that are residual from prior sampling or that might have outgassed from the sorbent. Conditioning specifications for sorbent tubes are provided by the manufacturer and should be executed per manufacturer protocol.

5.2 Prior to analysis, all sorbent tubes must be loaded within internal standards as described in SOP#: D-EMMD-AQB-015-SOP-01.

5.3 Two laboratory blanks should be analyzed at the beginning of every desorption sequence using a conditioned sorbent tube to remove any VOC contaminants from the trap that might have been adsorbed from the helium purge gas or outgassed from the sorbent in the trap.

5.4 Leak tests should be performed during each analytical run. If repeated leak test failures occur, see the Unity 2 Troubleshooting Guide, the Unity 2 Operators' Manual, the Markes International Leak Locating Guide, and the Markes International Thermal Desorption Training Guide.

5.5 The O-rings located inside of the DiffLok caps might need to be changed periodically due to wear. See the thermal desorption training guide for O-ring replacement.

5.6 The Peltier cooling will not function properly if the dry gas supply is not switched on. The dry gas supply must be set to 50–60 psi and have a dew point of less than -50°C or ice will form on the Peltier coolers.

5.7 Tubes and their associated DiffLok caps should be handled by the operator only when wearing either clean white cotton or nitrile gloves to prevent contamination from skin oils and the VOCs they contain.

Note: DiffLok caps used on sorbent tubes loaded with ***only*** VOCs should not be used interchangeably with sorbent tubes loaded with VOCs and PAHs to ensure there is no cross contamination of PAHs.

6.0 PERSONNEL QUALIFICATIONS

Personnel should have knowledge of the following:

- General laboratory safety practices including appropriate cylinder-handling procedures.
- Sorbents, breakthrough volumes, flowmeters, mass flow controllers (MFCs), computer spreadsheets, thermal desorption, gas chromatography, mass spectrometry, data analysis and validation, and general instrument troubleshooting.

7.0 EQUIPMENT AND SUPPLIES

The following equipment and supplies are needed:

- Markes International Thermal Desorber 50:50 (Markes International, Llantrisant, UK).
- Markes International Unity 2 (Markes International).
- Markes International BenchTOF-Select (Markes International).
- Markes International TOF-DS Software V 1.3 (Markes International).
- Markes International User Software V 5.1.103 (Markes International).
- Markes International CIA Advantage Software V 5.1.103 (Markes International).
- Computer with Windows 7 64-bit (English edition), Quad core, Intel Xeon E3-1225 v3 or equivalent processor, 8 GB DDR3 memory, 10 GB free space, 1920 x 1080 resolution graphics card and 1920 x 1080 monitor.
- TO-15/TO-17 air toxics focusing trap, part no. U0T15ATA-2S, for Unity 2 (Markes International).
- Clean cloth gloves (part no. 11-462-26B, Thermo Fisher Scientific, Waltham, MA) or nitrile gloves (part no. 55091, 55092, or 55093, Kimberly-Clark, Neenah, WI).
- Peek tubing (part no. SERZ-0108, 1/6" o.d. x 0.03" bore, 1M, Markes International).
- DiffLok Caps, ¼-inch stainless steel inert coated (part no. C-DLS10, Markes International).
- DiffLok Caps, ¼-inch stainless steel (part no. DL010, Markes International).
- Assorted wrenches.
- CapLok tools (Markes International).
- Low-emission Viton O-rings, sizes 006, 007, and 010 (part no. U-COV06, U-COV07, and U-COV10, respectively, Markes International).
- Ultra/TD-100 O-ring insertion tool (part no. SERMTD-1382, Markes International).

- Filter disk, sintered PFTE, 5.11 m, pack of 10 (part no. U-DISK1, Markes International,).

8.0 REAGENTS AND STANDARDS

- Cylinder gas, research-grade helium (AirGas, Morrisville, NC).
- Cylinder gas, ultra-high-purity nitrogen (AirGas).
- Daily external check standards loaded diffusively in the exposure chamber (SOP#: D-EMMD-AQB-007-SOP-01) or the climate-controlled exposure chamber (SOP#: D-EMMD-AQB-016-SOP-01). TO14A VOC Standards and internal standards may also be loaded using the active-loading system (see SOP#: D-EMMD-AQB-015-SOP-01).

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 The TD 50:50/Unity 2 automatically performs a leak test on the tube and the trap prior to each desorption to verify that the seals at these locations are tight to prevent sample loss.

9.2 The sorbent focusing trap is conditioned by analyzing two laboratory blanks using conditioned sorbent tubes at the beginning of every desorption sequence to remove contaminant VOCs.

9.3 Sorbent tubes are conditioned using a 1-hour tube conditioning cycle as described in SOP#: D-EMMD-AQB-008-SOP-01 (Markes TC-20) prior to sample collection to remove contaminant VOCs. Sorbent must be conditioned as specified in protocols provided by the manufacturer.

9.4 During diffusive sampling, samples can be exposed alone or in pairs depending on the data quality objectives (DQOs) of the study. The criteria for acceptable results for duplicate analytical precision, as defined in Compendium Method TO-17, Section 14, "Performance Criteria for the Solid Adsorbent Sampling of Ambient Air," require agreement within 20% for duplicate pairs (U.S. EPA, 1999b).

9.5 Depending on the DQOs of a study, laboratory and/or field blanks may be included at the beginning of the desorption sequence following the helium blank.

9.6 Depending on the DQOs of a study, two to three daily external check standards are included in each desorption sequence. These standards are used to gauge filament wear in the mass spectrometer and system stability. Daily external check standards are compared to the original external check standards that were analyzed with the initial calibration to determine if VOC concentrations are within ±30% of the current calibration range.

9.7 Internal standards are manually loaded on to each sorbent tube prior to analysis as described in SOP#: D-EMMD-AQB-015-SOP-01, to account for instrument drift. The responses of the internal standards are used by the GC-MS TOF software for calculation of compound concentrations and by the operator to monitor changes in the sensitivity of the analytical system. The responses of internal standard compounds should be monitored daily to ensure their response remains steady. Decreased response for these compounds indicates the system might need to be optimized and calibrated. See the Markes International Bench TOF-Select operators' manual for more information. Internal standards are loaded onto each tube to be analyzed by the instrument. The internal standard consists of four components: 4-bromofluorobenzene, chlorobenzene-d5, 1,4-difluorobenzene, and bromochloromethane.

10.0 STANDARDS PREPARATION

Standards are loaded diffusively onto the sorbent tubes by using an exposure chamber as described in SOP#: D-EMMD-AQB-007-SOP-01 or SOP#: D-EMMD-AQB-016-SOP-01. Standards may also be prepared using the active loading system as detailed in SOP#: D-EMMD-AQB-015-SOP-01.

11.0 PROCEDURES

11.1 *Establish Communication with the TD 50:50/Unity 2 Thermal Desorber*

Power on the TD 50:50 and Unity 2 by pressing the power switch located on the back of each instrument. After each instrument is powered on, the following procedures are used to establish communication between the TD 50:50 and Unity 2, instruments using the TD 50:50 instrument interface screen on the desktop computer designated for instrument control:

1. Turn on the desktop computer.
2. Double click the **Ultra TD-Tubes** icon on the desktop.
3. When the **Ultra TD** dialog box opens, the last sequence table that was analyzed will appear. On the upper toolbar, click **File > New > Sequence** or click the **New Sequence** icon.
4. When the blank sequence table appears on the screen (Figure 1), right click on any portion of the new sequence table and select **Add Set of Tubes** (Figure 2).

Figure 1. New sequence table.

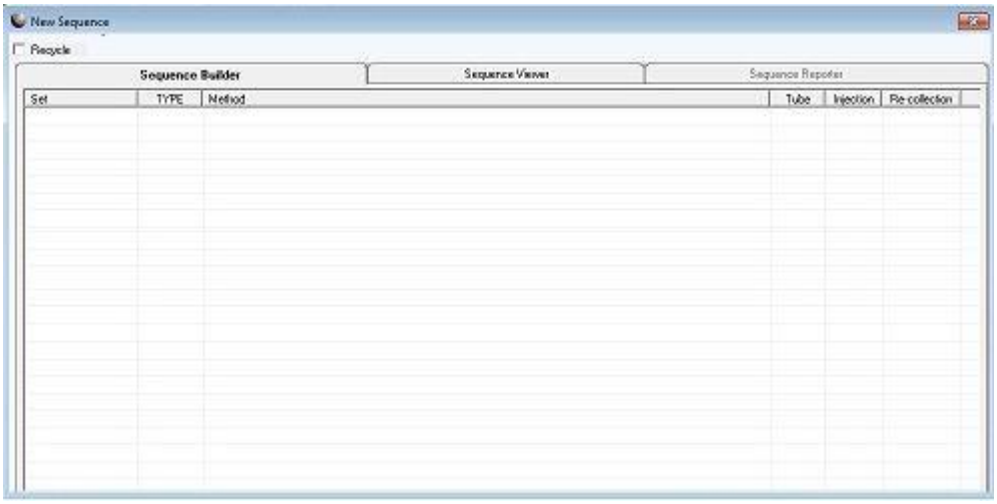
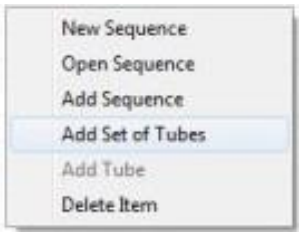
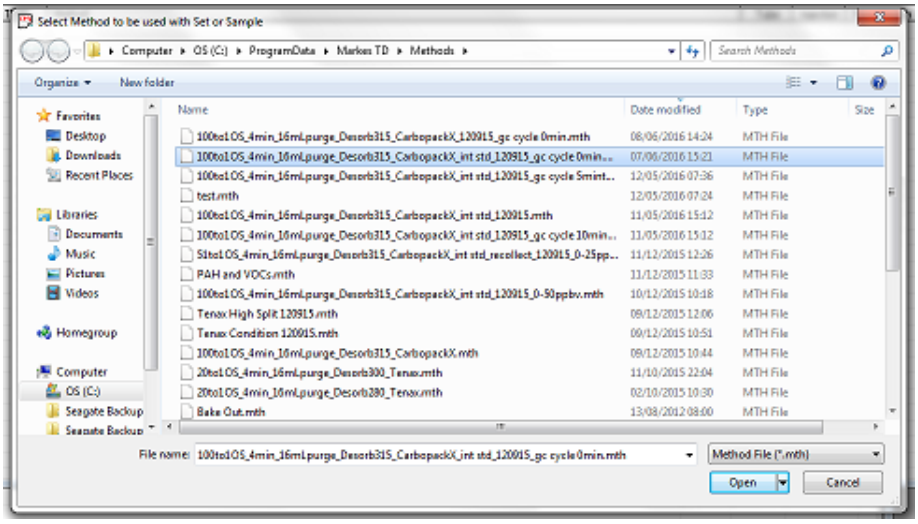


Figure 2. Adding a set of tubes.



5. When the **Select Method** dialog box (Figure 3) and "Add New Set" dialog box appears on the screen, select the desired method and click **Open**. (For the scope of daily laboratory activities, the VOC and non-targeted method is named "25to1OS_4min_16mLpurge_Desorb315_CarbopackX_no int std_100416_gc cycle time 47min.mth")

Figure 3. Selecting the desired method.

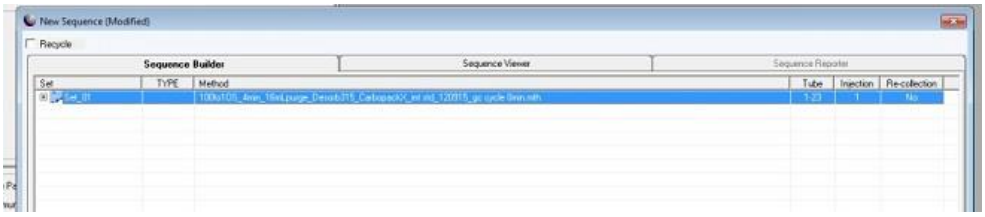


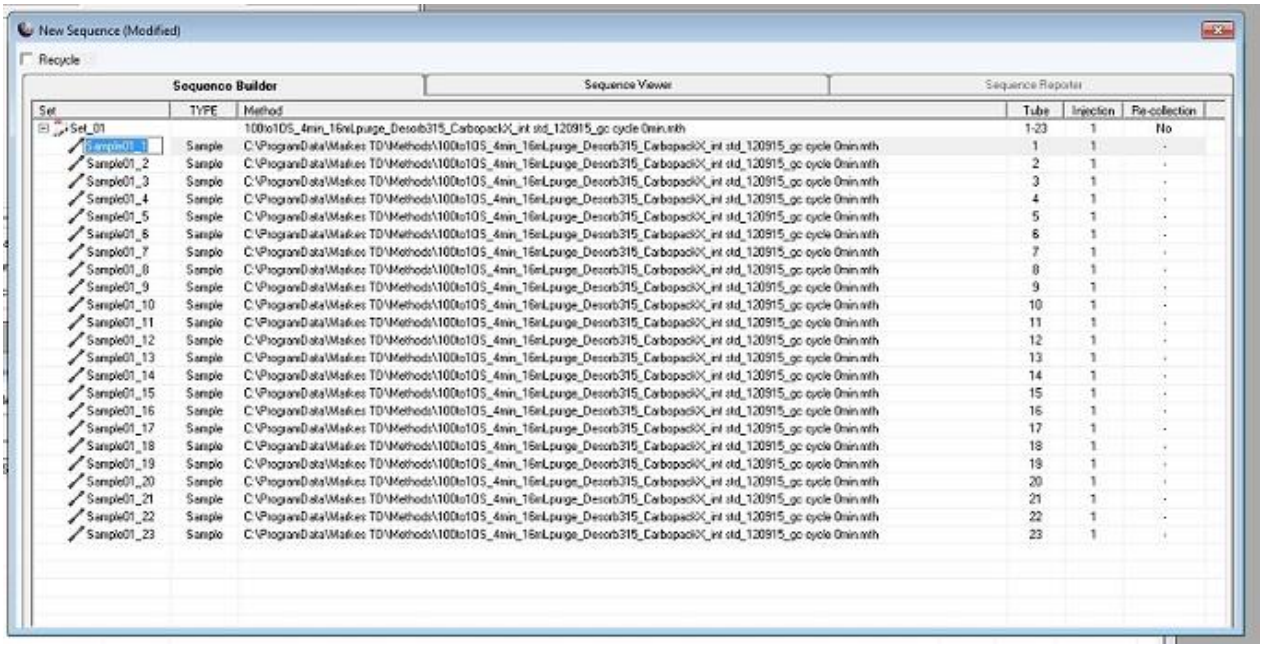
6. In the **Add New Set** dialog box (Figure 4), set the first and last tube numbers using the drop-down menus. For instance, if the operator wishes to analyze 23 tubes in slots 1–23, the first tube would be "1" and the last tube would be "23." ***Note:*** The **No. of Injection** is set to "1".

Figure 4. Selecting the number of tubes for analysis in the Add New Set dialog box.

7. In the Re-collection field, select "Same" from the drop-down menu to ensure sample is collected back onto the same sorbent tube from which it was desorbed.
8. Select **OK** after the first and last tubes have been set.
9. Click the + sign to expand the samples list for the new set of tubes. The sequence table will populate a row for each sample to be analyzed with the same name and method (Figure 5).

Figure 5. Expanding the sequence table.





10. In the **Set** column of the sequence table, click on the auto-generated sample identification (ID) code to highlight it in blue, and then enter the tube ID number in this

field (Figures 6a and 6b). Be sure to enter the tube ID numbers in the order that the tubes will be analyzed.

Figure 6a. Selecting the auto-generated tube ID.

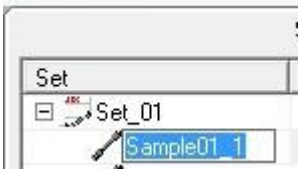
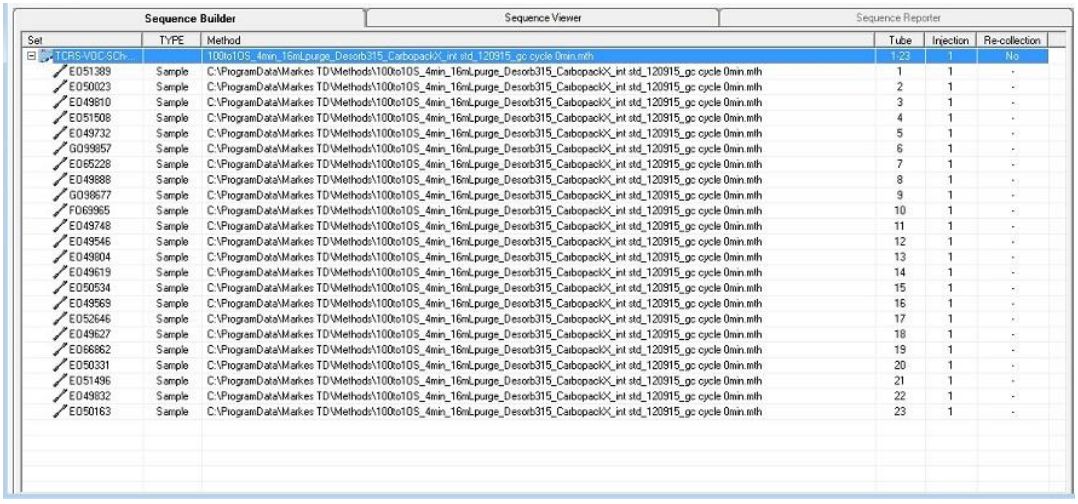
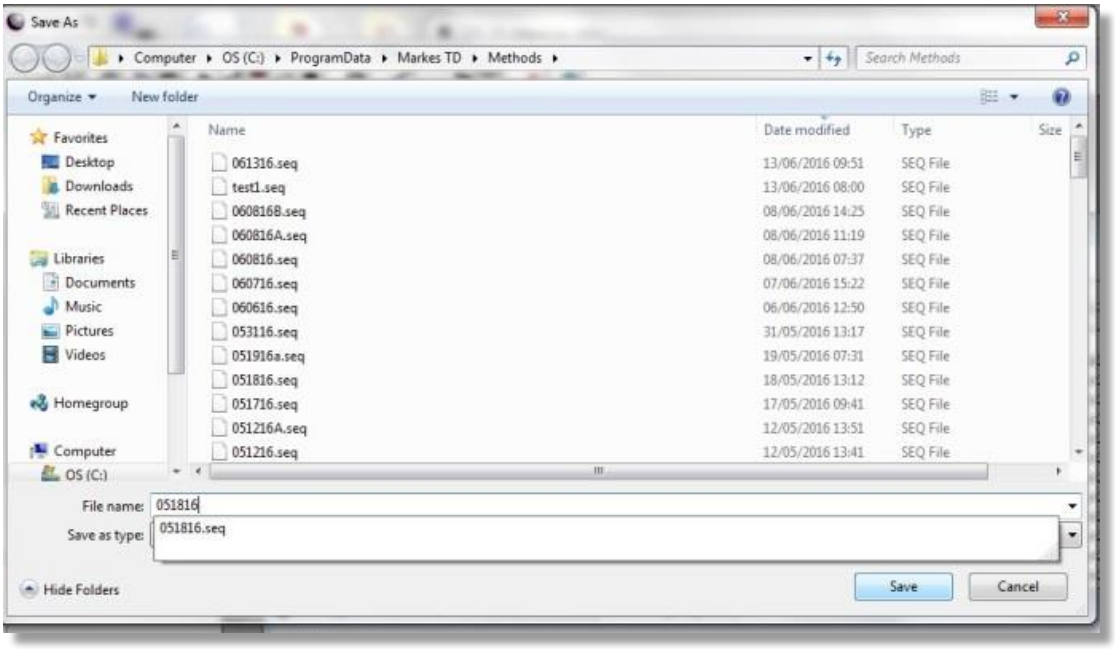


Figure 6b. Entering tube ID numbers into the sequence table.



11. Once the tube numbers have been entered, click **File > Save As** and enter the sequence name with the current days' date in the MMDDYY format, and then click **Save** (Figure 7). Note: If more than one sequence is run on a day, a unique identifier should be placed at the end of the sequence name such as MMDDYYA or MMDDYYB, as not to overwrite other sequences run on a particular day.

Figure 7. Saving the sequence name in the MMDDYY format.



12. Click the **Controlling Method** icon on the tool bar to load method parameters for the method specified in the sequence table.
13. Click **File > Save**.

11.2 *Set and Adjust Initial Flow Rates*

Gas flows are set and measured using the electronic carrier control (ECC) function. The ECC function uses an internal MFC to set and measure gas flows without operator intervention. Column head pressure is monitored by the GC at the head of the column, and any variations in pressure are compensated for at the supply. Total flow can be read from the GC. Flows can be confirmed with a flow meter.

Significant inconsistencies in flows tend to indicate problems with the TD 50:50 or the Unity 2. Due to the use of MFCs, flow inconsistencies can be more difficult to diagnose as all flow settings and readouts are digitally controlled. The operator should monitor chromatography carefully as this is the area in which flow inconsistences will have the greatest impact.

11.3 *Load the Carousel*

Although the number and type of samples, blanks, and standards will vary depending on the DQOs of the project, a rough ordering and brief explanation of tubes on the sample carousel is as follows:

- *Laboratory blank:* a conditioned, unexposed sampling tube that has remained in the laboratory. Two blanks are run at the beginning of the sequence to determine trap background levels.
- *Field blank (FB):* conditioned, unexposed sampling tube transported to the field and back.
- *Field spike (FS):* laboratory-exposed, 2.0 ppbv sampling tubes transported to the field and back.

- *Laboratory control (LC)*: the field.
- *Shipping blank (SB):* unused spare tube transported to the field and back.
- *Daily external check standard*: a sampling tube passively loaded with the calibration mixture at a designated concentration level for 24 hours (SOP#: D-EMMD-AQB-007-SOP-01 or SOP#: D-EMMD-AQB-016-SOP-01) actively loaded standards are also available for use (SOP#: D-EMMD-AQB-015-SOP-01). Daily check standards are used to monitor filament wear and system stability. The daily check standards are analyzed at the beginning, middle, and end of the sample batch to ensure the system remains within ±30% of the calibration range during analysis.

A typical sample batch consists of two laboratory blanks at the beginning of the sequence, two additional laboratory blanks within the sequence, 18 samples, and three standards for a total of approximately 27 analyses, which are completed in approximately 27 hours.

Tubes loaded into tube trays for analysis must be QA checked to ensure that tube numbers are correct and have been loaded in the correct order. The sequence table created in the TOF-DS software (see SOP#: D-EMMD-AQB-017-SOP-01) and written on the Markes Unity Almsco GC-MS TOF analysis sheet (Appendix C) must also be checked for correct tube order and numbers.

Use the following procedure to load the carousel:

1. Verify that the cylinder valves and the regulator outlet valves are open and that the regulator outlet pressure is set at the correct value (research helium = 50–60 psig).

2. Open the TD 50:50 door and remove the first tray to load the first 10 tubes in the analytical sequence (Figure 8). ***Note:*** The door of the TD 50:50 should never be opened during analysis.

Figure 8. Opening the TD 50:50 door to access tube trays.

3. While wearing either white cotton or nitrile gloves, load the top sample tray labeled 1–10, starting with the tube slot in the back of the tray, with the laboratory blanks, sample, and standard sorbent tubes (with DiffLok caps on both ends) by placing them horizontally in the numbered slots with the fritted (grooved) end of the tube (inlet)

pointing toward the right-hand side and the rear end of the tube (outlet) pointed towards the left-hand side of the tube tray (Figure 9).

Figure 9. Loading sorbent tubes into the TD 50:50 tube tray.

4. When all slots of the first tray have been loaded with tubes, insert the tray back into the top slot of the TD 50:50. Guide the tray into the slot slowly until a single click is felt and gently pull back on the tray. This click indicates the tray is properly seated (Figure 10).

Figure 10. Loading the sample tray into the TD when full.

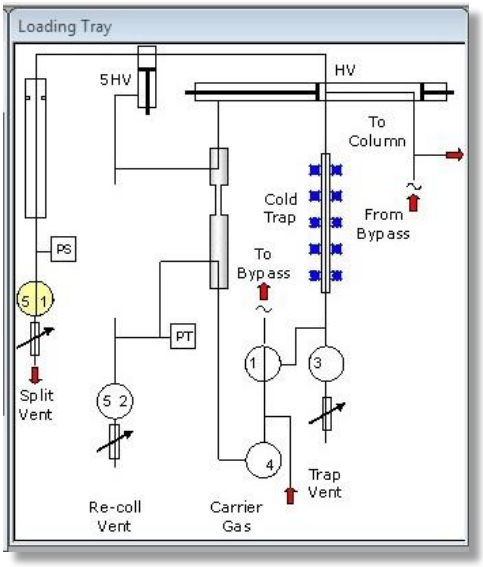
5. Repeat steps 2–4 with tube trays 2–10 in the TD until all sampling tubes are loaded. Be sure to remove and load one tray at a time in the order in which tubes trays are stacked inside the TD.

11.4 *Run the Ultra TD 50:50 and Unity 2 Analysis Sequence*

Use the TOF-DS analytical software to create the TOF GC-MS sequence table (sample list) and initiate an analytical sequence as described in detail in SOP#: D-EMMD-AQB-017-SOP-01. Initiate the analysis sequence on the system computer as follows:

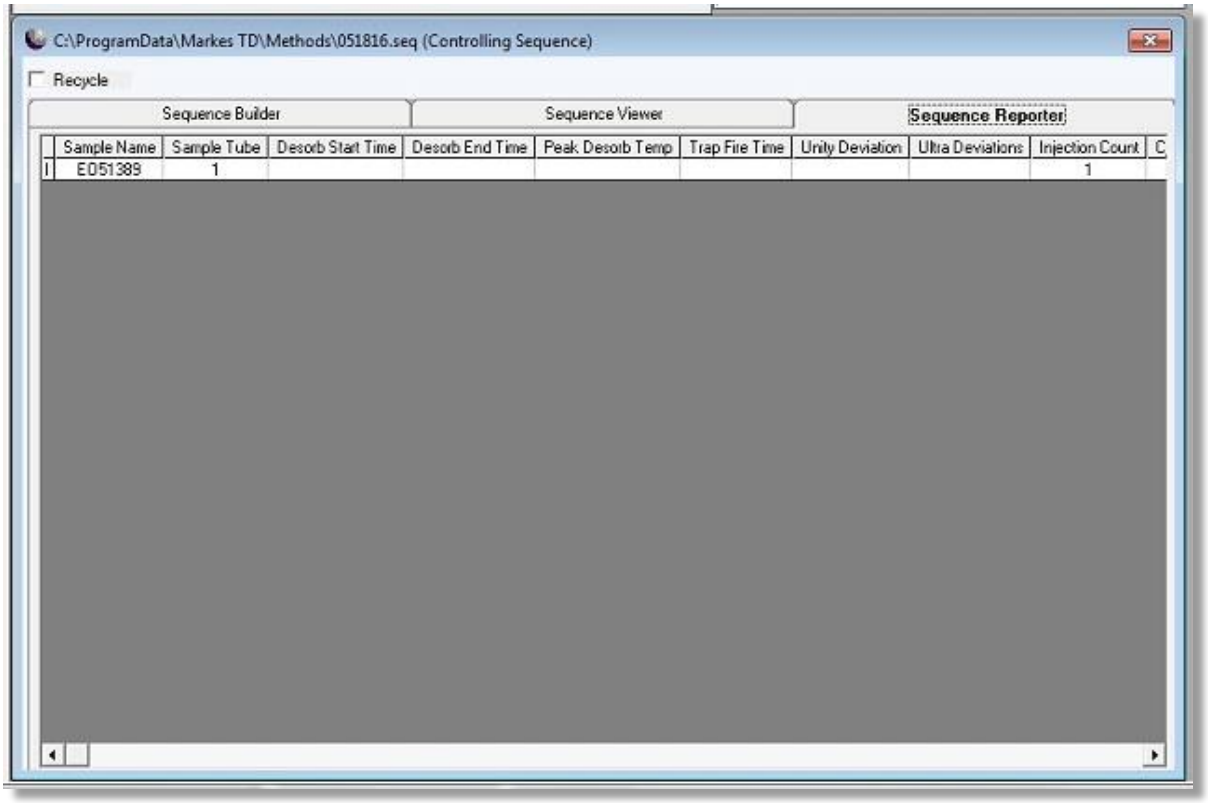
1. Follow the steps in section 11.1 to open the software and create the sequence table.
2. Click on the three tabs of the method dialog box: Pre-desorption, Tube/Sample desorption, and Trap Settings to ensure settings match those specified for the method. The VOC and non-targeted method parameters are detailed in Appendix A.
3. Once method settings are verified, click the first sample in the sequence table and click the start icon (▶) to begin the TD 50:50 and Unity 2 sequence. An internal schematic of the Unity 2 system will appear on screen when the sequence begins (Figure 11).

Figure 11. Unity 2 Schematic during desorption.



4. Click on the **Sequence Reporter** tab of the sequence table to review desorption parameters as sorbent tubes are desorbed (Figure 12). Throughout the analysis of samples, it is helpful to view this tab periodically to ensure no tube leak or system leaks occur. ***Note:*** If tube or system leaks do occur, contact the principal investigator to initiate troubleshooting efforts.

Figure 12. Sequence Reporter tab.



Note: After the samples have been desorbed, they are stored until data is reviewed. Prior to re-use, tubes must be conditioned and stored as described in SOP#: D-EMMD-AQB-008-SOP-01 (Markes TC-20) prior to use.

12.0 DATA AND RECORDS MANAGEMENT

12.1 The operator must maintain a laboratory notebook in which the experimental and sample details are recorded.

12.2 The operator must record the date that each cylinder gas is changed in the Markes International gas logbook.

12.3 Service on the Ultra TD 50:50 and Unity 2 must be documented in the maintenance notebook.

13.0 METHOD PERFORMANCE

13.1 Method detection limits (MDLs) on the order of 35 pptv for benzene have been achieved for 24-hour exposures.

13.2 Laboratory experiments to evaluate issues such as reverse diffusion, temperature and humidity effects, linearity of response, MDLs, and ozone effects, are discussed in McClenny et al. (2005). These experiments were used to determine the subset of the TO-14A VOCs listed in Section 1.0 that can be determined using Carbopack X diffusive sampling techniques.

14.0 MAINTENANCE AND TROUBLESHOOTING

14.1 The O-rings in the hot and cold nozzle of the TD 50:50 and the Viton O-rings on the Unity 2 sampling inlet must be periodically changed due to wear. Worn O-rings may result in leak test failures. See the operators' manual, leak locating guide, and thermal desorption training guide for O-ring replacement.

Note: The TD 50:50 and the Unity 2 must be powered off during routine maintenance.

14.2 The trap O-ring and filter on the cool non-valve end of the trap should be changed when trap changes occur or if poorly sealing O-rings are determined to be a leak source. Worn O-rings can result in leak test failures.

14.3 The focusing trap in the Unity 2 may need to be changed periodically due to wear. This change is generally performed by a Markes International technician during the annual performance maintenance service call as heated valve seals are also replaced. After installation of the new trap, trap conditioning is recommended using the parameters listed in Table 1. Instructions for changing and conditioning the focusing trap are detailed in the operators' manual.

Table 1. New Trap Conditioning Parameters

Trap Temperature (°C)	Hold Time (min)
200	10
250	10
300	10
350	30
350	30
350	30

14.4 The O-rings located inside of the DiffLok caps might need to be changed periodically due to wear. See the thermal desorption training guide for O-ring replacement.

14.5 For routine maintenance procedures and suggested troubleshooting procedures for the Ultra TD 50:50 and Unity 2, refer to the Unity 2 Troubleshooting Guide, the Unity 2 Operators' Manual, the Markes International Leak Locating Guide, and the Markes International Thermal Desorption Training Guide. A suggested maintenance schedule is given in Figure 14.

Figure 14. Suggested maintenance schedule.

Suggested maintenance schedule

Suggested maintenance frequencies are given below. However, in some cases (depending on the application), items may need replacing more frequently.

UNITY 1 and 2

Condition/change charcoal filter (split tube)	3 months[1]
Replace/repack cold trap	12 months[1]
Replace fused silica transfer line	12 months
Change sample tube O-rings/filters	12 months, or if damaged/leaking
Cold trap seals	12 months, or if damaged/leaking

TD-100

Condition/change charcoal filter (split tube)	3 months[1]
Replace/repack cold trap	12 months[1]
Replace fused silica transfer line	12 months
Change sample tube O-rings/filters	12 months, or if damaged/leaking
Cold trap seals	12 months, or if damaged/leaking
Replace O-rings in DiffLok caps	If damaged/leaking
Change nozzle seals	If damaged/leaking

ULTRA

Replace O-rings in DiffLok caps	If damaged/leaking
Change nozzle seals	If damaged/leaking

Air Server 3/8, CIA 8 and CIA *Advantage*

Replace filter disks	12 months

15.0 REFERENCES AND SUPPORTING DOCUMENTATION

15.1 *References*

D-EMMD-AQB-003-SOP-01 (alternative ID: ECAB-151.1). 2016. Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Carbopack X Diffusive Sampling Tubes Using the Agilent 6890N/5975 GC-MSD. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-005-SOP-01 (alternative ID: ECAB-153.0). 2013. Standard Operating Procedure for Carbopack X Sorbent Tube Conditioning using CDS Analytical Model 9600 Tube Conditioners. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-006-SOP-01 (alternative ID: ECAB-154.1). 2015. Standard Operating Procedure for Desorbing Volatile Organic Compounds from Carbopack X Sorbent Tubes Using the PerkinElmer TurboMatrix ATD. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-007-SOP-01 (alternative ID: ECAB-155.1). 2015. Standard Operating Procedure for Use of the Exposure Chamber for Loading Passive Sampling Devices with Volatile Organic Compounds. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-008-SOP-01 (alternative ID: ECAB-156.0E). 2013. Standard Operating Procedure for Carbopack X Sorbent Tube Conditioning Using the Markes International Model TC-20 Sample Tube Conditioner. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-015-SOP-01. 2016. Standard Operating Procedure for Actively Loading Sorbent tunes with Volatile Organic Compounds. Environmental Protection Agency. National Exposure Research Laboratory.

D-EMMD-AQB-016-SOP-01. 2016. Standard Operating Procedure for the Use of the Climate-Controlled Exposure Chamber for Loading Passive Sampling Devices with Volatile Organic Compounds. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-017-SOP-01. 2016. Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International BenchTOF-Select GC-MS TOF System. Environmental Protection Agency. National Exposure Research Laboratory.

McClenny, W.A., Oliver, K.D., Jacumin, H.H. Jr., Daughtrey, E.H. Jr., and Whitaker, D.A. 2005. 24 h diffusive sampling of toxic VOCs in air onto Carbopack X solid adsorbent followed by thermal desorption/GC/MS analysis–laboratory studies. *J. Environ. Monit.* 7:248-256.

U.S. EPA. 1999a. Compendium Method TO-14A: Determination of Volatile Organic Compounds (VOCs) in Ambient Air Using Specially Prepared Canisters with Subsequent Analysis by Gas Chromatography. In *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, 2nd ed., EPA/625/R-96/010b. Cincinnati, OH: Office of Research and Development.

U.S. EPA. 1999b. Compendium Method TO-17: Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling onto Sorbent Tubes. In *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, 2nd ed., EPA/625/R-96/010b. Cincinnati, OH: Office of Research and Development.

Unity 2 Operators' Manual. November 2011. Document no. QUI-1057 Version 1.4. (Markes International, Llantrisant, UK).

Unity 2 Trouble Shooting Guide. February 2008. Version 1.0. (Markes International).

Thermal Desorption Training Guide. Power Point. (Markes International).
Leak Locating Guide. Power Point. (Markes International).
Unity 2 (Digital MFCs already fitted) Hardware Installation Manual. March 2014. Document no. QSI-SERUTE-5156. (Markes International).

15.2 *Supporting Documentation*

Oliver, K.D., Jacumin, H.H. Jr., and Daughtrey, E.H. Jr. 2003. Initial Evaluation of PerkinElmer Carbopack X Diffusive Sampling Badges for Collection of Toxic VOCs. TR-4423-03-09. Research Triangle Park, NC: ManTech Environmental Technology, Inc.

Oliver, K.D., Jacumin, H.H. Jr., Daughtrey, E.H. Jr., and McClenny, W.A. 2000. Sample Integrity of Volatile Organic Compounds Collected and Stored on Multiadsorbent Tubes and Analyzed Using an AutoGC/MS System. TR-4423-00-07. Research Triangle Park, NC: ManTech Environmental Technology, Inc.

TurboMatrix ATD 650/TD Control Software User's Guide. June 2002. Part Number 09934591, Release A, PerkinElmer Instruments LLC, 710 Bridgeport Avenue, Shelton, CT 06484.

TurboMatrix Thermal Desorbers User's Guide. April 2000. Part Number M041-3331, Release B, PerkinElmer, Inc., 761 Main Avenue, Norwalk, CT 06859.

Appendix A: VOC Method Parameters

System: Ultra TD 50:50 and Unity 2
Method Filename: 25to1OS_4min_16mLpurge_Desorb315_CarbopackX_no int std_100416_gc cycle 47min.mth
TD mode: Two-stage desorption
Corresponds to Method: TO14_Tire Crumb_Final_100516_variable drops for the TOF-GC-MS

```
[UnityMethod]
Author =
MethodName =
Company =
Notes =
DateCreated =
DateModified = 05/10/2016 14:24:25
FileName = 25to1OS_4min_16mLpurge_Desorb315_CarbopackX_no int std_100416_gc cycle 47min
OperatingMode = Standard Two Stage
IdleSplit = TRUE
MinimumCarrierPressure = 5
PurgeTrapInLine = FALSE
PurgeSplit = TRUE
StandbyFlow =20
AirServerTrapFlow =20
PrePurgeSplitFlow =20
PrePurgeTrapFlow =20
PrimaryDesorb1SplitFlow =20
PrimaryDesorb2SplitFlow =20
PrimaryDesorb1TrapFlow =50
PrimaryDesorb2TrapFlow =20
TrapDesorbSpliFlow =36
PreTrpFirePurgeSplitFlow =36
PreTrpFirePurgeTrapFlow =50
TubeCondSplitFlow =20
AirServerLinePurge =20
DirectSamplingFlow =20
DryPurgeFlow =16
DirectModeFlushSplitFlow =20
StdInjFlow =50
AirServerTrapPurge =20
DirectModeTrapPurgeFlow =20
DirectModeFlushTrapFlow =20
PrePurgeTime = .1
AirServerPostSamplePurgeTime = 1
DryPurgeTime = 4
DirectPostSamplePurgeTime = 1
PreTrapFirePurgeTime = 2
AirServerPostSampleTrapPurgeTime = 1
AirServerPostSampleTrapPurgeDirect = 1
OvenTemperature1 = 315
DesorbTime1 = 15
Desorb1TrapInLine = TRUE
Desorb1Split = FALSE
OvenTemperature2 = 250
DesorbTime2 = 0
Desorb2Split = FALSE
StdInjTime = 1
LoopFillTime = .4
DryPurgeOrStdInj =DryPurge
TrapLow = 15
TrapHigh = 280
TrapHold = 5
TrapSplit = TRUE
QMB6Sample = 2
SensorTemperature = 65
TrapHeatRate = 0
ColumnFlow = 1.5
DesorbFlow = 20
TubeDesorbSplit = 0
TrapDesorbSplit = 36
InletSplitRatio =No Split
OutletSplitRatio =25.0 : 1
TotalSplitRatio =25.0 : 1
FlowPathTemperature = 160
GCCycleTime = 60
```

Appendix B: VOC_PAH Method Parameters

System: Ultra TD 50:50 and Unity 2
Method file name: PAH and VOCs.mth
TD mode: Two-stage desorption
Corresponds to Method: Method_TO17d for the TOF-GC-MS

```
[UnityMethod]
Author =
MethodName =
Company =
Notes =
DateCreated =
DateModified = 11/12/2015 11:27:38
FileName = C:\ProgramData\Markes TD\Methods\PAH and VOCs.mth
OperatingMode = Standard Two Stage
IdleSplit = TRUE
MinimumCarrierPressure = 5
PurgeTrapInLine = FALSE
PurgeSplit = TRUE
StandbyFlow =20
AirServerTrapFlow =20
PrePurgeSplitFlow =20
PrePurgeTrapFlow =20
PrimaryDesorb1SplitFlow =50
PrimaryDesorb2SplitFlow =20
PrimaryDesorb1TrapFlow =50
PrimaryDesorb2TrapFlow =20
TrapDesorbSpliFlow =23
PreTrpFirePurgeSplitFlow =23
PreTrpFirePurgeTrapFlow =50
TubeCondSplitFlow =20
AirServerLinePurge =20
DirectSamplingFlow =20
DryPurgeFlow =16
DirectModeFlushSplitFlow =20
StdInjFlow =50
AirServerTrapPurge =20
DirectModeTrapPurgeFlow =20
DirectModeFlushTrapFlow =20
PrePurgeTime = .1
AirServerPostSamplePurgeTime = 1
DryPurgeTime = 4
DirectPostSamplePurgeTime = 1
PreTrapFirePurgeTime = 1
AirServerPostSampleTrapPurgeTime = 1
AirServerPostSampleTrapPurgeDirect = 1
OvenTemperature1 = 340
DesorbTime1 = 10
Desorb1TrapInLine = TRUE
Desorb1Split = TRUE
OvenTemperature2 = 250
DesorbTime2 = 0
Desorb2Split = FALSE
StdInjTime = 3
LoopFillTime = .4
DryPurgeOrStdInj =StdInj
TrapLow =-10
TrapHigh = 315
TrapHold = 4
TrapSplit = TRUE
QMB6Sample = 2
SensorTemperature = 65
TrapHeatRate = 0
ColumnFlow = 2
DesorbFlow = 50
TubeDesorbSplit = 50
TrapDesorbSplit = 23
InletSplitRatio =2.0 : 1
OutletSplitRatio =12.5 : 1
TotalSplitRatio =25.0 : 1
FlowPathTemperature = 180
GCCycleTime = 26
```

16.0 SCOPE AND APPLICATION

16.1 This appendix applies to the exploration of the desorption of volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) from Carbograph 2TD and Carbograph 1TD dual-bed sorbent tubes using EPA Compendium Method TO-17 type procedures (U.S. EPA, 1999a). For this method, the Markes International Ultra TD 50:50 and Unity 2 are interfaced with a Markes International GC-MS TOF system.

16.2 This method is written as a companion to D-EMMD-AQB-017-SOP-01, *Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International BenchTOF-Select GC-MS TOF System*.

16.3 The standards are prepared by using flash vaporization to load PAHs and active loading to load VOCs onto sorbent tubes, as described in an SOP that is under development.

16.4 The following VOCs are the compounds of interest from the EPA Compendium Method TO-14A target list (U.S. EPA, 1999b). Target PAHs are the compounds of interest from the EPA Compendium Method TO-13A target list (U.S. EPA, 1999c).

VOCs:	**PAHs:**
Benzene	Naphthalene
Toluene	Acenaphthalene
Ethylbenzene	Acenaphthene
m,p-Xylene	Fluorene
Styrene	Phenanthrene
o-Xylene	Anthracene
4-Ethyltoluene	Fluoranthene
1,3,5-Trimethylbenzene	Pyrene

17.0 SUMMARY OF METHOD

VOCs and PAHs are desorbed from Carbograph 2TD and 1TD dual-bed sorbent tubes that have been previously loaded using flash vaporization techniques to load tubes with PAHs (SOP under development) followed by active loading with a gas-tight syringe to load VOCs (SOP#: D-EMMD-AQB-015-SOP-01) onto tubes conditioned using the Markes TC-20 (SOP#: D-EMMD-AQB-008-SOP-01) tube conditioner. The tubes, with DiffLok caps on both ends, are placed horizontally into the sampling tray with the fritted (grooved) end of the tube (inlet) pointing towards the right-hand side and the rear end of the tube (outlet) pointed towards the left-hand side of the sampling tray. An analytical sequence is created and initiated using the analytical software. The Ultra TD-Tube software is used to create a desorption sequence by selecting the "PAH and VOCs.mth" desorption method and then initiating the sequence.

18.0 REFERENCES

- D-EMMD-AQB-008-SOP-01 (alternative ID: ECAB-156.0E). 2013. Standard Operating Procedure for *Carbopack X Sorbent Tube Conditioning Using the Markes International Model TC-20 Sample Tube Conditioner*. U.S. Environmental Protection Agency, National Exposure Research Laboratory.
- D-EMMD-AQB-015-SOP-01. 2016. Standard Operating Procedure for *Actively Loading Sorbent tunes with Volatile Organic Compounds*. Environmental Protection Agency. National Exposure Research Laboratory.

- D-EMMD-AQB-017-SOP-01. 2016. Standard Operating Procedure for *Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International BenchTOF-Select GC-MS TOF System*. Environmental Protection Agency. National Exposure Research Laboratory.
- U.S. EPA. 1999a. Compendium Method TO-17: Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling onto Sorbent Tubes. In *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, 2nd ed., EPA/625/R-96/010b. Cincinnati, OH: Office of Research and Development.
- U.S. EPA. 1999b. Compendium Method TO-14A: Determination of Volatile Organic Compounds (VOCs) in Ambient Air Using Specially Prepared Canisters with Subsequent Analysis by Gas Chromatography. In *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, 2nd ed., EPA/625/R-96/010b. Cincinnati, OH: Office of Research and Development.
- U.S. EPA. 1999c. Compendium Method TO-13A: Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS). In *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, 2nd ed., EPA/625/R-96/010b. Cincinnati, OH: Office of Research and Development.

Appendix C: Markes Unity Almsco GC-MS TOF analysis sheet

DATE:
COMMENTS:
Lab D-277

ATD SLOT POSITION	ANALYSIS NUMBER	ANALYSIS DESIG. DATE	TYPE	#	EXT.	TUBE DESIG. #	DETAILS OF ANALYSIS
TUBE # 1	RUN # 1		HB	01	.d		Helium blank - empty glass tube
TUBE # 2	RUN # 2			02	.d		
TUBE # 3	RUN # 3			03	.d		
TUBE # 4	RUN # 4			04	.d		
TUBE # 5	RUN # 5			05	.d		
TUBE # 6	RUN # 6			06	.d		
TUBE # 7	RUN # 7			07	.d		
TUBE # 8	RUN # 8			08	.d		
TUBE # 9	RUN # 9			09	.d		
TUBE # 10	RUN # 10			10	.d		
TUBE # 11	RUN # 11			11	.d		
TUBE # 12	RUN # 12			12	.d		
TUBE # 13	RUN # 13			13	.d		
TUBE # 14	RUN # 14			14	.d		
TUBE # 15	RUN # 15			15	.d		
TUBE # 16	RUN # 16			16	.d		
TUBE # 17	RUN # 17			17	.d		
TUBE # 18	RUN # 18			18	.d		
TUBE # 19	RUN # 19			19	.d		
TUBE # 20	RUN # 20			20	.d		
TUBE # 21	RUN # 21			21	.d		
TUBE # 22	RUN # 22			22	.d		
TUBE # 23	RUN # 23			23	.d		
TUBE # 24	RUN # 24			24	.d		
TUBE # 25	RUN # 25			25	.d		
TUBE # 26	RUN # 26			26	.d		
TUBE # 27	RUN # 27			27	.d		
TUBE # 28	RUN # 28			28	.d		
TUBE # 29	RUN # 29			29	.d		
TUBE # 30	RUN # 30			30	.d		
TUBE # 31	RUN # 31			31	.d		
TUBE # 32	RUN # 32			32	.d		

SEM Analysis of Tire Crumb Particles for Sizing and Metals (D-EMMD-ECB-001-SOP-01)

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: SEM Analysis of Tire Crumb Particles for Sizing and Metals

Number: D-EMMD-ECB-001-SOP-01	Effective Date:

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: ECB-001.01

SOP Steward

Name: Ed Heithmar

Signature/Date:

Approval

Name: Tammy Jones-Lepp

Title: ECB Chief

Signature/Date:

Concurrence*

Name: Margie Vazquez

Title: EMMD QAM

Signature/Date:

*Optional Field

Revision History

Revision No.	Name	Date of Revision	Description of Change(s)

STANDARD OPERATING PROCEDURE FOR SEM ANALYSIS OF TIRE CRUMB PARTICLES FOR SIZING AND METALS – September 27, 2016

TABLE OF CONTENTS

1.0 SCOPE AND APPLICABILITY

The purpose of this procedure is to establish a method of SEM analysis that is as uniform as possible from sample to sample for the 2016 TCRS. The goal of analysis is to determine particle size range and overall size distribution of the particles. Also, to perform qualitative elemental composition determination of individual particles of interest.

This SOP was written by Emily Siska, student services contractor for RD/NERL/EMMD/ECB under the guidance of Ed Heithmar.

2.0 SUMMARY OF METHOD

Samples will be imaged with a scanning electron microscope (SEM) to determine the particle size and size distribution. Spectra will be obtained from an energy dispersive system (EDS) for qualitative metals analysis. Each sample will be prepared by placing a carbon adhesive tab on a metal stub and firmly placing the stub in the sample. All loose particles will be removed by blowing compressed air tangential to the sample. More information can be found in **Section 8: Specimen Preparation Guidance** in the user manual. At least five (5) separate positions on an SEM sample holder should be analyzed. Different detectors can be used to highlight different features of the sample. One could get a better idea of whether the metal particles are embedded into the rubber or free floating. More information on picking a detector and expected resolution can be found in **Section 2: Resolution** and **Section 6: Detectors** in the Carl Zeiss Sigma field emission SEM user manual. EDS may be taken point wise on particles suspected to be metals. If EDS is performed, a spectrum and a corresponding image should be included in the report. Principles behind SEM analysis and basic SEM operation can be found in the user manual.

3.0 DEFINITIONS/ACRONYMS

1. SEM: Scanning Electron Microscope
2. EDS: Energy Dispersive System
3. TCRS: Tire Crumb Research Study
4. QAPP: Quality Assurance Project Plan

4.0 HEALTH AND SAFETY WARNINGS

No hazardous chemicals are used in this procedure. However, it is necessary to wear safety googles while in the lab preparing samples to avoid eye contact. Samples should be prepared in the clean hood to avoid inhalation and sample contamination. Always follow all safety procedures mentioned during laboratory safety training. Special precautions should be taken while operating the SEM to avoid user injury and/or instrument damage as outlined in **Section 3: Safety Precautions (Warnings and Cautions)** and **Section 6: Do's and Don'ts** of the user manual.

5.0 CAUTIONS AND INTERFERENCES

To avoid cross contamination, each sample should be prepared separately and only one sample should occupy a sample tab. The sample(s) should be kept away from solvents to prevent sample alteration.

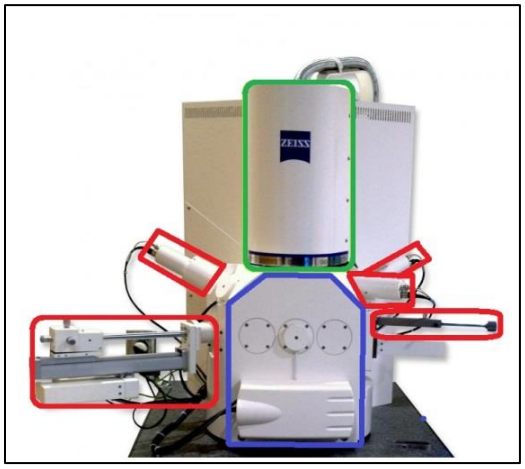
6.0 PERSONNEL QUALIFICATIONS AND RESPONSIBILITIES

To become a qualified user, you must perform 10 hours of imaging/data collection under the supervision of a qualified user. This training is to be documented using the SEM training sheet (Appendix A of this SOP) and kept in the user's training file.

Any person working in the lab must complete laboratory safety training.

7.0 EQUIPMENT AND SUPPLIES

Instrumentation:
The SEM (Carl Zeiss Sigma field emission SEM) is comprised of a source and optics (green), sample chamber (blue), and detectors (red).



It also has two monitors and two desktop computers, one set for the SEM software and the other for the x-ray detection software. Attached to the computer is a stage control for the SEM and a keyboard with various SEM controls.

Other attached equipment includes: Nitrogen gas (for venting the system and using variable pressure mode), compressed air, roughing pump, and chiller.

Consumables/Equipment:

1. Carbon planchet (e.g., Image Tab, Ted Pella, Inc.) for sample mounting (comes in various sizes and degrees of mirror finish
2. Aluminum sample mount (holds planchet and mounts on SEM sample stage)

8.0 REAGENTS AND STANDARDS

No reagents are used in this procedure

9.0 PROCEDURES

9.1 *Sample Collection, Handling, Preservation, and Preparation*

Sample collection is outside the scope of this SOP and is discussed in the project QAPP. Samples are stored at room temperature. There is no known maximum holding time for SEM imaging or EDS analysis of tire crumbs. Samples will generally be analyzed within six months of sampling.

Prior to SEM analysis, samples will have been size-fractionated by sieving. The fine particles (less than about 0.125-mm diameter) have the greatest surface area-to-volume ratio. Also, SEM is most appropriate for this size range. Therefore, SEM analysis will be performed on particles less than 0.125-mm diameter.

The fine particles will be placed on a carbon planchet on an aluminum sample mount in a manner that results in a roughly uniform single layer of particles. Either a 12.5-mm or a 25-mm planchet will be used, depending on the size of the fraction smaller than 0.125 mm.

9.2 *Instrument Operation*

9.2.1 Instrument Start Up

Open the Smart SEM software and login. To change the sample, make sure the nitrogen is on, select “Vent” under the vacuum tab, and remove the sample stage from the chamber. Mount the sample by loosening the hex screw for the mounting hole on the stage, inserting the stem of the aluminum sample mount, and retightening the hex screw. After the stage is replaced in the chamber, close the chamber and select “Pump”.

Check the system vacuum level at the bottom right displayed below. In 5-10 minutes, the green check mark indicates the chamber is at vacuum. If you wish to use variable pressure mode from high vacuum mode select the vacuum tab under SEM control. Then enter desired pressure (green), then select “Go To VP” (blue), and wait for the chamber pressure (red) to reach desired setting.

Next, set the “EHT Target” and make sure the voltage is appropriate for the sample (1.00 to 25.00kV). Then select “Beam State= EHT On.” Select the appropriate detector under the “Detectors” tab. The following detectors can be used for high vacuum mode: SE2, InLens, and NTS BSD. For variable pressure mode use either the VPSE G3 or NTS BSD. Next, move the stage to the appropriate height (usually 10-16mm) based on the detector that is being used. This can be done manually using the joystick or from the “Stage” tab.

9.2.2 Imaging

These samples will be prone to charging and it is suggested that the instrument be in variable pressure mode. Also, topology is important to sample analysis and therefore the back-scatter detector (BSD) should be used for imaging. To start, focus the image at the starting height. Then press the reduced button (above the magnification control), and increase the magnification to desired level and refocus. Once this is done, while still in reduced mode, turn on the wobble (directly below aperture controls). This alternates the magnification between the current and lower level. The image be will moving (left to right, up to down, or some combination in-between). Use the aperture controls to reduce this movement as much as possible, ideally stopping it completely. If done correctly the image should be coming in and out of focus with no movement.

Next, adjust the stigmation which can be done in or out of wobble mode. To reduce the stigmation in wobble mode, adjust the controls until the image does not appear to be “stretching.” Next, turn the wobble off and refocus the image. To correct stigmation outside of wobble mode, focus on an edge or spherical object. Adjust stigmation controls until the edge appears to be sharp or the sphere is completely round, then refocus. Depending on the sample being imaged you may have to repeat the aperture alignment and stigmation as you increase magnification, change the detector, change the acceleration voltage, or change the aperture size.

A common technique for imaging is to focus at a higher magnification then the one the image is being taken at. For example, if a particle is being imaged at 500X, it should be focused at a ~1000x or greater then imaged back at 500X. It is also common practice to focus and adjust all the parameters mentioned above in a separate area then the one that is being imaged. This prevents distortion that can be caused by burning or charging.

Higher quality images are obtained by adjusting and reducing the scan speed then increasing the noise reduction average N. However, a single image should not have a cycle time longer than ~1.5 min due to the quality increase being unnoticeable.

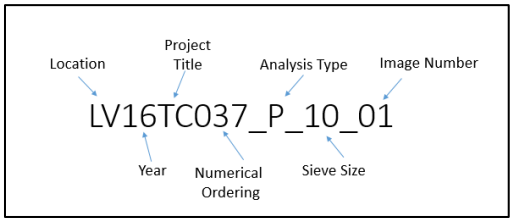
While the image is being collected, press the "Freeze" button (orange) which will stop the scan after the image is completed. To save the image, right click and save the file as a .tiff under the appropriate directory.

9.2.3 Shut Down

To shut down the SEM set the "Beam State = Off" and return the stage to its starting height (0 to 0.5mm). If in variable pressure mode, return the system to high vacuum mode (always leave the system in high vacuum mode when not in use). If the NTS BSD detector was used, return it to its retracted position. As courtesy to the next user, set the "EHT Target" to <5.00. To log off software, simply hit the "X" button in the upper right-hand corner.

10.0 DATA AND RECORDS MANAGEMENT

Samples file names will be created by using the sample name followed by consecutive image numbers.



The SmartSEM user interface is able to capture and store images for user analysis. The Quantax Esprit EDS software creates a report including instrument parameters and spectra. Images are stored on the instrument indefinitely on the C drive in the "Tire Crumb" folder and backed up on an external hard drive at the end of every week. No physical copies of images are created or stored. Documentation of the preparation and imaging of all samples will be written in a single laboratory notebook titled "Tire Crumb: SEM." This notebook is specific to the project and instrument; it will be written in by all researchers involved in SEM characterization.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

A high-resolution Au test specimen (Ted Pella) standard can be used to assess SEM imaging performance. A Ted Pella measuring chip is available to calibrate the SEM's scale bar/measuring tool. The SEM has an annual preventative maintenance contract to help keep the instrument in good working order. If there is reason to think the EDS is not working, copper tape can be used for trouble shooting purposes to determine if the EDX is working properly. If the signature lines do not match up exactly, a service engineer must be called to repair the instrument. There is nothing in house that can be done to fix the EDS if it is not showing the proper signal.

12.0 REFERENCES

- Zeiss EVO Series Operator User Guide
- Preventive Maintenance Checklist, SIGMA

Appendix A: SEM Training Sheet

SEM TRAINING CHECKLIST

Before Using the SEM_____

Sample Prep

- Tools
- Where to prepare sample

Checking the Instrument

- Nitrogen
- Chiller water
- Vacuum

Using the Log Book

- Different types of log books
- What to fill out where and how often

Loading the Sample

- Sample stages
- Tools

Using the SEM_____

Initial Start-Up

- Gun status
- Vacuum
- EHT
- Setting up your home screen
- Keyboard and joystick controls

Detectors

- Picking a detector
- Detector drop-down list
- Detector panel

Getting an Image

- Focus
- Wobble
- Apertures
- Stretching vs. image sharpness and what to use when
- Magnification
- Working distance
- Brightness and contrast
- Taking a picture

EDX

- Using EDX software
- Voltage increase
- Stage position

After Using the SEM

Shut Down

- What stays on?
- What gets turned off?

What to Do with the Sample

- When leaving it in the instrument
- When taking it out of the instrument

Date	Trainers	Hours

Trainer and Trainee Name/Signatures

Trainee

Trainer 1

Trainer 2

Trainer 3

Sieving Procedure for Tire Crumb Rubber Samples (D-EMMD-ECB-002-SOP-03) – October 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Sieving Procedure for Tire Crumb Rubber Samples

Number: D-EMMD-ECB-002-SOP-03	Effective Date: 10/01/16

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Steven Gardner

Signature:

Approval

Name: Tammy Jones-Lepp

Title: Branch Chief

Signature:

Concurrence*

Name: Margie Vazquez

Title: EMMD QA Mgr.

Signature:

*Optional Field

Revision History

Revision No.	Name	Date of Revision	Description of Change(s)
1	Steven Gardner	8/30/16	Changed weighing procedure to foil pans.
2	Steven Gardner	12/12/2016	Updated to include drying, use of coarse (#4) sieve pan. Old sieve pictures with 5 pans kept.

STANDARD OPERATION PROCEDURE FOR SIEVING PROCEDURE FOR TIRE CRUMB RUBBER SAMPLES – December 27, 2016

TABLE OF CONTENTS

1.0 SCOPE AND APPLICABILITY

This procedure was developed for the 2016 Tire Crumb Research Study (TCRS). The purpose of the procedure is to allow for a rough characterization of particle size for tire crumb samples and to collect fines for further analysis. This procedure may be suitable for use on other types of solid matrices.

2.0 SUMMARY OF METHOD

Samples are shaken through a number of stacked sieves. The fraction left in each sieve, along with the fines that filter through the entire stack, is weighed and reported.

3.0 DEFINITIONS AND ACRONYMS

- Fines: particles $\leq$ 150 µm in size
- TCRS: Tire Crumb Research Study

4.0 HEALTH AND SAFETY WARNINGS

Basic good laboratory hygiene and use of personal protective equipment is sufficient for most TCRS samples. Any samples that contain volatile compounds should be handled under a lab fume hood.

All lab staff are expected to have completed basic laboratory safety training prior to handling samples.

5.0 CAUTIONS AND INTERFERENCES

Not applicable.

6.0 PERSONNEL QUALIFICATIONS AND RESPONSIBILITIES

No specialized training is needed except for the lab safety training mentioned in Section 4.

7.0 EQUIPMENT AND SUPPLIES

See the Procedures section (Section 11) for pictures of the equipment listed below.

- A set of standard testing sieves in sizes in the following table:

US Sieve Size	Opening (mm)
No. 4	4.75
No. 10	2.00
No. 18	1.00

No. 60	0.250
No. 120	0.125
No. 230	0.063

- Lid and bottom pan for sieves.
- Lab balances, accuracy to 0.1 gm and to 0.1 mg
- Ziploc food storage bags used to store and weigh sample fractions
- Foil weighing pans
- Basic lab spatulas, funnels, brushes, and other implements for sample fraction transfer to pans and bags

8.0 REAGENTS AND STANDARDS

Not applicable.

9.0 SAMPLE COLLECTION AND SHIPMENT

Sample collection is outside the scope of this SOP.

10.0 SAMPLE HANDLING AND PRESERVATION

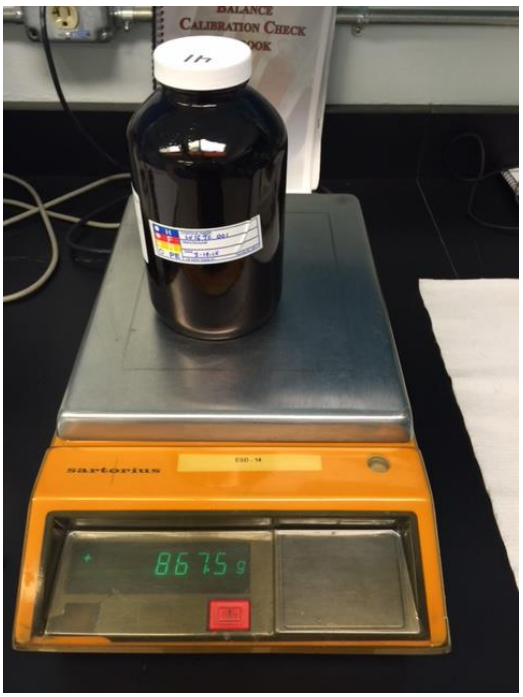
No special sample handling or storage is needed for TCRS samples. Sample storage and handling for samples that can degrade are outside the scope of this SOP.

11.0 PROCEDURES

11.1 Samples that are visibly wet are dried in a fume hood by placing in aluminum foil lined pans or trays for 24 hours before processing.

11.2 Build sieve stack with the following US standard sieve sizes: lid, 4, 10, 18, 60, 120, 230, then pan.

11.3 Weigh bulk sample, record. If creating a composite sample from one field, weigh each individual sample from the field and record.



11.4 Pour sample into top of sieve stack.

11.5 Shake the sieve stack for 15 minutes on the vibratory shaker to distribute the particles. Set the intensity of the shaker to level 5.

11.6 Open each sieve in sequence and determine the appropriate size foil pan and

plastic bag for the sample volume. Mark the bag with the sample number and the sieve size.

11.7 Tare the pan on the scale using the appropriate scale. For samples below 100 g, weigh on the 0.1 mg accuracy scale. Carefully transfer the material into the foil pan.

11.8 Weigh and record in notebook.

11.9 Transfer material from foil pan into the corresponding bag

11.10 *Calculations*

11.10.1 If analyzing a composite sample, calculate total sample weight (Step 11.3) by adding together individual location weights. This equals pre-sieved total sample weight.
11.10.2 Calculate total recovered weight by adding the weight of each size fraction.
11.10.3 Calculate % total recovery by taking the total recovered weight (Step 11.10.2)/total sample weight (Step 11.10.1) and multiply by 100. This is simply a check to see how much sample was recovered after sieving.
11.10.4 Calculate the % retained on each sieve by taking the weight for each fraction (Step 11.8), and dividing it by the total recovered weight (Step 11.10.2), then multiplying by 100.

12.0 DATA AND RECORDS MANAGEMENT

Results are recorded in the analyst's lab notebook and archived according to EPA records schedules as specified in project QA project plans.

13.0 QUALITY ASSURANCE/QUALITY CONTROL

Field duplicates may be done. Quality control will be specified in quality assurance project plans. Calculations will be checked, with at least 10% of calculations verified by a second analyst. This will be noted in the log book with the analyst's initials and date.

14.0 REFERENCES

Not applicable.

Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion (D-EMMD-ECB-003-SOP-01) – November 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion

Number: SOP # D-EMMD-ECB-003-SOP-01	Effective Date: 11/01/16

SOP was developed: ☐ In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Georges-Marie Momplaisir

Signature: Date:

Approval

Name: Tammy Jones-Lepp

Title: EDB Chief

Signature: Date:

Concurrence*

Name: Margie Vazquez

Title: EMMD QA manager

Signature: Date:

STANDARD OPERATING PROCEDURE FOR TOTAL NITRIC ACID EXTRACTABLE METALS FROM SOLID SAMPLES BY MICROWAVE DIGESTION

Prepared by and for:

Environmental Chemistry Branch USEPA/ORD/NERL-EMMD
944 East Harmon Avenue
Las Vegas, Nevada 89119

TABLE OF CONTENTS

1.0 DEFINITIONS AND ACRONYMS

ECB	Environmental Chemistry Branch
EMMD	Environmental Measurement and Monitoring Division
ICPMS	Inductively Coupled Plasma Mass Spectrometry
DI	Deionized
RO	Reverse osmosis
HNO_3	Nitric acid
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
SHEMP	Safety and Health Environmental Management Program
SOP	Standard Operating Procedure
MARS	Microwave Accelerated Reaction System
PPE	Personal Protective Equipment
QA	Quality assurance
QC	Quality control

2.0 DISCLAIMER

This standard operating procedure has been prepared for use by the Environmental Measurement and Monitoring Division (EMMD) of the U.S. Environmental Protection Agency (EPA) and may not be specifically applicable to the activities of other organizations. It is a modified version of ECB-012 SOP [Ref. 11.1]. It uses the multi-element nitric acid/hydrochloric acid-leach microwave-assisted digestion procedure described in EPA Method 3051a [Ref. 11.2] which renders a sample suitable for ICP-MS analysis. This procedure is limited to use by, or under direction of, chemists and technicians who have demonstrated proficiency with the procedure. **THIS IS NOT AN OFFICIAL EPA APPROVED SOP**. This document has not been through the Agency's peer review process or EMMD clearance process.

3.0 PURPOSE (SCOPE AND APPLICATION)

This document describes a procedure for the preparation of solid matrices such as tire crumb rubber, soils, sediments, or sludge and can be adapted for biological matrices and wipes, for analysis by inductively coupled plasma mass spectrometry (ICP-MS). It uses a mixture of nitric acid and hydrochloric acid to improve the extractability of metal analytes.

4.0 PROCEDURE SUMMARY

Organic material in the sample matrix is destroyed and metals that are extractable with a mixture of nitric and hydrochloric acid are solubilized by microwave digestion in a sealed, pressurized Teflon vessel. The sample is first allowed to pre-digest at room temperature, and then subjected to a microwave heating program that increased the temperature of the mixture slowly to 200°C and kept it at this temperature for another 30 minutes. The MARS-5 microwave unit (CEM Corporation, Matthews, NC) used is fitted with a fiber-

optic temperature sensor to monitor the temperature of the reference vessel. The instrument has the ability to regulate the temperature of the sample by adjusting the amount of applied power.

After cooling, the samples are diluted with deionized water and transferred to an acid cleaned polyethylene or Teflon container that can be centrifuged if needed to separate solid particles.

5.0 REAGENTS AND CHEMICALS

5.1 Deionized (DI) water: in-house 18.2 MΩ DI water.
5.2 Concentrated Nitric Acid Optima: high-purity, concentrated HNO_3 (65%-70% Fisher Scientific, 1-liter bottle, catalog #A467-2).
5.3 Concentrated Hydrochloric Acid Optima: high-purity, concentrated HCl (Fisher Scientific, 500 mL bottle, A466-500) use if needed only.
5.4 Hydrogen Peroxide Optima: 30% H_2O_2 Trace-pure (Fisher Scientific, 500 mL bottle, P170).
5.5 Matrix Spike Standard: Multi-Element Custom Standard of 48 elements Ag, Al, As, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Gd, Ge, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pd, Pt, Rb, Rh, S, Sb, Se, Si, Sm, Sn, Sr, Tb, Ti, Th, Tl, U, V, W, Y, and Zn in 15% HCL and 5% HNO_3, (SCP Science, catalogue # AQ0-008-122).
5.6 Concentrated HNO_3: Trace-metal clean concentrated HNO_3 (65%, Fisher Scientific, catalog #A509212) for washing labware.
5.7 10% Nitric Acid: Add 100 mL of concentrated nitric acid to 500 mL deionized water and dilute to 1 L.

6.0 EQUIPMENT/APPARATUS

6.1 CEM Corporation Microwave Accelerated Reaction System, Model MARS-5 which includes a microwave power system with selectable output of 0 – 1600 watts, a fluoropolymer-coated microwave cavity and rotating turntable. The instrument is fitted with a pressure sensor ESP-1500 Plus and fiber-optic temperature sensor model RTP-300 Plus (temperature range 40° to 250°C) that can be used to monitor the XP-1500 Plus vessels. Although both sensors are factory calibrated, it is strongly encouraged to check the instrument temperature reading against an external thermometer. A step-by-step procedure for calibrating the temperature probe of the MARS-5 instrument is reported in Appendix C. Please also refer to the MARS-5 Operation Manual [Ref 11.3] for more information on routine maintenance and cleaning.
6.2 MARS Digestion Vessels: CEM XP-1500 plus Control Vessel with a TFM liner
6.3 Centrifuge: Model IEC Centra MP4R International Equipment Company
6.4 Water Purification System: Water Pro Plus LABCONCO
6.5 Assorted micropipettes and appropriate tips Rainin E4^{TM} XLS, Mettler Toledo
6.6 0.45 µm PTFE filter membranes
6.7 Analytical balance: Mettler Toledo XP504, 4-decimal analytical balance, 520-gram maximum capacity, and minimum sensitivity of 0.1 mg
6.8 Calibrated Class 1 NIST Certified Reference Weights

6.9 Sample bottles and tubes of varying sizes

7.0 INTERFERENCES

A clean laboratory environment and trace-clean reagents are required to conduct trace inorganic analysis. Even when all of these are met, non-clean laboratory practices can lead to introduction of contaminants into a sample matrix and/or extract that could interfere with instrumental analysis. Attention to details and experience with clean lab practice procedures are necessary. The analyst should demonstrate and implement clean laboratory practices. QC measures are put in place to identify and reduce laboratory contaminants. During microwave digestion, decomposition of organic rich materials may create high vessel pressure. This may cause venting of the microwave vessel and result in loss of analytes and sample, which must be avoided. Digestion of such samples should be initially subjected to a reduced initial mass. Gradual and incremental increase of sample size is necessary when the digestion characteristics of a certain matrix are unknown. The concentration of reagents however should remain the same.

8.0 HEALTH & SAFETY PRECAUTIONS

8.1 Hydrochloric acid is a strong mineral acid that is highly corrosive. Nitric acid is a strong oxidizing agent and a strong acid. Hydrogen peroxide is also a very strong oxidizer. All these chemicals can cause skin irritation and burns, respiratory irritation, damage to eyes and organs if not handled properly.

8.2 The analyst should review the Safety Data Sheets (SDS) for each chemical in this procedure so that safe working procedure must be achieved. SDS are located in a properly labeled 3-ring binder in CHL-43 Laboratory and are also available on-line.

8.3 All of the hazardous chemicals used in this procedure should be handled only while using proper personal protective equipment (PPE) such as: gloves, lab coats, safety glasses, and appropriate close-toed shoes. Contact lenses may not be worn while working in the laboratory. Fume hoods must be utilized whenever possible to avoid potential exposure. Perform dilutions by adding acid to water.

8.4 The analyst should be familiar with the location and proper use of the fume hoods, eye washes, safety showers, and fire extinguishers.

8.5 Waste disposal should follow the recommended EMMD procedures for waste disposal whenever applicable. Contact the onsite EMMD's SHEMP Manager when the container is full for disposal.

8.6 Rapid and/or explosive generation of gases can occur during the digestion of samples with a high organic content such as oils, tissues, and rubber-based materials. The analyst should follow the advice given in paragraph 2 of Section 7.0.

8.7 When working with samples of unknown composition, always perform a pre-digestion step in an unsealed, open vessel, allowing a minimum of 15 minutes time for reaction of volatile or easily oxidized compounds to subside before sealing the vessel and microwave heating.

8.8 Never heat liquids in a sealed vessel that is not equipped with a pressure relief device.

8.9 Microwave digestion vessels can be highly pressurized and should be handled with care. To minimize internal pressure, the microwave digestion vessels should be allowed to cool to ambient temperature before opening. In addition, the vessels should be vented in a fume hood to release excess fumes.

8.10 Fumes from the microwave unit should be exhausted to a hood.

8.11 Organic solvents should not be subjected to microwave radiation as they may react explosively. Organic solvent such as ethanol – when mixed with concentrated acids like nitric acid – can react violently, even explosively, and this without applied heat.

8.12 All unused acids should be properly disposed in the acid waste collection container.

9.0 MICROWAVE EXTRACTION PROTOCOL

All digestion and volumetric vessels must be acid washed and rinsed with deionized water before use. Refer to Appendix C for the step-by-step procedures for cleaning vessels.

9.1 *Microwave Extraction of Tire Crumb Rubber Samples*

Use the sets of 12 XP-1500 Plus microwave vessels which also include a control vessel. The liner has a capacity of 100 mL and is made of Teflon® TFM, an advanced composite Teflon. These vessels are adequate to handle the high temperature required to digest the rubber material and can withstand a maximum pressure of 800 psi.

Bulk tire crumb rubber samples will be used without sieving or size reduction, to minimize contamination.

9.1.1 Place the microwave vessel and cap on a tared balance and record the weight in the digestion notebook. Mix the sample thoroughly. Remove the cap and transfer 0.25 g (within 0.02 g) of sample to the tared vessel. The sample must be placed in the bottom of the liner. The side walls of the liner must be free of sample deposits.

9.1.2 Determine the weight of concentrated nitric acid and hydrochloric acid that will make a slurry containing 9 mL HNO_3 and 3 mL HCl, taking their specific gravity into consideration (Section 11, no. 11.1). According to the manufacturer SDS, the Optima nitric acid has a specific gravity of 1.40 and the Optima HCl 1.18.

9.1.3 Tare the vessel, add concentrated nitric acid to the vessel and record the weight of the acid. Zero the vessel one more time, add the hydrochloric acid and record the weight. Make sure that the acid mixture covers the sample. Place a new rupture membrane in the vessel cap and seal firmly by hand. Weigh the vessel and record the weight before digestion.

9.1.4 Repeat the above procedure for a batch of up to 24 samples. Place a duplicate of the sample most likely to be more reactive in the MARS-5 control vessel. Insert the temperature probe in the control vessel sapphire well and secure the pressure line to the cap. The control sample is not normally used for data since it may become contaminated from the pressure monitoring line.

9.1.5 **MARS-5 System** – The microwave unit can only process a set of 12

samples in XP-1500 vessels at a time. Place the turntable into the microwave cavity. Place each vessel into the turntable. Connect pressure and temperature lines to their microwave ports.

9.1.6 Use program ECBTRC-1 for 1 – 4 vessels, ECBTRC-2 for 4 – 8 vessels, and ECBTRC-3 for 9 – 12 vessels. These programs are described in Appendix A. Record the temperature after ramp up and before the end of the digestion cycle.
NOTE: Due to the variable nature of the samples, some may be particularly reactive and require a gentle digestion to volatize potentially explosive compounds. For example, samples with larger and more irregular particles may be targeted for this extra step. Any change in the conditions used for digestion will be noted in the lab notebook used to document sample preparation.

9.1.7 After digestion is complete, allow the vessels to cool until the temperature drops to less than 30°C. There is no need to open the microwave door to help cool the vessels. Once the method run is complete, the MARS-5 system will go through an automatic cooling cycle.

9.1.8 Vent the vessels in a fume hood and weigh to verify that no significant amount of solution was lost.

9.1.9 Place the microwave vessels in the fume hood. Add 250 µL of hydrogen peroxide in each vessel and allow enough time for the hydrogen peroxide to oxidize remaining organic material. The extract should be quite clear at the end of the process.

9.1.10 Transfer the solution quantitatively to a 60 mL LDPE polyethylene sample bottle and bring to a final weight of 50 g of solution using deionized water. Allow solids to settle and if needed, centrifuge the sample. This sample will be further diluted and analyzed by ICPMS. In addition, a filtration step can be included if judged necessary, using a 0.45µm membrane filter.

9.1.11 Use the in-house developed Excel program to do all calculations and print digestion log to be placed in the digestion log binder or paste in the project laboratory book. The Excel program will compute the Calculated Weight before Digestion, Percent Difference, Recovery Percent, and Dilution Factor.

10.0 QUALITY ASSURANCE AND QUALITY CONTROL

10.1 **Blank:** A preparation blank will accompany each digestion batch of 24 or fewer samples. The mixture of acid reagents (Section 9.1.1) will be used. The blank is used to track potential contamination during sample preparation and extraction. The blank is treated as a regular sample. If a sample is filtered before analysis, then a blank will also need to be filtered to assess if the filter is contributing any contamination.

10.2 **Laboratory Control Sample (LCS):** Another reagent blank will be prepared and to this, will be added 250 µl of the custom spike standard solution (Section 5.5) before digestion. The LCS results will be used to determine if the laboratory can

perform the analysis in a clean matrix.

10.3 **Laboratory Duplicate (Dup):** One tire crumb sample will be digested in duplicate with each batch of 24 or fewer samples to check the precision of the digestion method.

10.4 **Matrix spike (MS):** With each digestion batch of 24 or fewer samples, an additional subsample taken from a randomly selected tire crumb container will be prepared. This will be spiked with 250 µl of the standard mixture of analytes and digested. The MS is used to document the effect of the sample matrix on analyte recovery.

10.5 **Standard reference material:** A standard reference sample (if available for the sample matrix type to be analyzed) should be included with each batch of samples processed. As of now there is no standard reference material available for tire crumb rubber.

10.6 **Digestion Percent Difference:** The percent difference between the weight after digestion and the calculated weight before digestion should not exceed 10%. If the percent difference does exceed 10%, investigate the reason, correct, and re-digest the sample.

10.7 **Pressure and Temperature Monitoring for the MARS-5 system:** Examine the pressure and temperature monitoring graph. If the pressure or temperature deviates from the set point by 15% or more after ramp up and before the end of the digestion, investigate the problem, and consult the group leader to determine if re-digestion is necessary. Determine if the sample in the monitoring vessel was the cause and if not, the microwave may need recalibration (refer to Appendix B and C).

11.0 CALCULATIONS

11.1 **Acid Weight** = desired volume x density
Optima Nitric acid weight = 9 mL x 1.40 g/mL = 12.60 g
Optima Hydrochloric weight = 3 mL x 1.18 g/mL = 3.54 g

11.2 **Calculated Weight before Digestion:** The Excel program will add the vessel weight, the sample weight, and the acid weight to give the expected weight before digestion in grams.

11.3 **Percent Difference:** The Excel program will divide the weight after digestion by the calculated weight before digestion and multiply by 100 to give a percent difference.

11.4 **Dilution Factor:** The Excel program will add the sample weight and the acid weight adjusted for specific gravity, and divides the result by the sample weight to obtain the dilution factor used in data calculations.

12.0 MISCELLANEOUS NOTES

12.1 **MARS-5 System:** Monitor the pressure and temperature during the digestion process. If the expected temperature or pressure is not maintained during the digestion, investigate the cause, and consult the group leader to determine if re-digestion is necessary.

12.2 Follow instructions in Appendix B and the MARS-5 instruction manual if

recalibration of the pressure sensor or temperature sensor is required. The pressure sensor calibration constant is 4315-4316-0621-0623-6637-2241.

12.3 The digestion data will be stored on the individual analyst computer and on a designated EPA shared drive. Additionally, hard copies of the digestion data will be placed in the Tire Crumb digestion binder located in CHL-43.

13.0 REFERENCES

13.1 USEPA SW-846 Method 3015A. *Microwave-assisted acid digestion of sediments, sludges, soils, and oils.*

13.2 SOP-ECB-012.0 *Total nitric acid extractable metals from aqueous samples by microwave digestion*, 2012.

13.3 Mars-5 Users Guide, CEM Corporation.

Appendix A: Microwave Extraction Programs

Two microwave-accelerated reaction systems (MARS-5) are located in CHL-25 laboratory. The digestion programs use a ramp-to-temperature approach and are available on both systems. Depending on the number of vessels used, the analyst should choose the appropriate ECBTRC-method.

1. **Program Name: ECBTRC-1**
   Max Power = 400W
   % Power = 100%
   Ramp = 20 minutes
   Pressure = 0 psi
   Temperature = 200^{o}C
   Hold Time = 30 minutes
   Number of Vessels = 1 to 4

2. **Program Name: ECBTRC-2**
   Max Power = 800W
   % Power = 100%
   Ramp = 20 minutes
   Pressure = 0 psi
   Temperature = 200^{o}C
   Hold Time = 30 minutes
   Number of Vessels = 5 to 8

3. **Program Name: ECBTRC-3**
   Max Power = 1600W
   % Power = 100%
   Ramp = 30 minutes
   Pressure = 0 psi
   Temperature = 200^{o}C
   Hold Time = 20 minutes
   Number of Vessels = 9 to 12

Appendix B: Quality Control Test for MARS-5 Vessels and Sensors

This quality control test should be performed only if deemed necessary.

1. Place 50 mL DI water in the control vessel (XP1500+ or HP500+), and assemble as you normally do with the temperature and pressure sensors attached.
2. Place the control vessel in the turntable and connect the sensors (no other vessels are run during this test).
3. Go into the CEM Directory and load the preprogrammed "QC ESP/EST" method.
4. Allow the method to run, observing the temperature and pressure readings. When you get to stage 5, record the temperature once the pressure has begun controlling at 200 psi. The temperature should be 200°C +/- 10°C at 200 psi. If it is not, either the temperature or pressure sensor is out of calibration.
5. Allow the vessel to cool to < 50°C. Measure the volume of water remaining in the vessel. If you put 50mL in, you should get 50mL back out. If the volume is < 50mL, then there is a leak. Note that a vessel can be leaking and still pass the QC test (200 psi = 200°C). Look for volume loss instead.
6. If the QC test fails (i.e., you do not get 200°C +/-10°C at 200 psi), then recalibrate the temperature probe and repeat the QC test.

Appendix C: Procedure for Microwave Calibration
Prepared by Jason Sylva

Pressure Calibration

1. From the home screen on the instrument, press “Setup” then follow the outlined procedure.
2. Use the “Select” button to select the option of choice. “Select Sensor” > “Pressure Sensor” >
3. “ESP-1500” > “Yes” > “Zero Sensor” then press “Back” and select “Display Calibration Constant”.
4. If the calibration constant reads: “0000-0000-0000-0000-0000-0000” refer to operation manual pages 60-66 or contact manufacturer for proper calibration constant for your model. The calibration number used for this model is: **4315-4316-0523-6637-2241**

Temperature Calibration

1. From the home screen of instrument, press “Setup” then follow the outlined procedure.
2. Use the “Select” button to select the option of choice. “Select Sensor” > “Temperature” > “RTP-300 plus” > “GF number (GF number is located on thermometer) > “Calibrate RTP-300 Plus”.
3. Place microwave thermometer into the reference top of microwave cell, then insert into a beaker of water. A second external thermometer is placed into the same bath and this is taken as the actual temperature and entered into system.
4. Refer to operation manual pages 67-68 for further details.

Appendix D: Procedure for Cleaning Vessels

This cleaning sequence has been determined to be adequate to minimize contamination in glass, polyethylene, polypropylene, or PTFE vessels.

1. Prepare cleaning solution by adding a small amount (approximately 5 mL) of concentrated Citranox (Alconox Inc.) liquid soap to a tub of reverse osmosis (RO) water (~6 L). Remove all markings and residue from vessels using a designated lab brush or methanol if necessary. Submerge vessels in soap solution and allow to soak for a few minutes.
2. Clean the vessels with a lab brush. Rinse three times with reverse-osmosis water and one last time with deionized water.
3. Place the vessels in an acid bath containing 10% (v/v) Trace-metal clean concentrated HNO3 in DI water, and let them soak overnight (and up to 48 hours).
4. Remove vessels, rinse three times with deionized water.
5. Allow vessels and lids to air dry (open end down) on a clean surface.
6. This step applied only to the XP-1500 microwave digestion vessels that already went through the above cleaning steps.
    a. Use the Optima grade acid to prepare 1 liter of 10% nitric acid.
    b. Fill the vessels approximately half way with acid (~50 mL) and secure the lids. Don't forget to place a safety membrane in the vent cap.
    c. Place the vessels in the carousel and microwave the solution using extraction program ECBCM-1, -2, or -3 (Appendix E) depending on the number of vessels.
    d. After cooling to room temperature, pour the nitric acid solution back into the Teflon cleaning solution container. Rinse vessels and caps three times with deionized water, and allow to air dry.
7. All sample bottles and microwave vessels should be capped and stored in sealable plastic bags or plastic containers to prevent contamination.
8. Label bag or container with the cleanup date and batch number.
9. When cleaning polyethylene conical tubes with external graduation, soaking in an acid bath is not recommended as the material used to graduate the tubes can be removed upon contact with acid and therefore can become a source of contamination.
    a. Remove the caps and let the tubes stand in the Styrofoam base.
    b. Pour DI water into each tube and secure the caps. Let the sealed tubes soak for a few minutes, shake well, and discard the water. Repeat this step two more times.
    c. Fill each tube with 10% nitric acid and let sit overnight or at least 12 hours at room temperature in the Styrofoam container with their lids on.
    d. The next morning, the sealed tubes are reversed to give the cap a chance to get in contact with the acidic solution. Leave the tubes in that position for at least four hours.
    e. Pour the acid content into an acid cleaning bottle.
    f. Rinse tubes and lids several times with deionized water and allowed to air dry.
    g. Cap the tubes, place them back in their Styrofoam base and keep them sealed until usage.

Appendix E: Microwave Assisted Cleanup Programs

1. **Program Name: ECBCM-1**
   Max Power = 400W
   % Power = 100%
   Ramp = 10 minutes
   Pressure = 100 psi
   Temperature = 170^{o}C
   Hold Time = 30 minutes
   Number of Vessels = 1 to 4
   Average Sample Volume = 50 mL

2. **Program Name: ECBCM-2**
   Max Power = 800W
   % Power = 100%
   Ramp = 10 minutes
   Pressure = 100 psi
   Temperature = 170^{o}C
   Hold Time = 30 minutes
   Number of Vessels = 5 to 8
   Average Sample Volume = 50 mL

3. **Program Name: ECBCM-3**
   Max Power = 1600W
   % Power = 100%
   Ramp = 10 minutes
   Pressure = 100 psi
   Temperature = 170^{o}C
   Hold Time = 30 minutes
   Number of Vessels = 9 to 12
   Average Sample Volume = 50 mL

Appendix F: Bench Sheets for Sample Processing

See the following pages for bench sheets used to help track sample processing, including reagent and standard lot numbers.

ECB Microwave Digestion Sheet

Date: _____ Sample Matrix: _____ Batch ID: _____ No. of Samples: _____

Analyst (s): _____

Reagents and Standards:	**Manufacturer:**	**Cat. No.:**	**Lot No.:**	**Exp. Date:**
Nitric Acid				
Hydrochloric				
Hydrogen Peroxide				
Deionized Water 18.2 MΩ	Water PRO PS			
Spike Standard Solution				

Microwave System: _____ **Microwave Program:** _____ **Start Time:** _____ **End Time:** _____

Table 1: Microwave Assisted Extraction of Metals in

ECB-EMMD Sample ID	Vessel No.	Vessel Wt (g)	Sample Wt (g)	Nitric Acid Wt (g)	Hydrochloric Acid Wt (g)	Wt of* spike Std. (g)	Wt Before Digestion (g)	Wt After Digestion (g)	Vol. of** H_2O_2 (uL)	Wt of Dil. Digestate (g)	Wt of Dil. Digestate sent to RTP (g)
Reference	**1**										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
	9										
	10										
	11										
	12										

▪ **dup**: duplicate sample, ▪ **spk**: spiked sample, ▪ **BLK:** procedure blank, ▪ **LCS**: laboratory control sample, ▪ **Std**: standard, ▪ **Dil**: diluted, ▪ **Wt**: weight, ▪ **H_2O_2**: Hy

▪ Weight of Nitric acid needed: _____ ▪ Weight of Hydrochloric acid needed: _____ [wt(g) = V(mL) **X** density (g/mL)]

***** Volume of spike standard solution: _____ ▪ Reference sample ID: _____ ** Hydrogen peroxide was added after digestion.

Use this digestion sheet if number of samples in a batch is ≤ 12

ECB Microwave Digestion Sheet 1 of 2

Date: _____ Sample Matrix: _____ Batch ID: _____ No. of Samples: _____ No. of Sets: 2

Analyst (s): _____

Reagents and Standards:	**Manufacturer:**	**Cat. No.:**	**Lot No.:**	**Exp. Date:**
Nitric Acid				
Hydrochloric				
Hydrogen Peroxide				
Deionized Water 18.2 MΩ	Water PRO PS			
Spike Standard Solution				

Microwave System: _____ **Microwave Program:** _____ **Start Time:** _____ **End Time:** _____

Table 1: Microwave Assisted Extraction of Metals in

ECB-EMMD Sample ID	Vessel No.	Vessel Wt (g)	Sample Wt (g)	Nitric Acid Wt (g)	Hydrochloric Acid Wt (g)	Wt of* spike Std. (g)	Wt Before Digestion (g)	Wt After Digestion (g)	Vol. of** H_2O_2 (uL)	Wt of Dil. Digestate (g)	Wt of Dil. Digestate sent to RTP (g)
Reference	**1A**										
	2A										
	3A										
	4A										
	5A										
	6A										
	7A										
	8A										
	9A										
	10A										
	11A										
	12A										

▪ **dup**: duplicate sample, ▪ **spk**: spiked sample, ▪ **BLK:** procedure blank, ▪ **LCS**: laboratory control sample, ▪ **Std**: standard, ▪ **Dil**: diluted, ▪ **Wt**: weight, ▪ **H_2O_2**: Hy

▪ Weight of Nitric acid needed: _____ ▪ Weight of Hydrochloric acid needed: _____ [wt(g) = V(mL) **X** density (g/mL)]

***** Volume of spike standard solution: _____ ▪ Reference sample ID: _____ ** Hydrogen peroxide was added after digestion.

Use digestion sheets 1 and 2 if the number of samples in a batch is > 12 and ≤

ECB Microwave Digesion Sheet 2 of 2

Date: _____ Sample Matrix: _____ Batch ID: _____ No. of Samples: _____ No. of Sets: 2

Analyst (s): _____

Reagents and Standards:	**Manufacturer:**	**Cat. No.:**	**Lot No.:**	**Exp. Date:**
Nitric Acid				
Hydrochloric				
Hydrogen Peroxide				
Deionized Water 18.2 MΩ	Water PRO PS			
Spike Standard Solution				

Microwave System: _____ **Microwave Program:** _____ **Start Time:** _____ **End Time:** _____

Table 1: Microwave Assisted Extraction of Metals in

ECB-EMMD Sample ID	Vessel No.	Vessel Wt (g)	Sample Wt (g)	Nitric Acid Wt (g)	Hydrochloric Acid Wt (g)	Wt of* spike Std. (g)	Wt Before Digestion (g)	Wt After Digestion (g)	Vol. of** H_2O_2 (uL)	Wt of Dil. Digestate (g)	Wt of Dil. Digestate sent to RTP (g)
Reference	**1B**										
	2B										
	3B										
	4B										
	5B										
	6B										
	7B										
	8B										
	9B										
	10B										
	11B										
	12B										

▪ **dup**: duplicate sample, ▪ **spk**: spiked sample, ▪ **BLK**: procedure blank, ▪ **LCS**: laboratory control sample, ▪ **Std**: standard, ▪ **Dil**: diluted, ▪ **Wt**: weight, ▪ **H_2O_2**: Hy

▪ Weight of Nitric acid needed: _____ ▪ Weight of Hydrochloric acid needed: _____ [wt(g) = V(mL) **X** density (g/mL)]

***** Volume of spike standard solution: _____ ▪ Reference sample ID: _____ ** Hydrogen peroxide was added after digestion.

Use digestion sheets 1 and 2 if the number of samples in a batch is > 12 and ≤

Innov-X XRF ANALYSIS PROCEDURES: For Tire Crumb Samples (D-EMMD-ECB-004-SOP-01) – September 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Innov-X XRF ANALYSIS PROCEDURES: For Tire Crumb Samples

Number: D-EMMD-ECB-004-SOP-01	Effective Date: 09/29/16

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: ECB-004.01

SOP Steward

Name: Steven Gardner

Signature:

Approval

Name: Tammy Jones-Lepp

Title: ECB Chief

Signature:

Concurrence*

Name: Margie Vazquez

Title: EMMD QA manager

Signature:

*Optional Field

REVISION HISTORY

Revision No.	Name	Date of Revision	Description of Change(s)

STANDARD OPERATING PROCEDURES FOR INNOV-X XRF ANALYSIS PROCEDURES FOR TIRE CRUMB SAMPLES

TABLE OF CONTENTS

1.0 SCOPE AND APPLICABILITY

The XRF technology described in this SOP is an Innov-X Field Portable XRF. It is a field screening instrument that returns values for some metals with parts per million sensitivity. This analyzer has a mode for soil media that is being adapted for use with tire crumb rubber media.

2.0 SUMMARY OF METHOD

Air dried tire crumb samples are placed into analysis cups, and analyzed for 300 seconds in standard mode for heavy metals, and 300 seconds for light element analysis. The analyzer then combines the data from the two modes and gives concentration data for a range of elements.

3.0 DEFINITIONS AND ACRONYMS

TCRS: Tire crumb research study
XRF: X-ray fluorescence

4.0 HEALTH AND SAFETY WARNINGS

This instrument emits ionizing radiation in order to do the analysis. Operators should have radiation safety training and wear a radiation detector when using the instrument. For this study, the instrument will only be used while mounted in the test stand, which automatically shuts off if the cover is opened, greatly reducing the chance of the operator getting a radiation dose.

5.0 CAUTIONS AND INTERFERENCES

The technology has known interferences. One is that high levels of lead will cause arsenic levels to be underreported, or have higher uncertainty. The instrument corrects for this internally.

The containers housing the sample can also cause a metal to be under estimated. In a previous study, it was found that the use of thick plastic bags can cause reporting of lower concentrations for copper and chromium. Because of this, it was decided in the tire crumb study to use laboratory XRF cups covered with a thin Mylar® sheet for the analysis.

6.0 PERSONNEL QUALIFICATIONS AND RESPONSIBILITIES

Personnel should be trained by an experienced operator for eight (8) hours before using the instrument by themselves.

7.0 EQUIPMENT AND SUPPLIES

- Innov-X X-Ray Fluorescence Analyzer
- XRF sample cups
- Mylar film

8.0 REAGENTS AND STANDARDS

- NIST Standards 2709, 2710a, 2711a
- SiO2 Blank

9.0 SAMPLE COLLECTION AND SHIPMENT

Sample collection and shipment is covered in another SOP.

10.0 SAMPLE HANDLING AND PRESERVATION

TCRS samples are held at room temperature in HDPE containers. No special sample storage or preservation is needed. Holding time for tire crumb samples is taken from SW-846 Chapter 3, Table 3-2 for sample analysis reported for the TCRS project.

11.0 PROCEDURES

11.1 Replace the battery daily and insert iPAQ in housing on stand.

11.2 Run calibration with manufacturer-supplied metal calibration disk.

11.3 Set XRF for 300-second runtime for both LEAP (light element) and standard analysis.

11.4 Run the SiO2 blank, and Standards 2709 (San Joquin soil, baseline metals) and 2710a (Montana soil, high metals) reference materials. For each standard and blank, enter the shot description into the analyzer.

11.5 Run unknown samples. Each size fraction will be placed in an analysis cup with a Mylar cover. One shot per sample, 10-minute runtime. For each unknown, enter the sample number into the analyzer.

11.6 Once a day, shoot one sample seven (7) times by setting the instrument on repeat. Do not move the sample during the process.

11.7 Once a day, shoot a duplicate of one of the day's samples, and shoot Standard 2711a (Montana soil, moderate metals) reference material.

11.8 At the end of the day, re-run one of the Standards 2709, 2710a, or 2711a.

11.9 Download the data file daily and store on the EPA network under O:\Public\TCRS-XRF as a .csv unaltered file. The file will be stored with a file name with the format "results-YRMODA" where YR, MO, and DA are two-digit numbers for the year, month, and date.

12.0 DATA AND RECORDS MANAGEMENT

All calculations are done within the instrument. Data and records management will be covered by the TCRS QAPP.

13.0 QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control procedures for this analysis include the analysis of blanks, NIST Standard Reference Material Soils, and the running of a duplicate and seven (7) replicates for precision daily. See the Procedure section for the sequence of these analyses.

14.0 REFERENCES

1. SW-846: *Test methods for evaluating solid waste: physical/chemical methods compendium*, https://www.epa.gov/hw-sw846

2. InnovX User Manual, Chapter 4 Operation

3. SW-846: *Test Method 6200: field portable X-Ray Fluorescence Spectrometry for the determination of elemental concentrations in soil and sediment*, https://www.epa.gov/hw-sw846/sw-846-test-method-6200-field-portable-x-ray-fluorescence-spectrometry-determination

Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples (D-EMMD-PHCB-033-SOP-01) – September 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples

Number: D-EMMD-PHCB-033-SOP-01	Effective Date: 9/12/16

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: M. Scott Clifton
Signature: Date:

Approval

Name: Myriam Medina-Vera
Title: Branch Chief, PHCB
Signature: Date:

Concurrence*

Name:
Title:
Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

STANDARD OPERATION PROCEDURES FOR THE EXTRACTION AND ANALYSIS OF SVOCs IN TIRE CRUMB RUBBER SAMPLES

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

Tire crumb rubber (TCR) is a material made from recycled tires that is used as infill for synthetic turf athletic fields. In order to investigate the potential health effects from human exposure to the tire crumb material, it is essential to be able to measure the chemicals from the rubber itself. This method will be used for extraction and analysis of semi-volatile organic compounds (SVOCs) from the TCR.

2.0 SUMMARY OF METHOD

Samples of TCR are stored in a freezer at -20ºC after receipt at the EPA lab. The samples are allowed to warm to room temperature, then the sample is homogenized inside of the collection jar by shaking in a manner to cycle the contents from the bottom to the top of the jar. Two separate 1 g aliquots will be removed from each sample, shaking between each aliquot, with each being transferred to a clean 50 mL polypropylene centrifuge tube. Internal Standard solution is added to each tube along with a ceramic homogenizer. Ten mL of 1:1 acetone:hexane is then added to each sample tube. The tubes are capped and are vortex mixed for 1 minute, allowed to sit for 2 minutes, then vortex mixed for an additional minute. The tubes are then centrifuged at 4,000 RPM for 5 minutes. The solvent is removed and is transferred to a 15 mL vial. A 1 mL aliquot of the extract is transferred to an autosampler vial for GC/MS/MS analysis. Another aliquot is transferred to a vial where it is solvent exchanged to methanol for LC/TOF analysis. Since there is no appropriate surrogate matrix for TCR, quality assurance/quality control (QA/QC) samples will consist of a duplicate preparation, a reagent spike, reagent blank, and a TCR sample prepared from a TCR designated as a reference sample.

3.0 DEFINITIONS

3.1 Tire crumb rubber samples – Samples of tire crumb collected at a processing facility or from a synthetic turf field.
3.2 Spiking solution – Solution containing stable isotope-labeled chemicals representative of target analytes. Aliquots of this solution are transferred to blank media to prepare lab spikes.
3.3 Internal standard solution (IS) – Solution containing compounds used to normalize instrument response. This solution is added to all standards, spikes, and blanks as indicated in the procedure.
3.4 Check standard – A mid-level calibration standard that is analyzed between 10 sequential sample extracts to determine the continued accuracy of the calibration curves.
3.5 Reagent blank – A matrix free extract that is prepared and analyzed to assess contamination and interferences from the materials and method.
3.6 Reagent spike – A matrix-free extract that has been fortified and been through the extraction procedure to determine losses or interferences from the method.
3.7 TCR Control – An extract prepared from a previously characterized TCR sample that is used to evaluate the performance of select chemicals that are native to that sample.

3.8 TCR reference sample – A TCR sample that will have aliquots removed and analyzed for each sample batch to determine the continuing performance of the analytical method.

4.0 HEALTH AND SAFETY WARNINGS

4.1 Follow the procedures detailed in the Health and Safety Research Protocol for your study or task.
4.2 Follow proper operating procedures for all equipment and instruments used.

5.0 MATERIALS AND EQUIPMENT

5.1 50 mL polypropylene centrifuge tubes with caps, Falcon Tubes, or equivalent (Corning part No. 352098)
5.2 Spiking and internal standard (IS) solutions, (See Tables 1 and 2).
5.3 TCR reference sample
5.4 Adjustable repeating pipette, Eppendorf Repeater, XStream, or equivalent
5.5 Ceramic homogenizers (Agilent part no. 5982-9313)
5.6 Acetone, pesticide residue grade or equivalent
5.7 Hexane, pesticide residue grade or equivalent
5.8 Methanol, HPLC grade or equivalent
5.9 Top-loader pipettes, adjustable volume
5.10 Vortex mixer (Vortex Genie II, multi-tube vortex mixer, or equivalent)
5.11 Centrifuge, capable of timed runs at 4000 RPM
5.12 15 mL amber vials w/PTFE-lined caps (Supelco part no. 27088-U or equivalent)
5.13 Autosampler vials, 2 mL, caps with PTFE-lined septa
5.14 Gas chromatograph with triple quadrupole mass spectrometer (GC/MS/MS), Agilent 7890/7010 or equivalent

6.0 INTERFERENCES

Interferences are any component that interferes with the quantitative analysis of the compounds used in this study. Interferences will be identified and evaluated as part of this study's ongoing QA/QC plan.

7.0 PERSONNEL QUALIFICATIONS

This SOP is written to be used by personnel familiar with the equipment and procedures that are used. Personnel should be adequately trained and display proficiency with those techniques prior to using this SOP for sample analysis.

8.0 METHOD CALIBRATION

The GC/MS/MS instrument should be calibrated for the analytes using the parameters listed in Tables 3 through 6 prior to sample analysis. Calibrate the GC/MS/MS system in

a range from 0.1 ng/mL to 500 ng/mL using the following calibration levels (ng/mL): 0.1, 0.5, 1.0, 2.5, 5, 10, 25, 50, 100, 250, 500. The calibration will be monitored by running a mid-level check standard between every 10 samples. A check standard must be within ±25% of its prepared concentration for the calibration to remain valid. If an analyte does not pass the ±25% criterion, but is not found in any of the relevant samples, the analysis may continue unless the response has decreased to the point of compromising the ability to detect that analyte. If a check standard fails, investigate the problem and take corrective action. Recalibrate the instrument and begin sample analysis from the point of the last good calibration check.

9.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Tire crumb rubber samples will be stored in a freezer (-20ºC) at the EPA laboratory. Samples will be allowed to warm to room temperatures prior to removing aliquots for analysis and will be returned to freezer storage immediately following aliquot removal. Sample extracts will also be stored under freezer conditions (-20ºC) in cases where analysis cannot be performed immediately. Recap and store the autosampler vials after the analysis for later use, if needed. Since PAHs are being analyzed, protect the extracts from light.

10.0 ANALYTICAL PROCEDURE

10.1. Sample Preparation, Extraction, and Processing

Remove the tire crumb rubber samples from the freezer and allow to warm to room temperature (approximately 30 minutes). Mix the tire crumb material well by shaking and rotating. For each sample, weigh three 1 g aliquots into individual 50 mL polypropylene tubes, mixing well between aliquots. Record the weights.

One sample batch will consist of triplicate aliquots from 24 tire crumb rubber samples prepared in duplicate along with the following QA/QC samples:

- 1 Reagent spike
- 1 Reagent blank
- 1 Reference TCR sample

10.1.1 Reagent Spike Preparation

1. Label a clean 50 mL polypropylene tube as the “Reagent Spike.”
2. Add a ceramic homogenizer and 10 mL of 1:1 acetone:hexane to the tube.
3. Fortify the solvent in the tube with 100 µL of spiking solution and 100 µL of internal standard solution.
4. Cap and process with TCR samples.

10.1.2 Reagent Blank Preparation

1. Label a clean 50mL polypropylene tube as the "Reagent Blank."
2. Add a ceramic homogenizer and 10 mL of 1:1 acetone:hexane to the tube.
3. Fortify the solvent in the tube with 100 µL of internal standard solution.
4. Cap and process with TCR samples.

10.1.3 TCR Reference Sample Preparation

1. Weigh a 1g aliquot of the TCR material selected as the reference sample into a clean 50 mL polypropylene tube and record the weight.
2. Prepare along with other TCR samples using the steps in 10.1.4.

10.1.4 Samples

1. Transfer a 100 µL aliquot internal standard solution to the surface of the TCR in each sample.
2. Add a ceramic homogenizer and 10 mL or 1:1 acetone:hexane.
3. Vortex for 1 minute.
4. Allow to sit for 2 minutes.
5. Vortex for an additional minute.
6. Centrifuge at 4000 RPM for 5 minutes.
7. Transfer the solvent layer to a 15 mL amber tube with PTFE-lined cap.
8. Transfer a 1 mL aliquot to an autosampler vial for GC/MS/MS analysis.
9. Transfer a second 1 mL aliquot from each sample extract to a clean autosampler vial.

10.2 Sample Analysis by GC/MS/MS

Analyze samples by GC/MS/MS using the conditions specified in Tables 3 and 4.

10.3 Sample Analysis by LC/TOF

Analyze samples by LC/TOF using the conditions specified in SOP#: D-EMMD-PHCB-034-SOP-01 (*Analytical method for non-targeted and suspect screening in environmental and biological samples using Time-of-Flight Mass Spectrometry [TOFMS]).*

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

Data will be reviewed by EMMD QA staff. The data quality objectives and review procedures from the Quality Assurance Project Plan (QAPP) for the study being conducted will dictate specific quality assurance practices. All QA practices will be consistent with the NERL Quality Management Plan.

The reagent spike, reagent blank, and reference sample will serve to measure method performance for each sample. Target recovery for the reagent spike should be ± 30% of the nominal concentration for each analyte. Any deviations will be recorded and investigated. The measured concentrations in the reference sample will be recorded and compared among batches to evaluate any potential issues related to a specific batch as well as to evaluate long-term accuracy of the method as a whole.

12.0 RECORDS

Chain of custody records will be maintained to document the removal and extraction of each TCR sample. Those records will be maintained by the study coordinator upon completion of the analysis. Records of sample preparation and analysis will be maintained in a three-ring binder which will be transferred to the study coordinator upon completion of sample analysis.

Table 1. Stock Solutions

	Catalog Number		Concentration
Spiking Solution			
PAH mix	S-70846-02[a]		10 µg/mL
Phthalate mix	S-70846-01[a]		10 µg/mL
TCR mix	S-70846-03[a]		10 µg/mL
Internal Standard Solution			
PAHs			
Chrysene D_{12}	ES-5164[b]		480 ng/mL
Phenanthrene D_{10}	ES-5164		480 ng/mL
Acenaphthene D_{10}	ES-5164		480 ng/mL
Benz[a]anthracene D_{12}	ES-5164		480 ng/mL
Naphthalene D_8	ES-5164		480 ng/mL
Perylene D_{12}	ES-5164		480 ng/mL
Fluoranthene D_{10}	ES-5164		480 ng/mL
Benzo[b]fluoranthene D_{12}	ES-5164		480 ng/mL
Benzo[a]pyrene D_{12}	ES-5164		480 ng/mL
Benzo[g,h,i]perylene D_{12}	ES-5164		480 ng/mL
Indeno[1,2,3-cd]pyrene D_{12}	ES-5164		480 ng/mL
Dibenz[a,h]anthracene D_{14}	ES-5164		480 ng/mL
Acenaphthalene D_8	ES-5164		480 ng/mL
Fluorene D_{10}	ES-5164		480 ng/mL
Pyrene D_{10}	ES-5164		480 ng/mL
Benzo[k]fluoranthene D_{12}	ES-5164		480 ng/mL
Phthalates			
Diethyl phthalate D_4	DLM-1629-1.2[b]		480 ng/mL
Di-N-hexyl phthalate 1,2 $^{13}C_2$	CLM-4669-1.2[b]		480 ng/mL

Bis(2-ethylhexyl)phthalate D_4	DLM-1368-1.2[b]		480 ng/mL
Benzyl butyl phthalate D_4	DLM-1369-1.2[b]		480 ng/mL
Other			
Dibenzothiophene D_8	DLM-2206-0.1[b]		1050 ng/mL

Suppliers:
[a] Accustandard
[b] Cambridge Isotope Labs

Table 2. GC/MS/MS Parameters

Parameter	Value
GC System	Agilent 7890 Gas chromatograph
Injector	Capillary injector in split-less mode Pulsed split-less at 25 psi for 0.5 min, then split at 50 mL/min at 1 min. Temperature: 250ºC Liner: Single gooseneck glass, deactivated Injection volume: 1 µL
Column	Agilent VF-5ms, 30 M x 0.25 mm x 0.25 µm, Column flow: 1.2 mL/min
Temperature Program	50ºC for 2 min to 325ºC at 10ºC/min, hold 5 min.
Detector	Agilent 7010 Triple Quadrupole Mode: Electron Impact (EI) operating in MRM/Scan mode Electron Multiplier Voltage by Gain Curve Transfer Line: 300º

Table 3. Compounds and Data Collection Parameters

Time Segment	Compound	Class	RT	Pre1	Prod 1	Prod1 CE	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
1	Cyclohexaneamine	TCR	4.471	69.8	43.1	15	99.8	56	10			
1	Analine	TCR	6.521	92.7	66.1	15	65	39.1	15			
1	n-Butylbenzene	TCR	7.858	90.5	65.1	20	134	91.2	25			
2	Naphthalene d8	PAH	9.956	136	108.1	10	136	84.1	30			
2	Naphthalene	PAH	10.003	127.9	102.1	20	127.9	78.1	20			
3	Benzothiazole	TCR	10.62	135	82.1	30	135	108	20			
3	Cyclohexylisothiocyanate	TCR	10.713	81.9	67	10	140.6	55.1	25			
4	Resorcinol	TCR	11.241	109.8	82.1	15	109.8	69	20			
4	2-Methylnaphthalene	PAH	11.638	142.3	141.2	20	142.3	115.1	45	114.7	89.1	20
4	1-Methylnaphthalene	PAH	11.863	142.3	141.2	20	142.3	115.1	45	114.7	89.1	20
5	Dicyclohexamine	TCR	13.229	137.5	56.1	10	137.5	83.1	15			
5	Dimethyl phthalate	Phthalate	13.584	163	77	30	163	135	15			
5	Acenaphthalene d8	PAH	13.712	159.9	158.1	20	159.9	132.1	20			
5	Acenaphthalene	PAH	13.743	151.9	126.1	30	151.9	102.1	30			
5	Phthalimide	TCR	13.889	146.8	103.1	10	146.8	76.1	35			
5	Acenaphthene d10	PAH	14.092	164.1	162.1	30	162.1	160.1	15			
5	Acenaphthene	PAH	14.169	152.1	126.1	30	152.1	102.1	30			
5	2,6-Di-tert-butyl-p-cresol	TCR	14.241	144.5	105.1	15	144.5	129.1	20			
5	N,N-Dicyclohexylmethylamine	TCR	14.33	151.5	70.1	10	151.5	55.1	25			
6	Diethyl phthalate d4	Phthalate	15.252	153	69.1	50	153	97.1	30			
6	Diethyl phthalate	Phthalate	15.278	149	65	30	149	93	20			
6	n-Hexadecane	TCR	15.322	85.1	43.1	10	98.9	57.1	10			
6	Fluorene d10	PAH	15.33	176	174.2	20	175	172.1	50			
6	Fluorene	PAH	15.401	166.1	165.1	15	165.1	164.1	15			
6	4-tert-Octylphenol	TCR	15.453	106.8	77.1	20	134.3	107.1	15			
7	2-Bromomethylnaphthalene	PAH	16.29	140.6	115.1	20	219.8	141.1	10			
7	2-Hydroxybenzothiazole	BT	16.396	150.7	96.1	25	150.7	123.1	20			
8	Dibenzothiophene d8	BT	17.34	191.5	146.1	50	191.5	160.1	30			
8	Dibenzothiophene	BT	17.385	183.4	139.1	25	183.4	152.1	50			
8	Phenanthrene d10	PAH	17.622	188.3	160.2	40	188.3	186.3	30			
8	Phenanthrene	PAH	17.678	177.9	152.1	25	176.1	150.1	25			
8	Anthracene	PAH	17.8	177.9	152.1	25	176.1	150.1	25			
9	Diisobutyl phthalate	Phthalate	18.249	149	65	25	149	93	15			
Time Segment	Compound	Class	RT	Pre1	Prod 1	Prod1 CE	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
9	3-Methylphenanthrene	PAH	18.843	192.2	191.2	20	188.7	163.1	40	192.2	165.1	45

9	2-Methylphenanthrene	PAH	18.906	188.7	163.1	40	192.2	191.2	20	192.2	165.1	45
9	1-Methylphenanthrene	PAH	19.153	188.7	163.1	40	192.2	191.2	20	192.2	165.1	45
9	Dibutyl phthalate	Phthalate	19.212	149	65	30	149	93	20			
10	2-Mercaptobenzothiazole	TCR	19.45	166.5	123	10	166.5	109	30			
11	Fluoranthene d10	PAH	20.467	211.9	210.2	20	211.9	208.1	20			
11	Fluoranthene	PAH	20.51	202.1	200.1	30	202.1	152.1	30			
11	Pyrene d10	PAH	20.994	211.9	210.2	20	211.9	208.1	20			
11	Pyrene	PAH	21.036	201.1	200.1	15	200.1	174.1	30			
12	Di-N-hexylphthalate (2)13C2	Phthalate	22.647	153	66	25	153	95.1	20			
12	Benzyl butyl phthalate d4	Phthalate	22.756	153	69.1	25	153	97.1	5			
12	Benzyl butyl phthalate	Phthalate	22.77	149	65	25	91	65	15			
13	bis(2-Ethylhexyl) adipate	Phthalate	23.017	147	55.1	25	111	55.1	15			
13	Benz(a)anthracene d12	PAH	23.858	240.2	236.2	50	118.1	116.1	15			
13	Benz(a)anthracene	PAH	23.911	228.1	226.2	30	114	101.1	10			
13	Chrysene d12	PAH	23.929	240.2	236.2	50	118.1	116.1	15			
13	Chrysene	PAH	23.989	228.1	226.2	30	114	101.1	10			
13	Bis-2-ethylhexyl phthalate d4	Phthalate	24.165	153	69.1	25	153	97.1	20			
13	Bis-2-ethylhexyl phthalate	Phthalate	24.184	149	65	30	149	93	20			
13	1-Hydroxypyrene	PAH	24.195	217.5	189.1	40	188.5	163.1	40			
14	Di-n-octyl phthalate	Phthalate	25.65	149	65	30	149	93	20			
15	Benzo(b)fluoranthene d12	PAH	26.243	263.9	260.2	50	132.2	118.1	10			
15	Benzo(b)fluoranthene	PAH	26.297	126.1	113.1	10	252.1	250.2	35			
15	Benzo(k)fluoranthene d12	PAH	26.311	263.9	260.2	50	132.2	118.1	10			
15	Benzo(k)fluoranthene	PAH	26.355	252.1	250.2	35	126.1	113.1	10			
15	Benzo(e)pyrene d12	PAH	26.84	264	260.2	40	132.2	118.1	15			
15	Benzo(e)pyrene	PAH	26.86	252	250.2	50	125	112	20			
15	Benzo(a)pyrene d12	PAH	26.907	264	260.2	40	132.2	118.1	15			
15	Benzo(a)pyrene	PAH	26.959	252.1	250.1	35	125	124.2	10			
15	Perylene d12	PAH	27.08	264	260.1	40	130.1	116.1	15			
16	Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	TCR	28.153	123.6	107.1	10	97.6	42.1	20			
16	Indeno[1,2,3-cd]pyrene d12	PAH	28.988	288	284.2	50	288	286.2	40			
16	Dibenz(a,h)anthracene d14	PAH	29.022	288	284.2	50	288	286.2	40			
16	Indeno(1,2,3-cd)pyrene	PAH	29.03	138.1	137.2	10	137	136.1	15			
Time Segment	Compound	Class	RT[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	CE	Pre3	Prod 3	CE
16	Dibenz(a,h)anthracene	PAH	29.075	138.1	137.2	10	125	124.2	10			
16	Benzo(g,h,i)perylene d12	PAH	29.43	288	284.2	50	288	286.2	20			
16	Benzo(g,h,i)perylene	PAH	29.473	276.1	274.1	45	138	125.1	15			

17	Coronene	PAH	32.449	299.4	298.1	30	299.4	298.1	30			

[a] RT = Retention Time
[b] Pre1 = Precursor Ion 1
[c] Prod1 = Product Ion 1
[d] CE = Collision Energy

Preparation of Air Samples Collected on PUF Plugs for GC/MS Analysis (D-EMMD-PHCB-036-SOP-01) – September 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Standard Operating Procedure for Preparation of Air Samples Collected on PUF Plugs for GC/MS Analysis

Number: D-EMMD-PHCB-036-SOP-01	Effective Date: 09/08/2016

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: M. Scott Clifton
Signature: Date:

Approval

Name: Myriam Medina-Vera
Title: Branch Chief, PHCB
Signature: Date:

Concurrence*

Name:
Title:
Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

STANDARD OPERATING PROCEDURE FOR PREPARATION OF AIR SAMPLES COLLECTED ON PUF PLUGS FOR GC/MS ANALYSIS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This SOP details the extraction and work-up procedures for air samples collected on pre-cleaned polyurethane foam (PUF) plugs. This method is applicable for extraction of both indoor and outdoor field samples as well as laboratory-generated samples, including collection from emissions experiments. This SOP is written to encompass a wide range of analytes and to be applicable across many studies. Analytical performance will need to be assessed for specific analytes prior to use.

2.0 SUMMARY OF METHOD

There are two acceptable methods for extraction and processing of PUF plugs that will be detailed in this SOP that involve either Soxhlet or ultrasonic extraction. The method chosen will depend on availability of materials and equipment, resources, and analytical performance. For both methods, samples are allowed to equilibrate to room temperature after removing from freezer storage.

With the Soxhlet extraction method, samples are transferred to clean 150 mL Soxhlet extractors. Internal standard solution is spiked onto the surface of the samples. Boiling flasks are filled with 300 mL of 1:1 acetone:hexane along with several boiling chips. The extractors are assembled on a heating mantle with condensers and heat is applied so the extraction rate is ~20 cycles per hour. The samples are extracted for 16 hours (overnight is convenient). The extracts in the boiling flasks are allowed to cool and are then concentrated to 2-5 mL on a rotary evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL hexane rinses of the boiling flask. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.

For ultrasonic extraction, samples are transferred to clean 60 mL amber jars. Internal standard solution is added to the PUF. Each jar is filled with 50 mL of 1:1 acetone:hexane and is then sealed with a PTFE-lined cap. The jars are placed in an ultrasonic cleaner with water level well below the level of the jar cap. The ultrasonic cleaner is then turned on for 15 minutes. Sample jars are removed from the cleaner and the extracts are transferred through funnels into 250 mL narrow-mouth bottles. The funnels are rinsed with hexane from a wash bottle after the extracts are added. The solvent addition, extraction, and transfer is repeated two more times. The extracts in the bottles are then evaporated to 2-5 mL using a parallel evaporator. The concentrated extracts are then transferred to a 15 mL graduated glass tube along with two 2 mL hexane rinses of the bottle. The extracts are then concentrated to a final volume of 1 mL under nitrogen. The extracts are then transferred to autosampler vials for analysis.

3.0 DEFINITIONS

3.1 PUF – Polyurethane foam sample media.
3.2 SRS – Surrogate recovery standards which are used to evaluate analyte recovery.

3.3 IS – Internal standard solution which is used in quantification to establish response ratios.
3.4 Method blank – Unfortified media that is extracted to evaluate interferences and possible contamination in the media or lab.
3.5 Method spike – Media that is fortified and extracted to evaluate analyte recovery from the extraction process.
3.6 Recovery spike – Unfortified media that is extracted and processed like the method blank. The extract is fortified after sample preparation is complete. This is used to simulate 100% analyte recovery so matrix effects that can influence the measured concentrations can be evaluated.

4.0 HEALTH AND SAFETY WARNINGS

4.1 Follow the procedures detailed in applicable Health and Safety Research Protocols.
4.2 Follow proper operating procedures for all equipment and instruments used.
4.3 Exercise caution when using syringes and avoid inhalation or dermal contact with all solvents and solutions used in this procedure.
4.4 Exercise caution when working with and around heating mantles used for Soxhlet extraction. Perform extractions inside of a fume hood and ensure that all connections are secure before leaving the extractors unattended. Allow flasks used in extraction to cool before handling.
4.5 The ultrasonic cleaner and the water bath inside can become very hot, so exercise caution when removing containers from the bath and allow the bath to cool or replace the water with cool water before continuing if the heat is excessive.

5.0 MATERIALS AND EQUIPMENT

5.1 *Soxhlet method*

5.1.1 Clean PUF plugs (Supelco 20600-U or equivalent)
5.1.2 Stainless-steel forceps
5.1.3 Spiking solution, applicable to analytes being measured
5.1.4 Internal standard solution (IS), applicable to analytes being measured
5.1.5 Pipette or syringe capable of accurately delivering 50 µL of solution
5.1.6 Soxhlet extractors with condensers and chillers, 150 mL
5.1.7 Heating mantles
5.1.8 Boiling flasks, 500 mL
5.1.9 Hexane, pesticide grade or equivalent
5.1.10 Acetone, pesticide grade or equivalent
5.1.11 Boiling chips
5.1.12 Rotary evaporator
5.1.13 Glass serological or volumetric pipette capable of 2 mL
5.1.14 Pasteur pipettes – 9”
5.1.15 Graduated tubes, glass, 15 mL

5.1.16 Nitrogen evaporator with heated water bath (N-Evap or equivalent) or dry block
5.1.17 Autosampler vials, 2 mL, caps with PTFE-lined septa

5.2 Ultrasonic Extraction Method

5.2.1 Clean PUF plugs (Supelco 20600-U or equivalent)
5.2.2 Stainless-steel forceps
5.2.3 Spiking solution, applicable to analytes being measured
5.2.4 Internal standard solution (IS), applicable to analytes being measured
5.2.5 Pipette or syringe capable of accurately delivering 50 µL of solution
5.2.6 Wide-mouth glass jars with PTFE-lined caps, 60 mL
5.2.7 Ultrasonic cleaner with water bath
5.2.8 Hexane, pesticide grade or equivalent
5.2.9 Acetone, pesticide grade or equivalent
5.2.10 Analytical funnels, glass
5.2.11 Narrow-mouth bottles, Boston round, 250 mL
5.2.12 Parallel evaporator, Buchi Multivapor P-6 or equivalent
5.2.13 Glass serological or volumetric pipette capable of 2 mL
5.2.14 Pasteur pipettes – 9”
5.2.15 Graduated tubes, glass, 15 mL
5.2.16 Nitrogen evaporator with heated water bath (N-Evap or equivalent) or dry block
5.2.17 Autosampler vials, 2 mL, caps with PTFE-lined septa

6.0 INTERFERENCES

Interferences are any component that interferes with the quantitative analysis. Interferences should be evaluated prior to applying this method to study samples. This method may be modified to deal with interferences if necessary as long as modifications are documented and are acceptable within a study’s quality assurance plan. If interferences not identified during method evaluation are discovered with study samples, they will be identified and evaluated as part of a study’s ongoing quality assurance/quality control (QA/QC) plan.

7.0 PERSONNEL QUALIFICATIONS

This SOP is written to be used by personnel familiar with the equipment and procedures that will be used. Personnel should be adequately trained and display proficiency with those techniques prior to using this SOP for sample analysis.

8.0 SAMPLE PRESERVATION AND STORAGE

Study samples will be collected at a field location or laboratory and stored at freezer conditions (-20ºC) until they can be extracted. Sample stability should be assessed for the analytes in a given study if extended (>30 days) storage time is anticipated. At the time of extraction, the samples will be removed from the freezer and will be allowed to warm to room temperature. Sample

extracts will be stored under freezer conditions (-20ºC) in cases where analysis cannot be performed immediately. The extracted PUF plugs will be discarded.

9.0 EXTRACTION PROCEDURE SOXHLET METHOD

9.1 Remove PUF air samples from the freezer and let warm to room temperature.

One sample batch will consist of the following:

- Air samples (from freezer)
- 1 Lab spike
- 1 Lab blank
- 1 Laboratory 100% recovery spike

9.2 While the samples from the freezer are warming to room temperature, the method spike, method blank, and recovery spike can be prepared for extraction.

9.3 Method Spike Preparation

9.3.1 Place a clean PUF plug into a 150 mL Soxhlet extractor so that it's in a U-shape and sits under the siphon tube on the extractor.
9.3.2 Transfer a 50 µL aliquot of spiking solution to the PUF plug and allow the solvent to evaporate (~1 minute).
9.3.3 Transfer a 50 µL aliquot of internal standard solution to the PUF plug and allow the solvent to evaporate (~ 1 minute).

9.4 Lab blank Preparation

9.4.1 Place a clean PUF plug into a 150 mL Soxhlet extractor so that it's in a U-shape and sits under the siphon tube on the extractor.
9.4.2 Transfer a 100 µL aliquot of internal standard to the PUF plug and allow the solvent to evaporate (~1 minute).

9.5 Recovery Spike Preparation

9.5.1 Place a clean PUF plug into a 150 mL Soxhlet extractor so that it's in a U-shape and sits under the siphon tube on the extractor.
9.5.2 After extraction and concentration, add 50 µL of spiking solution to the sample.
9.5.3 Add 100 µL of internal standard solution, cap, and vortex along with the other samples processed in the sample batch.

9.6 Air Sample Preparation

9.6.1 Place the PUF sample into a 150 mL Soxhlet extractor so that it's in a U-shape and sits under the siphon tube on the extractor.

9.6.2 Fortify each of the PUF plugs with 100 µL of internal standard solution and allow the solvent to dry.

9.7 Sample Extraction (Spikes, Blanks, and Air Samples)

9.7.1 Label a 500 mL boiling flask for each sample to be extracted.
9.7.2 Add 300 mL of 1:1 acetone:hexane to each flask along with several boiling chips.
9.7.3 Assemble each Soxhlet extractor with the corresponding boiling flask and attach the condenser. Verify that each condenser has cool liquid passing through it. The liquid should be between 10°C and 15ºC to prevent water condensation into the extractor and to prevent vaporization of the solvent.
9.7.4 Place the assembled extraction apparatus onto a heating mantle or on an extraction bank and begin heating the solvent in the flasks.
9.7.5 Once the solvent vapor begins condensing and dripping onto the PUF plugs, adjust the heat so that all of the extractors are working at approximately the same rate (~ 10-20 cycles/hour).
9.7.6 After the majority of the samples have performed one extraction cycle, begin timing the extraction. Allow the extraction to continue for 16 hours.

9.8 Extract Processing

9.8.1 Turn off the mantle or extraction bank providing heat to the extraction solvent. Turn off cool water supply to the condensers after the solvent stops boiling. Allow the extracts to cool before handling (~ 30 minutes).
9.8.2 Concentrate the extract on a rotary evaporator to a volume of 2-5 mL.
9.8.3 Transfer the concentrated extract to a 15 mL graduated tube. Rinse the boiling flask twice with 2 mL aliquots of hexane. Transfer the rinsate to the graduated tube.
9.8.4 Concentrate to a volume of 1 mL under nitrogen.
9.8.5 Transfer the sample solution to an autosampler vial using a Pasteur pipette.
9.8.6 Cap the autosampler vial and analyze by GC/MS.
9.8.7 If the sample cannot be analyzed immediately, store in a freezer at -20ºC until they can be analyzed.

10.0 EXTRACTION PROCEDURE - ULTRASONIC METHOD

10.1 Remove PUF air samples from the freezer and let warm to room temperature.

One sample batch will consist of the following:

- Air samples (from freezer)
- 1 Lab spike
- 1 Lab blank
- 1 Laboratory 100% recovery spike

10.2 While the samples from the freezer are warming to room temperature, the method spike, method blank, and recovery spike can be prepared for extraction.

10.3 *Method Spike Preparation*

10.3.1 Place a clean PUF plug into a 60 mL wide-mouth glass jar.
10.3.2 Transfer a 50 µL aliquot of spiking solution to the PUF plug and allow the solvent to evaporate (~1 minute).
10.3.3 Transfer a 100 µL aliquot of internal standard solution to the PUF plug and allow the solvent to evaporate (~ 1 minute).
10.3.4 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.4 *Lab blank Preparation*

10.4.1 Place a clean PUF plug into a 60 mL wide-mouth glass jar.
10.4.2 Transfer a 100 µL aliquot of internal standard solution to the PUF plug and allow the solvent to evaporate (~ 1 minute).
10.4.3 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.5 *Recovery Spike Preparation*

10.5.1 Place a clean PUF plug into a 60 mL wide-mouth glass jar.
10.5.2 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.
10.5.3 After extraction and concentration, add 50 µL of spiking solution to the sample.
10.5.4 Add 100 µL of internal standard solution, cap, and vortex along with the other samples processed in the sample batch.

10.6 *Air Sample Preparation*

10.6.1 Place the PUF sample into a 60 mL wide-mouth glass jar.
10.6.2 Fortify each of the PUF plugs with 100 µL of internal standard solution and allow the solvent to dry.
10.6.3 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.7 *Sample Extraction (Spikes, Blanks, and Air Samples)*

10.7.1 Place samples into an ultrasonic cleaner and start the cleaner.
10.7.2 Allow the cleaner to run for 15 minutes.
10.7.3 Carefully remove the samples from the ultrasonic cleaner, drying the outside of each jar with a paper towel as it is removed.

10.7.4 Assemble an analytical funnel on a 250 mL glass Boston round bottle and label one bottle for each sample extract.
10.7.5 Carefully pour the solvent content of each sample jar into the corresponding bottle through the funnel. Allow the jar to sit in the funnel until solvent stops dripping.
10.7.6 Carefully remove the jars and add another 50 mL of 1:1 acetone:hexane. Recap the jars.
10.7.7 Rinse the inside of each funnel into its bottle with hexane from a wash bottle.
10.7.8 Repeat steps 10.7.1 to 10.7.7 two more times, omitting step 10.7.6 after the third extraction.

10.8 *Extract Processing*

10.8.1 Concentrate the sample extracts inside of the bottles to a volume of ~2-5 mL on a parallel evaporator.
10.8.2 Transfer the concentrated extract to a 15 mL graduated tube. Rinse the bottle twice with 2 mL aliquots of hexane. Transfer the rinsate to the graduated tube.
10.8.3 Concentrate to a volume of 1 mL under nitrogen.
10.8.4 Transfer the sample solution to an autosampler vial using a Pasteur pipette.
10.8.5 Cap the autosampler vial and analyze by GC/MS.
10.8.6 If the sample cannot be analyzed immediately, store in a freezer at -20ºC until they can be analyzed.

11.0 RECORDS

Chain of custody records will be maintained to document the removal and extraction of each air sample. Those records will be stored as indicated in the applicable study's QA plan. The performance of this procedure will be documented in a NERL laboratory notebook. This documentation will include details and observations for each sample batch analyzed.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

Data will be reviewed by the EMMD QA Manager. The data quality objectives and review procedures from the QAPP for the study being conducted will dictate specific quality assurance practices. All QA practices will be consistent with the NERL Quality Management Plan.

The method blank, method spike, and recovery spike will serve to measure method performance for each batch of samples.

Determination of Moisture Content in Tire Crumb Rubber (D-EMMD-PHCB-041-SOP-01) – September 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Determination of Moisture Content in Tire Crumb Rubber

Number: D-EMMD-PHCB-041-SOP-01	Effective Date: 9/2/16

SOP was developed: ☒ In-house ☐ Extramural

SOP Steward

Name: M. Scott Clifton
Signature: Date:

Approval

Name: Myriam Medina-Vera
Title: Branch Chief, PHCB
Signature: Date:

Concurrence*

Name:
Title:
Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

* Optional Field
NERL-SOP.1 (7/2003)

STANDARD OPERATION PROCEDURE FOR THE DETERMINATION OF MOISTURE CONTENT IN TIRE CRUMB RUBBER – September 2, 2016

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

Tire crumb rubber samples collected from playing fields may contain varying amounts of water based on environmental conditions at the sampling location. Since many of the analyses of tire crumb rubber is based on mass of analyte/mass of rubber, the percentage of moisture is very important in being able to accurately quantify the levels of analyte in the rubber sample. Moisture analysis is comprised of not only the amount of water in a sample, but the amount of other materials that are volatile at 110ºC. Since the mass of other materials is likely to be below the weight sensitivity of the moisture analyzer, weight loss is assumed to be mostly water. This SOP describes the procedure used to determine the amount of moisture in tire crumb rubber samples using the Mettler-Toledo HE53 Moisture Analyzer based on industry standard procedures prescribed in ASTM Method D1509-15.

2.0 SUMMARY OF THE METHOD

To determine the moisture content, the sample will be allowed to reach room temperature while the moisture analyzer is set up. Prior to measurement, the balance calibration will be verified using certified check weights. When the sample has equilibrated to room temperature, the moisture analysis process can begin. A disposable sample pan is placed onto the moisture analyzer and tared. A tire crumb sample (2 g) is then spread in a thin, even layer across the total surface of the pan and the weight is recorded on a Moisture Analysis Form (Appendix A). The moisture analysis is then started and will continue until mass loss is less than 1 mg/30 s. The HE53 Moisture Analyzer will display the percent moisture content which should be recorded on the form.

3.0 DEFINITIONS

- Moisture analyzer – Also called a moisture balance. An instrument that combines a balance with a heating element to determine weight loss over time at a set temperature. This is used to calculate the percent moisture in a sample.
- TCR – tire crumb rubber
- SOP – standard operating procedure
- HDPE – high-density polyethylene
- COC – chain of custody

4.0 CAUTIONS

4.1 The moisture analyzer operates at 110ºC. Care should be taken to keep flammable materials away from the analyzer to reduce the risk of fire. Care should also be taken when operating the instrument and handling sample pans after analysis as they will be hot and can burn skin. Hot pans should be handled with metal forceps or tongs only.

4.2 The moisture analyzer should be operated with adequate ventilation as vapor and fumes from the drying process may contain volatile compounds which may be noxious and/or toxic.
4.3 Laboratory staff performing the moisture analysis will wear appropriate protective equipment, including nitrile gloves and safety glasses.

5.0 RESPONSIBILITIES

5.1 The laboratory staff performing the moisture analysis will complete custody documents as appropriate as well as appropriately document the analysis in the Moisture Analysis Form and EPA/NERL laboratory notebook.

6.0 MATERIALS

6.1 Mettler Toledo HE53 Moisture Analyzer
6.2 Clean plastic spatulas or spoons
6.3 Laboratory wipes – Kimwipes or equivalent
6.3 Aluminum sample pans – Mettler Toledo P/N 13865
6.4 Moisture Analysis Form

7.0 PROCEDURES

Remove the samples to be analyzed from freezer storage and allow to equilibrate to room temperature. The METALS COMPOSITE sample from the fields and the METALS Batch 1 sample from the recycling plants will be used for this determination. This should take at least 1 hour. While samples are warming, prepare the equipment for the analysis.

7.1 *Equipment Preparation*

7.1.1 Clean plastic spatulas or spoons with deionized water and laboratory wipes if necessary. Pre-cleaned, sterile spatulas or spoons may also be used.
7.1.2 Turn on the moisture analyzer and let it warm up for at least 1 hour prior to use. Make sure the analyzer is set for 110ºC analysis temperature. Note: The temperature inside the analyzer will not go to 110ºC until the analysis has begun.

7.2 *Moisture Determination*

7.2.1 Place a clean sample pan into the moisture analyzer. Close the lid and tare.
7.2.2 Place a 2 g and a 10 mg weight onto the sample pan and record the weight. Remove the 10 mg weight and record the weight displayed on the balance. The weight must be 2g ± 1%. Note: It is only necessary to perform this check once per day.

7.2.3 Open the lid and weight 2 g ±0.1g of TCR into the sample pan. Make sure that the TCR is evenly distributed in the pan in an even layer. Close the moisture analyzer lid and record the weight.

7.2.4 Press the "Start" key on the analyzer and wait for the instrument to perform the measuring process.

7.2.5 The moisture analyzer will indicate when the measurement is complete. Record the percent moisture from the analyzer display.

7.2.6 Repeat steps 7.2.1 and 7.2.3-7.2.5 for a duplicate measurement after thoroughly mixing the TCR sample in its original jar by shaking and stirring.

8.0 RECORDS

Record data from moisture analysis directly into a Moisture Analysis Form (Appendix A). Place a copy of the form into a NERL laboratory notebook and maintain the original forms in a binder for transfer to the study coordinator. Enter the data into an Excel spreadsheet that indicates the sample ID, individual measurements, and the average of the two measurements. Record the use of the sample on the appropriate custody sheet which are maintained by the study coordinator.

9.0 REFERENCE

- ASTM D5603 – 01: *Standard classification for rubber compounding materials – recycled vulcanizate particle rubber* (June 15, 2015)
- ASTM D1509-15: *Standard test methods for carbon black – heating loss* (November 1, 2015)

APPENDIX A – Tire Crumb Rubber Moisture Analysis Form

Moisture Analysis Form
D-EMMD-PHCB-041-SOP-01

Analyst: ________________________ **Moisture Analyzer**: _________________

Date: ___________________________ **2g + 10mg =** __________

2g =__________ **Pass:** _____

Sample ID	Weight (g)	% Moisture	Average % Moisture
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			
TCRS - F - _ _ -1-1-M-0			

Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument (D-EMMD-PHCB-042-SOP-03) – February 2014

U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
National Center for Computational Toxicology
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: **Standard Operating Procedure for Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument**

Number: D-EMMD-PHCB-042-SOP-03 | Effective Date: February 2014

SOP was developed ☒ In-House ☐ Extramural

Alternative Identification:

SOP Steward

Name: Kasey D. Kovalcik

Signature: Date:

Approval

Name:
Title:
Signature: Date:

Concurrence*

Name:
Title:

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

* Optional Field
NERL-SOP.2 (11/2005)

Revision Changes from Previous Version

HR-ICPMS Analysis

Version	Date	Revisions/Changes
98.0		Original Effective Date. (previously titled SOP-WDE-08-08)
98.1	1/12/11	Update Reagent Blank Concentration Update Calibration Preparation and Concentrations Update Instrument Analytical Method
98.2	2/1/14	Update QA/QC Guidelines
042-SOP-03	9/1/16	Update to name due to new division/branch identification

STANDARD OPERATING PROCEDURE FOR OPERATION AND MAINTENANCE OF THE ELEMENT 2 HIGH-RESOLUTION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY INSTRUMENT

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

Inductively coupled plasma mass spectrometry (ICPMS) is a widely accepted analytical tool for trace and ultra-trace elemental analysis. It has been the technique of choice for accurate and precise measurements needed for today's demanding applications. In ICPMS, an inductively coupled plasma (a gas consisting of ions, electrons, and neutral particles) is formed from argon gas under an intense electromagnetic field. The plasma is used to atomize and ionize the sample matrix. The resulting ions are then passed through a series of apertures into the high-vacuum mass analyzer. Isotopes of the elements are identified by their mass-to-charge ratio (m/z), and the intensity of a specific peak in the mass spectrum is proportional to the amount of that isotope (element) in the original sample.

Because of the enormous number of possible interferences, the ability to isolate analytes from interfering species is critical to any analytical ICPMS instrument. Double-focusing magnetic sector field ICPMS, often called high-resolution ICPMS (HR-ICPMS), provides a straightforward solution to most of the polyatomic and isobaric interferences by separating the analyte of interest from its interfering species. EPA's National Exposure Research Laboratory (NERL) purchased an HR-ICPMS instrument, the Element 2 (E2), from Thermo Finnigan (now Thermo Fisher Scientific, Waltham, MA). This instrument has been installed in NERL's Class 100 clean lab.

This standard operating procedure (SOP) is intended to provide the following information:

- Descriptions of various components of E2 and its control software (v3.0)
- The basic operational settings of the E2
- Autosampler control procedures
- Procedures to prepare reagents and calibration standards
- Procedures for verifying performance measures of the E2 on a daily basis
- Instructions for preparing various logs
- Instructions for setting up a sequence for automated use of the instrument with QC checks
- Need-based instrument conditioning and maintenance procedures such as changing recirculating fluid, establishing mass calibration, and changing the entrance slit assembly
- Instructions for flagging sequence results using the "ICPMS Data Flagger" application

2.0 SUMMARY OF METHOD

2.1 This SOP is intended for either (1) analysis of SEAS slurry samples after preparing samples according to SOP-WDE-08-01 *Standard Operating Procedure for Preparation of SEAS Samples for HR-ICP-MS Analysis*, or (2) analysis of ambient air particles collected on Teflon filter media after aqueous or dilute acid-phase extraction (SOP# ECAB-114.0 *Standard Operating Procedure for the Three-Stage Extraction of Filter Media for Ion Chromatography and High-*

Resolution Inductively Coupled Plasma Mass Spectrometry) and (3) ECAB 149.0 *Processing Hydraulic Fracturing Field Samples for Trace Metal Analysis*.

2.2 An internal standard is added on-line at the time of analysis using a second channel of the peristaltic pump and a low dead-volume mixing manifold.

2.3 Data acquisition and evaluation parameters for this method have already been established in the clean lab. Appendix 1 lists all parameters for the multi-elemental method used in this SOP.

2.4 This method employs the ASX-510 autosampler (CETAC Technologies, Omaha, NE) for analysis.

3.0 DEFINITIONS

ALM	Auto Lock Mass
cps	counts per second
DI	deionized water from ion-exchange resin cartridges, plastic tap water
E2	Element 2, the second-generation Element HR-ICPMS instrument
HEPA	high-efficiency particulate air filter
HR	high resolution ($\Delta m = 10,000$)
ICPMS	inductively coupled plasma mass spectrometry
IDL	instrument detection limit
LR	low resolution ($\Delta m = 300$)
mL	milliliter
mm	millimeter
MR	medium resolution ($\Delta m = 4000$)
MSDS	Material Safety Data Sheet
NIST	National Institute of Standards and Technology
plasma	mixture of ionized gases and free electrons
ppb	parts per billion (µg/L)

ppm	parts per million (mg/L)
QA	quality assurance
QC	quality control
reagent water	sterilized (UV-treated) DI water with a resistivity of 18.2 MΩ·cm (at 25 °C) or greater (DI water produced by Millipore or Barnstead system)
RF	radio frequency
RO	reverse osmosis (water used for tube-cleaning purposes)
RPD	relative percent difference
RSD	relative standard deviation
SD	standard deviation
SEM	secondary electron multiplier

4.0 GENERAL SAFETY CONSIDERATIONS

4.1 To avoid personal injury or damage to the instrument, do not perform any servicing other than that contained in the E2 hardware manual unless you are qualified to do so.

4.2 High voltages capable of causing personal injury are used in the instrument. Some maintenance procedures require that the mass spectrometer be shut down and disconnected from the power before service is performed.

4.3 Do not operate the mass spectrometer with the top or side covers off. Do not remove protective covers from printed circuit boards. Safety labels are used on the instrument to show potential safety risks. Read the labels carefully.

4.4 Ensure that you read and understand the hazards of the chemicals used.

4.5 Refer to MSDSs for information on the hazards and toxicity of specific chemical compounds and for the proper handling of compounds, first aid for accidental exposure, and procedures for remedying spills or leaks.

5.0 INTERFERENCES

E2 can be operated to achieve mass resolutions, R (**Δ**m/m), of up to 10,000 by means of entrance and exit slit assemblies. By varying resolution settings, it is possible to tailor an analysis in such a way that each isotope is analyzed at a resolution that will enable it to be fully resolved from any interference with minimal compromise of its sensitivity.

Table 1 lists common interferences on the most abundant isotopes that require resolution settings (provided list is not complete). If the needed R value is between 300 and 4,000, the MR setting is used for data acquisition. If R is in the 4,000 to 10,000 range, the HR setting is recommended. If the calculated R value is over 10,000, that interference cannot be resolved by E2. Under such circumstances, an alternative isotope and/or interference correction equation, or combination of both, must be applied.

Table 1. Interferences and Resolving Power Required

Analyte	Interference	R = m/|Δm|	Measurement Mode	Alternate Isotope and Required Mode	Correction
^{40}Ca	^{40}Ar	190,476	($)	^{39}Ca in MR	
^{40}K	^{40}Ca	28,369		^{39}K in HR	
^{115}In	^{115}Sn	212,963	($)		^{115}Sn
^{87}Sr	^{87}Rb	300,000		^{88}Sr in LR (*)	
^{112}Cd	^{112}Sn	54,369	($)		
^{111}Cd	$^{95}Mo^{16}O$	32,362	($)		^{111}MoO (*)
^{56}Fe	$^{40}Ar^{16}O$	2,506	^{56}Fe in MR		
^{51}V	$^{35}Cl^{16}O$	2,576	^{51}V in MR		
^{52}Cr	$^{12}C^{40}Ar$	2,378	^{52}Cr in MR		
^{75}As	$^{40}Ar^{35}Cl$	9,500	^{75}As in HR		
^{80}Se	^{80}Kr, $^{40}Ar^{40}Ar$	>10,000		^{77}Se in HR	
^{74}Ge	$^{56}Fe^{18}O$, ^{74}Se	>10,000		^{72}Ge in HR	
(*) Correction will not be required if the sample does not have a significant amount of the interferent. ($) Interference unavoidable at all resolution settings.					

6.0 PERSONNEL QUALIFICATIONS

The operator/analyst must have the following qualifications:

- High-level experience using an ICPMS instrument
- A thorough understanding of the fundamentals of inorganic analytical chemistry
- User-level knowledge of the Windows XP operating system

7.0 EQUIPMENT AND SUPPLIES

7.1 *Equipment*

Equipment	Model No. and Manufacturer	Located in	Usage
Element 2	E2, Thermo Fisher Scientific	D456	Elemental analysis
Autosampler	ASX-510, Cetac Technologies	D456	Sample introduction system
Ultrasonic bath	1875HTA, Crest	D461-B	Sample preparation
Convection oven	737F, Fisher Scientific	D461-A	Sample tube cleaning
Weighing balance	1) SP202, Scout Pro 2) ME235SD, Sartorius Genius	D456	Making reagents, gravimetrically

7.2 *Supplies and Ordering Information*

Classification	Items	Vendor	Part No.
E2 usage-dependent	1. Entrance slit assembly 2. Torch 3. RF load coil 4. SEM ICP2 plug-in 5. Platinum skimmer cone 6. Platinum sampler cone 7. Platinum guard electrode	Thermo ESI Thermo Thermo ESI ESI ESI	1047360 ES-1002 1139230 1114170 ES-3000-1809 ES-3000-1807 ES-1001-0004
E2 annual maintenance	1.Skimmer valve rebuild kit 2. F5 oil, 1 L 3. P3 oil, 1 L 4. Turbopump wicks	Thermo Pfeiffer Pfeiffer Thermo	1120650 PF 001 852-T PK 001 106-T
E2 sample intro.	1. 100-µL Teflon neb 2. Orange-green 2-stop PP 3. Gray-gray	ESI Cetac Cetac	ES-2040-27 020-030-004 020-030-011
Sample containers	1. 15-mL PP vials 2. 50-mL PP vials	Nalgene FisherBrand	366036 06-443-20

8.0 ANALYTICAL STANDARDS AND QA/QC SOLUTIONS

8.1 *General Guidelines*

8.1.1 Always use reagent water for making analytical standards and for sample preparation.

8.1.2 Any acid concentration must be less than 4% for ICPMS analysis to

minimize damage to the interface and to minimize isobaric molecular interferences.

8.1.3 The concentrations of dissolved solids in analysis solutions should be less than 2% to protect the sample interface on the instrument. Higher concentrations may plug the sample cone orifice.

8.1.4 Know your sample. Protect the SEM from high chemical concentrations (high ion currents). SEM suffers from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this period, response factors are constantly changing, which causes instrument instability that invalidates the calibration curve, thereby invalidating all associated sample results. For instance, sodium bicarbonate ($NaHCO_3$) sample matrix is known to cause this problem.

8.1.5 Use acid-cleaned sampling tubes and containers for standards and samples using the following procedure:

1. Rinse in reverse osmosis (RO) water, and then fill with RO water.
2. Let sample containers/tubes leach in RO water for 1 hour.
3. Empty tubes and fill with 4% HNO_3 (v/v) + 2% HCl (v/v) solution.
4. Place filled tubes in a convection oven at 90ºC for 3 hours.
5. Let tubes and contents cool, and then pour out the acid solution (save and reuse this acid solution mix).
6. Fill tubes with reagent water for 1 hour.
7. Pour out the rinse solution, and triple rinse with ultra-clean reagent water. Store tubes completely full with reagent water until use.
8. Tubes do not need to be completely dry before use.

8.2 *Chemicals and Reagents*

- Concentrated analytical-grade Baseline nitric and hydrochloric acids (SeaStar Chemicals Inc., Sidney, BC, Canada)
- High-purity primary standard solutions
- Reagent water (> 18.2 MΩ·cm) for making calibration standards
- Liquid argon, high-purity grade (99.99%)

8.3 *Preparation of Reagents and Analytical Standards*

8.3.1 Reagent Blank
Prepare 0.2% (v/v) nitric acid and 0.1% (v/v) hydrochloric acid solution by adding 4 mL of conc. nitric acid and 2 mL of conc. hydrochloric acid to a 2-L Teflon bottle and make up the volume with reagent water.

8.3.2 Tune Solution
The tune solution contains elements representing all of the mass regions of interest, thereby allowing verification that the resolution and mass

calibration of the instrument are within the required specifications. The solution is also used to verify that the instrument has reached thermal stability. Use individual primary standards from High Purity Standards (Charleston, SC, USA) to make a stock tune solution according to procedures in Table 2, and then dilute it 100 times in 1% HNO_3 to create a working tune solution of 1 ppb.

8.3.3 Autosampler Rinse Solution

Prepare 0.4% (v/v) nitric acid and 0.2% (v/v) hydrochloric acid solution by adding 4 mL of conc. nitric acid and 2 mL of conc. hydrochloric acid to a 1-L Teflon water bottle and making up the volume to 1000 mL with reagent water.

8.3.4 Stock Solutions

Depending on availability, Stock Solutions A, B, and S are made from certified primary standard solutions procured from High Purity Standards. Use acid-washed, wide-mouth Teflon bottles to make stock solutions. Table 3 summarizes the procedure to make all stock solutions. Appendix 5 shows an alternate method based on Custom Multi-Element Mixed Stock Solutions.

Table 2. Preparation of Tune Solution

Element PSS[a]	Conc.[b] (ppm)	Volume Needed (mL)	Final Conc. (ppb)	Matrix
Na	10	1	100	
Mg	10	1	100	
K	10	1	100	
Ca	10	1	100	
Al	10	1	100	
Fe	10	1	100	
Si	10	1	100	
Zn	10	1	100	
Ba	10	1	100	
Reagent water [c]		90		
HNO_3 100% (v/v)		1		1.18% HNO_3

[a]PSS: certified primary standard solution, High Purity Standards (Charleston, SC, USA).
[b]PSS made in 2% nitric acid matrix.
[c]Makeup for reagent water, gravimetrically. (Procedure: After all individual elements are pipetted into a wide-mouth labeled Teflon bottle, place bottle carefully on weighing scale. Tare weight and add reagent water from a narrow-tip fit, squeeze-type reagent water bottle until weight measurement reaches volume shown in table.)

Table 3. Preparation of Stock Solutions

Stock A				
Element PSS[a]	**Conc.[b] (ppm)**	**Volume Needed (mL)**	**Final Conc. (ppb)**	**Matrix**
Na	10	10	1000	
Mg	10	10	1000	
K	10	10	1000	
Ca	10	10	1000	
Al	10	3	300	
Fe	10	3	300	
Si	10	10	1000	
Zn	10	3	300	
Ba	10	3	300	
Water[c]		38		
HNO_3 100% (v/v)				1.2% HNO_3

Table 3. Preparation of Stock Solutions (continued)

Stock B				
Individual Element PSS[a]	**Conc.[b] (ppm)**	**Volume Needed (mL)**	**Final Conc.**	**Matrix**
Ag, As, Be, Bi	10	1 mL from every PSS	100 ppb each element	
Cd, Ce, Co, Cr, Cs, Cu				
Dy, Gd, Ge, La, Li				
Mn, Mo, Nd, Ni				
P, Pb, Pd, Pt, Rb, Rh				
Sc, Sb, Se, Sm, Sn, Sr				
Tb, Ti, Th, Tl, U, V, W, Y				
Water[c]		60.3		
HCl 100% (v/v)		0.50		0.5 % HCl

HNO_3 100% (v/v)		0.22		1.0% HNO_3
Stock S				
Element PSS[a]	**Conc.[d] (ppm)**	**Volume Needed (mL)**	**Final Conc. (ppm)**	**Matrix**
S	1000	1.0	10	
Water[c]		99.0		

[a]PSS: certified primary standard solution, High Purity Standards (Charleston, SC, USA).
[b]PSS made in 2% nitric acid matrix.
[c]Makeup for reagent water, gravimetrically.
[d]Sulfur PSS is made in just reagent water (no acid in it).

8.3.5 Standard Reference Materials
National Institute of Standards and Technology (NIST) traceable materials SRM1640e and SRM1643 should be diluted to 10 and 50 times, respectively, with reagent water.

8.3.6 Working Standards for HR-ICPMS Calibration
Use the guidelines in Table 4 to make working standard (WS) solutions to establish HR-ICPMS analytical calibrations. Appendix 2 shows concentrations at which each species is present in every working standard.

Table 4. Working Multi-element Standards for HR-ICPMS Calibration

Stock solution	**WS0**	**WS1**	**WS2**	**WS3**	**WS4**	**WS5**	**WS6**
Stock S		40 µL	400 µL	1.0 mL	2.0 mL	3.0 mL	10.0 mL
Stock A		40 µL	400 µL	1.0 mL	2.0 mL	3.0 mL	10.0 mL
Stock B		20 µL	200 µL	0.5 mL	1.0 mL	1.5 mL	5.0 mL
Reagent blank[c]	100 mL	99.9 mL	99 mL	97.5 mL	95 mL	92.5 mL	75 mL

[c]Add reagent blank gravimetrically.

9.0 INSTRUMENT AND SOFTWARE

9.1 *E2 Instrument*

The various components of the E2 are shown in Figure 1. The E2 users are strongly urged to familiarize and follow nomenclatures given in this figure. The design principle of E2 is a double-focusing sector field analyzer based on a reverse Nier-Johnson geometry in which the magnetic sector is located in front of

the electrostatic sector. Details of components and operating principles can be found in the E2 hardware manual.

9.2 *Overview of E2 Software*

Version 3.0 of the E2 software is currently in use. A program group is provided on the desktop for quick access by clicking the "Thermo ELEMENT" icon (see Figure 2). The applications can also be started by selecting the program in C:\Program Files \Thermo\Element. The program group has 14 different application task windows. Table 5 summarizes the applications for each task. Three of the 14 tasks—Diagnostic, PCL, and MakePex—are not shown here because they are not to be explored at the user level.

Figure 1. Components of the Element 2 instrument.

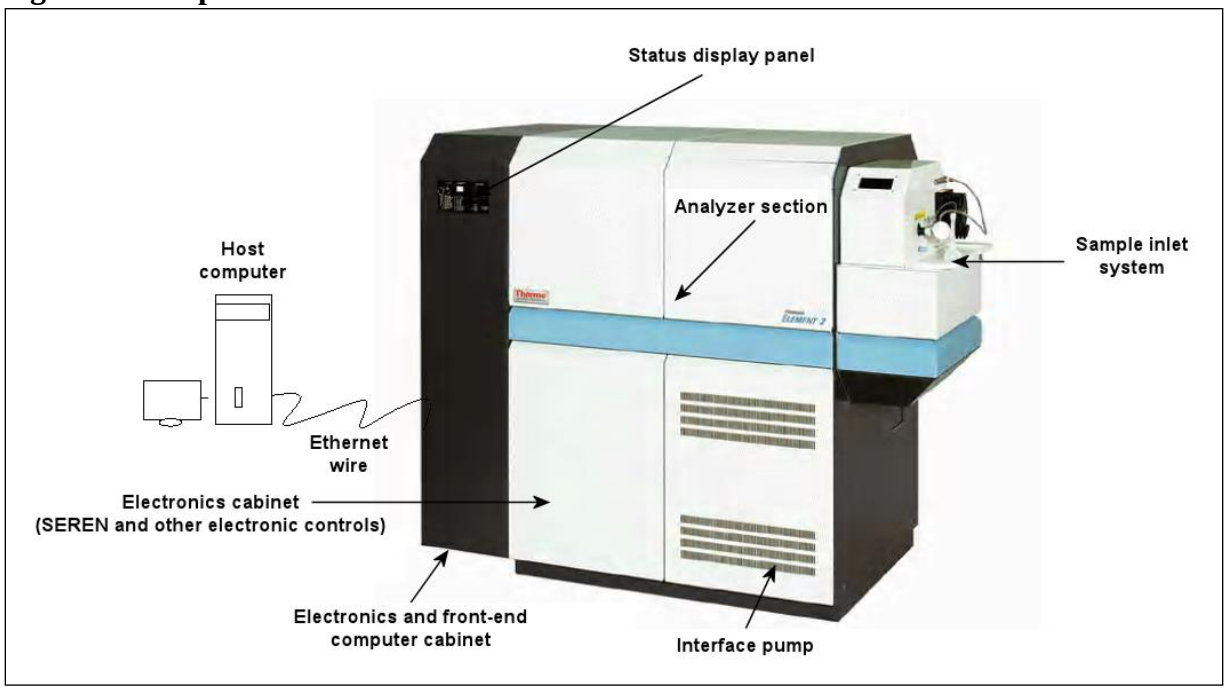


Figure 2. Task window folder.

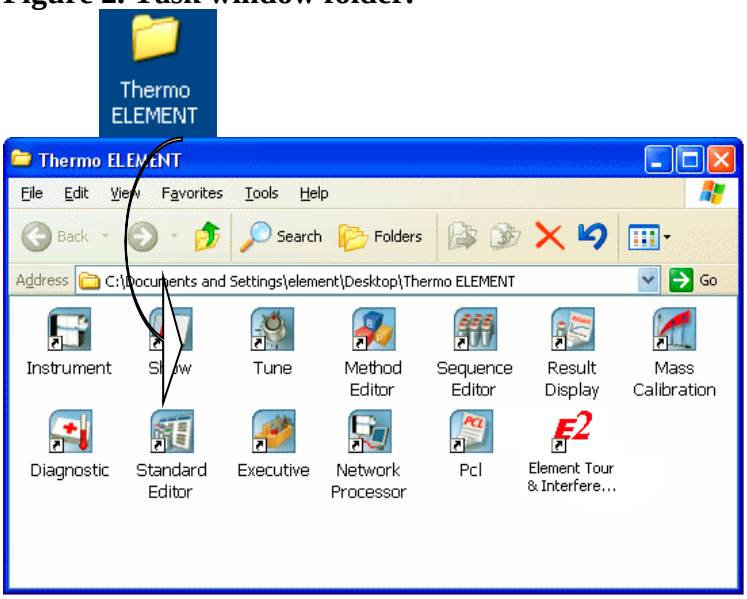


Table 5. Summary of Tasks for Each Application

Icons	Task Window	Application
	Element Tour and Interferences	Gives access to the interference workshop and other useful information.
	Network Processor	Responsible for the network connection between the PC and the instrument (LAN connection).
	Executive	Gives access to general instrument settings and is used to configure the autosampler, default directories, etc.
	Instrument	Used to acquire basic spectra and test methods.
	Tune	Display and optimize mass spectrometer parameters (plasma settings, lens voltages, etc.).
	Method Editor	Define the elements to be measured (e.g., what data to acquire, duration of the analysis and how it should be evaluated).
	Standard Editor	Generate standard concentration files for E2 analytical calibration.
	Sequence Editor	Used to setup a series of analyses. Enables the user to run a number of sample analyses, evaluate and quantify the data, and report the results. The sequence application is the key application of the software.
	Show	Display and process the acquired spectra or time-resolved data.
	Result Display	Display information concerning the results from analyses already performed. The report styles are also controlled here.
	Mass Calibration	Check, create, update, or modify mass calibrations.

10.0 BASIC E2 INSTRUMENT OPERATIONS (STARTUP, STANDBY, AND SHUTDOWN)

The procedure for starting an analysis is detailed below in Sections 10.1 and 10.2. At the end of analysis, E2 should be taken to standby (Section 10.3). Standby means the status of all components is known and the components are okay. When E2 is in standby, only the plasma is switched off. The shutdown procedure (Section 10.4) should be performed only after consulting the laboratory manager. Shutdown status implies the E2 system is vented and the power for all components, including the front-end computer, is switched off.

10.1 *Bringing the E2 Instrument from Shutdown to Standby*

1. Switch on the main power for the instrument (switch S1 on back of unit in Figure 3).
2. Turn on the argon gas so the overall head pressure > 110 psi. Turn on the recirculating chiller unit (the chiller should remain on unless performing maintenance that specifies otherwise – see section 12.1).
3. Turn the key-operated switch (status panel) to ON position to start the high vacuum (HV) for the backing pump and the four turbo pumps.
4. Switch on the power for the electronics and front-end computer (switch S2 on front of unit in Figure 3).

Note: It is recommended that the front-end PC be rebooted using the RESET button on the status display panel. The instrument is in standby position when the high vacuum reaches 10^{-7} mbar. This will take approximately 24 h.

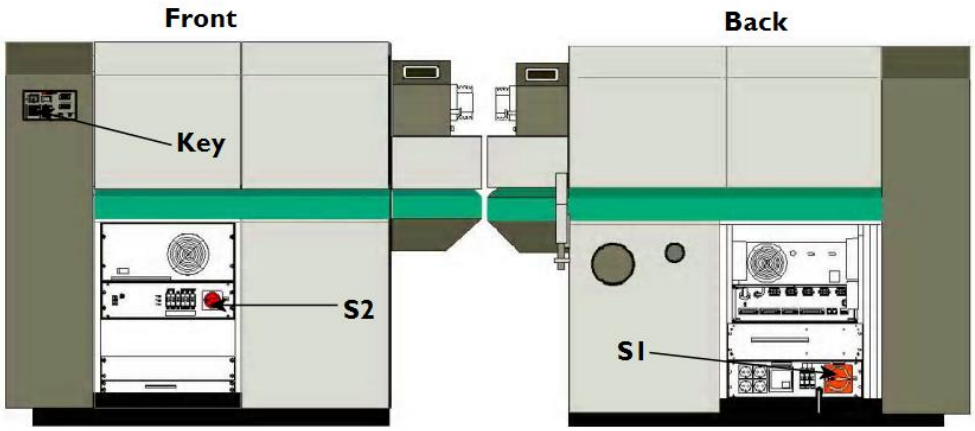


Figure 3. Front and back views of E2.

10.2 *Bringing E2 from Standby to "Ready to Measure"*

1. Open and fill in the Daily Startup Log (see Appendix 3.1, and refer to Section 12 for the complete procedure). Once checks are passed, proceed to the next step.
2. Connect the drain line of the cyclone spray chamber to the peristaltic pump head. Connect sample lines if needed.
3. Open the instrument task window, and switch on the plasma by activating the PLASMA ON button.
4. When the status box signals "Ready," switch on the high voltage by clicking the HV button in the tool bar of the instrument task window.

After 2 hours of thermal equilibration, the instrument is now ready for tuning.

10.3 *Bringing E2 from "Ready to Measure" to Standby*

1. When measurements are finished, rinse the sample inlet for 5 minutes by inserting the sample inlet in a 0.2% acid rinse solution.
2. Go to the instrument task window and switch off the plasma by clicking the

PLASMA OFF button. Wait until the stop sequence is completed.

3. If the peristaltic pump was not stopped, stop it manually by clicking "Peri.pump on/off." Open the brackets at the peristaltic pump and release the tubing.
4. Switch off the high voltage by clicking the HV button in the instrument tool bar. If the instrument is in continuous daily usage, it is better not to switch off HV.

10.4 *Bringing E2 from Standby to Shutdown*

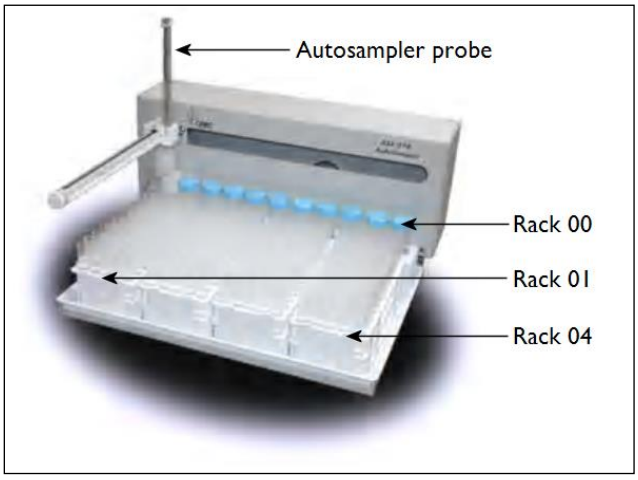
This operation should be done only when a power shutdown is announced. Always consult the laboratory manager before shutting down the instrument.

1. Turn the key switch of the status panel counterclockwise. This turns off the pumps.
2. Turn off switch S2 (front).
3. Turn off switch S1 (back).
4. Turn off the recirculating chiller and argon gas lines.

11.0 AUTOSAMPLER CONTROL

An ASX-510 autosampler is connected to the host computer and controlled through the E2 software. The ASX-510 is equipped with one built-in sample tray (Rack 00) capable of holding ten (10) 50-mL polypropylene sample vials and four removable trays (Racks 01–04) that are each capable of holding up to sixty (60) 15-mL polypropylene sample tubes (see Figure 4). This SOP covers only the required procedures for operation of the ASX-510. Refer to the autosampler operator's manual for detailed procedures for installation, usage, maintenance, and troubleshooting.

Figure 4. ASX-510 autosampler (source: Cetac web site).



Check the following before you operate the autosampler via the host computer:

1. Ensure the autosampler power is on. The green LED indicator (above the flow-through rinse station) stays lit while the power is on. Also make sure the RS-232

cable is securely connected at both ends.

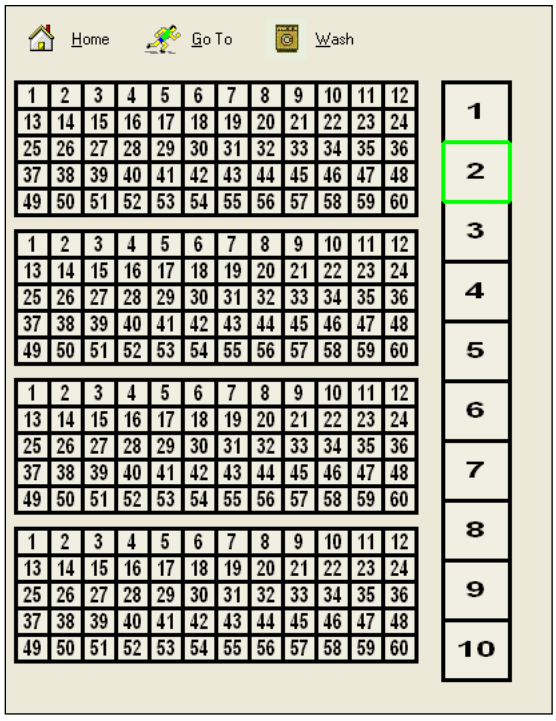
2. Ensure the rinse station is properly connected. No air bubbles should be visible in the rinse uptake tubing before you run samples. The rinse solution container should be filled with rinse solution, and the drain line of the autosampler should be connected to the rinse collection tank.

11.1 *Manual Operation*

The bottom left portion of the instrument task window displays the ASX-510 autosampler graphical user interface (GUI), as shown in Figure 5. The RR/PPP format, where RR refers to the rack number and PPP refers to position numbers, is used for moving the autosampler probe to a specific location. Selecting a spot with the mouse creates a red square around the sample. Next, press the GO TO button at the top of the GUI, which moves the sampler probe to the selected location and starts sampling. At this point, the box turns green.

The HOME button at the top of the GUI returns the autosampler probe to the home position (does not sample). Pressing the WASH button pumps rinse solution into the flow-through rinse cell. The sampler probe is dipped into the rinse cell so it gets washed externally while rinse solution is sampled as well.

Figure 5. GUI of ASX-510 sample trays with position numbers.



11.2 Automated Operation

Vial positions have to be used in connection with the Sequence Editor to operate the ASX-510 in a fully automated fashion (Section 12 covers this in greater detail). Adhere to the following vial positions while building an analytical sequence so that available sequence templates can be manipulated easily for new sequence setups.

Rack 00:

- Position 1 (00/001): Rinse solution
- Position 2 (00/002): Tune solution
- Positions 3–8 (00/003–00/008): Calibration standard solutions
- Position 9 (00/009): Check sample

Rack 01: Positions 48 and 49 are used for placing SRM samples. Other positions are generally left unused.

Racks 02–04: Any number of these positions can be used depending on the number of samples. For convenient sequence building, the last two positions of every row are left empty (see section 12.6). Once sample vials are loaded, make sure the arrangement is correctly defined in the Sequence Editor.

12.0 ROUTINE OPERATIONAL SEQUENCE OF E2

The following sequences of steps assume that the instrument is in standby mode. If not, follow the steps in Section 10 for bringing the instrument from shutdown to standby.

12.1 *Getting Ready*

1. Open the Daily Startup Log (Appendix 3.1). Fill in the form as you go through the following steps.
2. Check two argon gas tanks (located in the XRF laboratory in room D455-A). Check the liquid argon level indicators and output pressure in the gas cylinders. At least one cylinder should have greater than 50% Ar and approximately 200 psi output pressure. The secondary control (mounted on the wall) is set to output 115–140 psi to the instrument.

Special note: In case of a limited argon gas supply, the slit assembly and skimmer valve assemblies can be controlled by an additional gas line such as nitrogen. (Refer to p. 4-11 of the E2 hardware manual for configuration). We do not recommend this setup unless the situation is unavoidable.

3. Check the recirculating chiller unit located next to the gas tanks. Write down the display temperature (should be ~ 18ºC) and water level.
4. Make sure the backup power supply unit, located in the service corridor, is operational. No warning messages should be displayed. Write down the percentage of backup power (usually 80%).
5. Check the system status panel of the E2 (top left). Make sure you see green LEDs as shown in Figure 6 on the following: TORCH IN POS, ARGON PRESSURE, INTERFACE COOL, HV ELECTRONICS, INTERLOCK

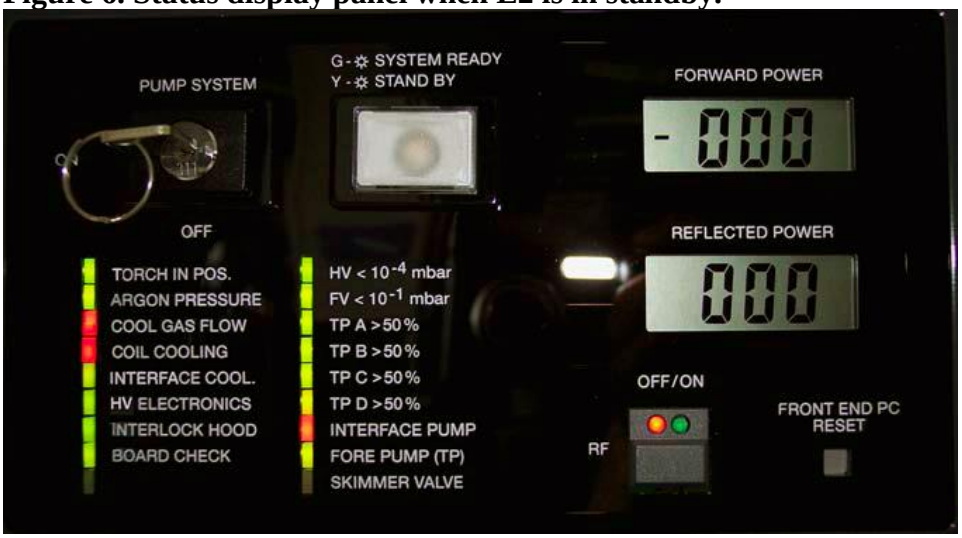
HOOD, BOARD CHECK, HV, FV, TP A, TP B, TP C, TP D, and FORE PUMP. If not, go to Section 15 for troubleshooting references.

6. Start the host computer and log on as "SEAS". Currently, the SEAS user group is set up for executing multi-elemental analysis of fine PM samples in a 0.2% nitric acid and 0.1% hydrochloric acid (v/v) matrix. Leave the password space empty and click LOGIN.
7. Open the folder labeled Thermo ELEMENT on the desktop.
8. Double click Network Processor, which will establish communication between E2's front-end computer and the host computer. This will automatically open and activate the EXECUTIVE task window.

9. Select EXECUTIVE task window, if not selected already, and click the CUSTOMIZE menu.

10. In AUTOSAMPLER settings, the ASX-510 should be selected and COM2 enabled.

Figure 6. Status display panel when E2 is in standby.



11. In INSTRUMENT settings, you should see the following:

DEADTIME: ACTIVE	FIELD REGULATOR TYPE: FAST
COOL GAS: 18 LPM	SEM TYPE: TYPE 2
ADDITIONAL GAS 1 AND 2: 1 LPM	SCAN OPTIMIZATION: MASS ACCURACY
RF GENERATOR: SEREN	NO OF PRE-SCANS: 0
MATCHBOX: SEREN	PELTIER COOLING: UNSELECT

If any settings differ, notify the laboratory manager and correct it.

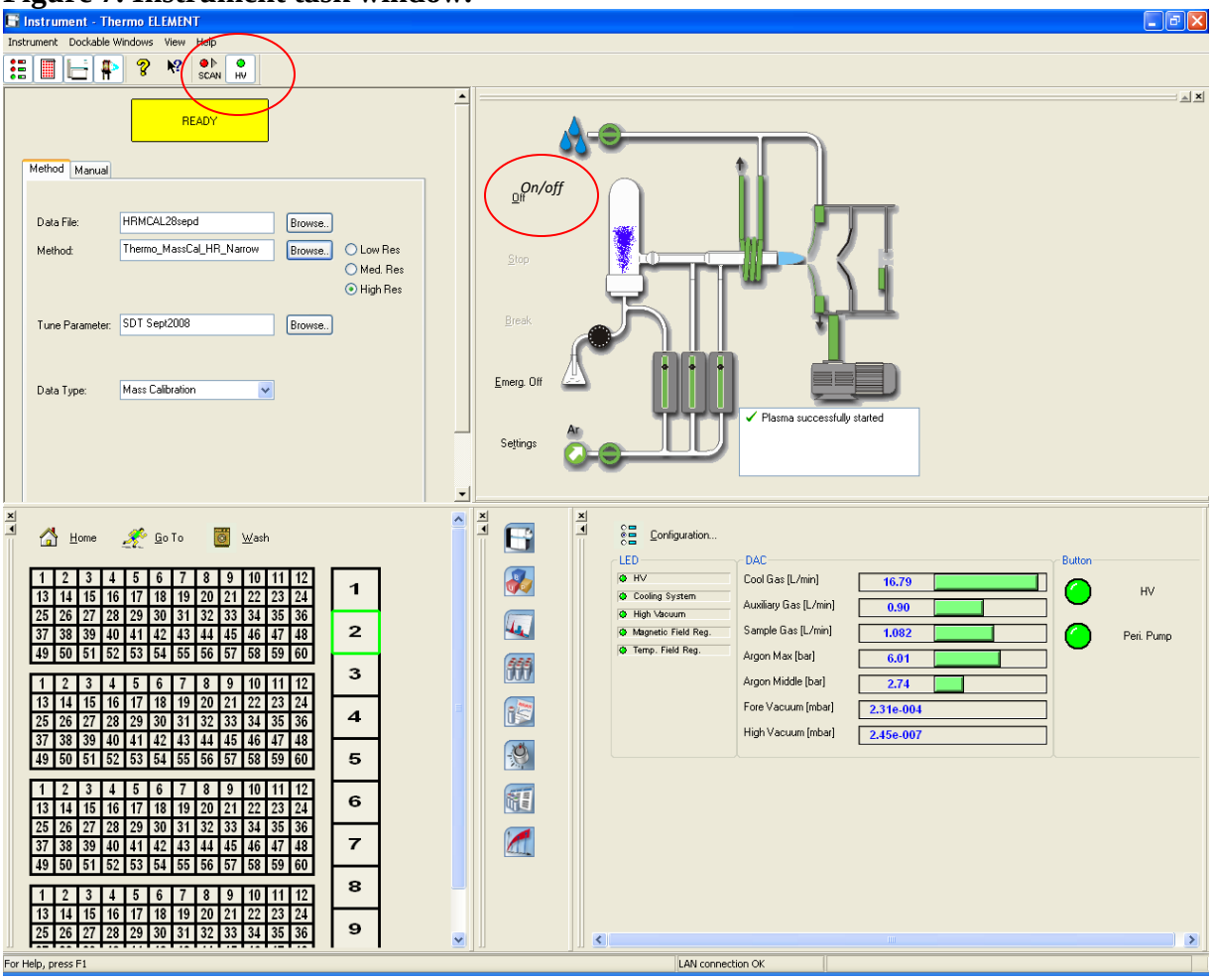
12. Connect sample (autosampler end) and internal standard (IS) line outlets to E2's first and second peristaltic pump channels using orange-green peristaltic tubings. Combine the outlet of tubings using a T-joint. Connect the perpendicular end of the T-joint to the nebulizer with green-coded (100 µL) capillary.

13. Connect the cyclone spray chamber drain to the peristaltic pump using gray-gray polypropylene tubing, and connect its outlet to the ICPMS rinse collector underneath the E2's sample tray.
14. Set the autosampler and IS sample inlet lines to draw reagent blank (0.2% HNO_3 and 0.1% HCl solution).

12.2 *Igniting Plasma*

1. Select the Thermo ELEMENT folder on the desktop and click the INSTRUMENT icon. The instrument task window is shown in Figure 7.
2. Start high voltage (HV) if not already on (see top left red circle in Figure7).

Figure 7. Instrument task window.



3. Under the Instrument dropdown menu, select "peristaltic pump turn c.w.," and then "peristaltic pump" on. E2's peristaltic pump should then rotate clockwise. Adjust tension knobs in the pump such that solutions from sample and IS lines flow smoothly. Also ensure that the cyclone continuously drains.
4. Start Plasma by clicking the ON button in the plasma schematic (top right

quadrant in the instrument task window (top middle red circle in Figure 7). Plasma will start after approximately 5 min. The following sequence should be observed during the ignition process: interface pump starts, RF generator starts, plasma appears, and sample gas slowly ramps up to set value.

Caution: Once the RF generator starts and "Forward Power" is displayed on the front status panel of the E2, plasma should have been lit. Otherwise, a condition called "Cold Plasma" has developed which can harm the torch. Under this circumstance, press the RF ON/OFF button on the status display panel to turn off RF power, and terminate the startup sequence by pressing the STOP button in the plasma schematic (see Figure 7).

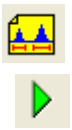
5. Record the High Vacuum pressure prior to plasma ignition.
6. After plasma ignition, fill in the fore and high vacuum readings in the Daily Startup Log (Appendix 3.1). ***Note:*** Typically, fore vac is on the order of 10^{-4} mbar and high vac is ~ 1.7×10^{-4} mbar. If the vacuum readings differ significantly, contact the laboratory manager.

At this point, the Daily Startup Log should be completed and the E2 successfully started. Next follow the instructions below for tuning the instrument.

12.3 *Tuning the Instrument*

1. Open the Tune task window. The most recent tune file (with extension .tpf) automatically loads. Otherwise, find an earlier day's file and load it (older tune files are stored in folder "C:\Element\user\SEAS\Tune Parameters\").

Note: If there have been hardware changes such as new cones, torch, or slit assembly, an extensive tuning will be required. Detailed tune operations can be found in chapter 2 of E2 manual version 3.



2. Press <F2> to open the Scan List menu (alternatively, click the yellow spectrum in the tool bar), and load THERMO_LR_TUNE.SCL. Press START SCAN (green button in the tool bar). You should now see Li, In, and U panes active. Make sure you are still sampling reagent blank through both channels.
3. Let the instrument warm up for approximately 30–60 min.
4. By default, tune parameters are locked for all resolutions. Make sure that "All Parameters Resolution Dependent" under the Tune menu is *not* enabled.
5. Set the autosampler probe (sample line) to the tune solution (refer to section 11), normally placed at position 00/002 of the ASX-510 autosampler. Select position 00/002 in the autosampler GUI (instrument task window) and click GO TO. The IS sample line should still sample reagent blank. The take-up time of tune solution by the ASX-510 setup is approximately 4 min.
6. Stop the scan and restart the LR tune for intensity and stability.
7. Check signal stability and intensity for Li, In, and U. The instrument specification (spec) for In is one million cps. If the performance is not satisfactory, check and adjust the sample gas (by opening slider controls with a right mouse click on the gas-flow box) and torch Z positions (in torch position box). If the performance is satisfactory, click Stop Scan (red square). Click the Save As button in the tool bar and create today's tune file. A

suggested format is yourlastname_ddmonthyyyy (e.g., pancras_12April2008).

8. Next perform MR tune for optimum peak shape and resolution. Open the scan list again and select Thermo_MR_Tune_Fe_ArO_Resolution.scl. Start the scan. Lower the y scale so the ^{56}Fe peak can be seen. The current resolution for MR is displayed at the top left of the intensity box. You may need to slightly adjust the Focus Quad 1 (FQ1) of the MR lens to achieve optimum resolution. For MR, the spec is 4000.
9. Save the tune parameters and proceed to HR tune.
10. Open the scan list and select Thermo_HR_Tune_K_ArH_Resolution.scl. Tune for high-resolution lenses.
11. If the mass separation between K39 and ArH38 is not satisfactory, adjust FQ1 of the HR lens such that an R value of 10,000 is achieved.
12. Once tuned, save the tune parameters under today's tune file name.
    At this point, E2 is ready for performance evaluation.

12.4 *Performance Evaluation*

1. Open the Sequence Editor task window. Make sure ALM on the Actions menu is enabled. A mass calibration is deemed valid when mass drift values do not exceed 500 ppm. The E2 operator can see current drift values by going to Executive/Log files/EDAC.
2. Use the Open button in the tool bar of the Sequence task window to open the sequence named "Daily Performance". The sequence settings can be viewed in two ways: spreadsheet (Figure 8) and GUI (Figure 9). The spreadsheet is recommended for setting up or editing and the GUI for verifying and running a sequence.

Figure 8. Spreadsheet view.

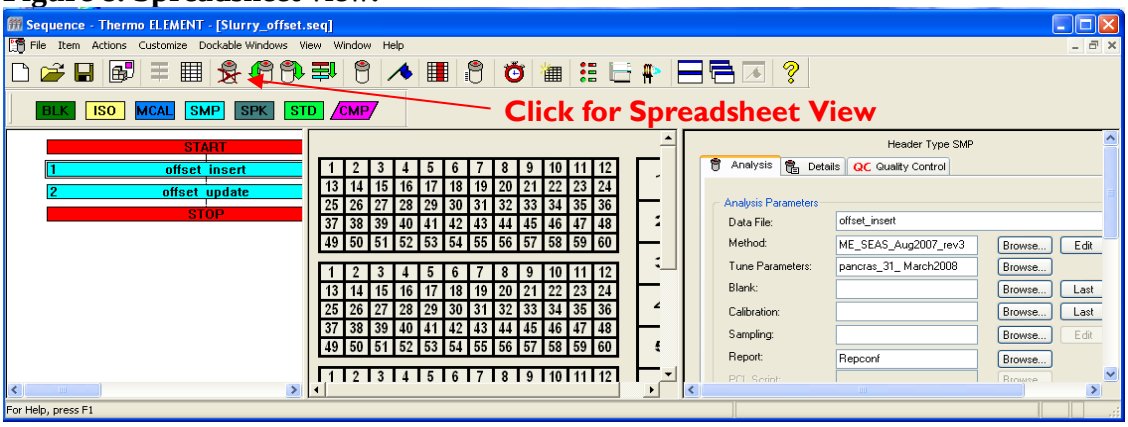
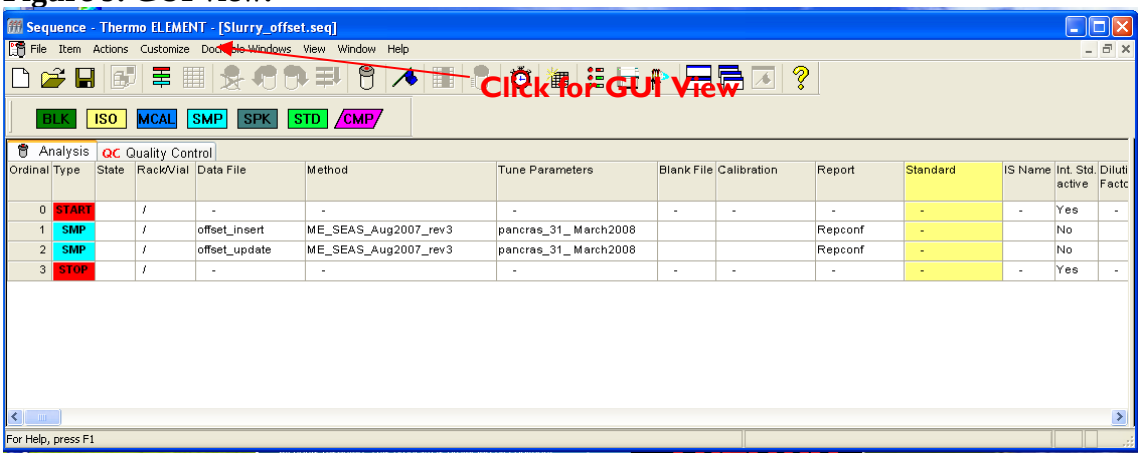


Figure 9. GUI view.

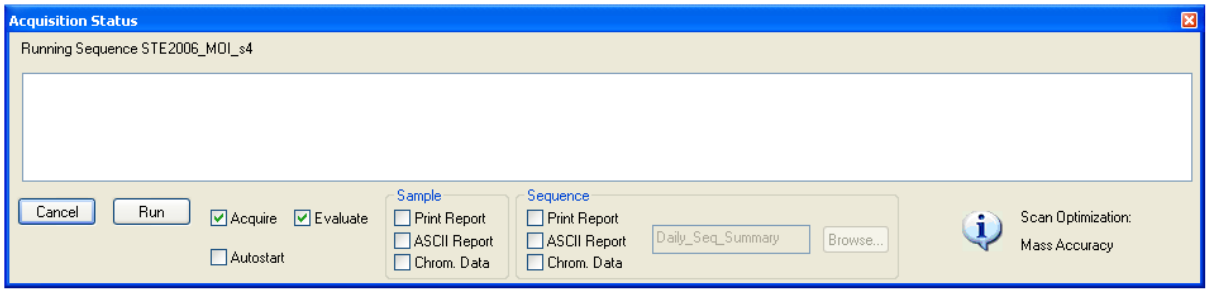


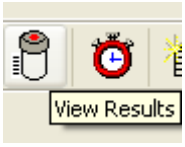
3. Switch to spreadsheet view, and replace the old tune file with the current file.

Note: The autosampler probe still samples tune solution, and the IS line samples reagent blank.

4. Click the Start Flag icon in the top center of the GUI page. This will open the Run task window (showing acquisition status) at the bottom of the Sequence task window (see Figure 10). Enable the Acquire, Evaluate, and ASCII Report boxes and browse to find the Daily_Seq_Summary sequence, and then click the Run button. This run takes 2.5 min to complete.

Figure 10. Run task window.





5. Once this sequence is completed, select the analyzed sample in the daily performance sequence, and then click View Results. This action will open the Results task window. Open the Daily Settings and Performance Log (Appendix 3.2), and fill in the available data from the Results page.
6. Compare your values with the established performance criteria (refer to Table 6). If your results do not meet these criteria, tune again until the set criteria are met.

Table 6. Tune Performance Criteria for NERL's E2

Parameter	Criteria	RSD	Resolution
In115 LR	>1,000,000 cps	< 1–2%	≥300
In115 MR	~8–12% of LR	< 2%	≥4000
In115 HR	~1–2% of LR	< 4%	≥10,000
Ba137O16/Ba137(LR)	< 0.5%		
Ba137++/Ba137(LR)	< 5 %		
U238O16/U238(LR)	< 20%		

Once the performance criteria are met, the E2 is ready for analysis.

12.5 *Sequence Start—Mass Offset*

Check mass offsets every time a new analysis sequence is started. Generally, mass offset values are method specific. Set the autosampler probe (sample line) to rinse for a few minutes to clean the sample lines, but use a mid-level calibration standard to check the offsets.

1. Open the Sequence Editor task window, and make sure ALM on the Actions menu is enabled. From the File menu, open the file "slurry_offset.seq".
2. Go to spreadsheet view, and type or browse by double-clicking the cell for the analytical method for which method offsets are to be computed (for SEAS sample analysis, the method is "MultiElement_SEAS_Aug2008"). Type or browse for the current tune file.
3. Click the Start Flag icon in the top center of the GUI page. This action will open a Run task window at the bottom of the Sequence task window. Enable the "Acquire" and "Evaluate" boxes if not already enabled, then click Run. Click "Continue" on the pop-up menu.
4. After the first sample is analyzed, a pop-up screen will ask if you want to continue to the next sample. Press the Stop sequence.
5. Select the sample that was just analyzed, and click the Results task window icon in the menu bar. The Results task window will open and display the results for the run just completed. Under the File menu in the Results window, click "Update the offset values from the results into the active method file" and press "OK."
6. Close the Results task window, and start the sequence again. Now monitor peak positions of the second sample in the Show Task window while the sample is being analyzed. This provides visual proof that the centroid of a mass peak is now centered within the chosen mass range.

At this point, mass offsets are computed and inserted into the analytical method. Next an analytical sequence is created from an existing sequence. This template is specific for the ASX-510 autosampler.

12.6 *Sequence Start—Analysis*

1. In the Sequence task window, open the sequence

"Slurry_sample_Seq_Template" by choosing File/Open as Template/.

2. Go to the spreadsheet view and change sample names and method files as needed. Follow Table 7 to fill in the needed columns/cells. You can select and drag cells to fill all of the rows in a column.
3. Standard files are named WS0, WS1, WS2, WS3, WS5, and WS6. Corresponding standards concentration tables are created and stored in C:\Element\user\SEAS\Standards\folder.
4. This sequence has autosampler positions, so therefore the sequence automatically starts when the Run button is pressed.

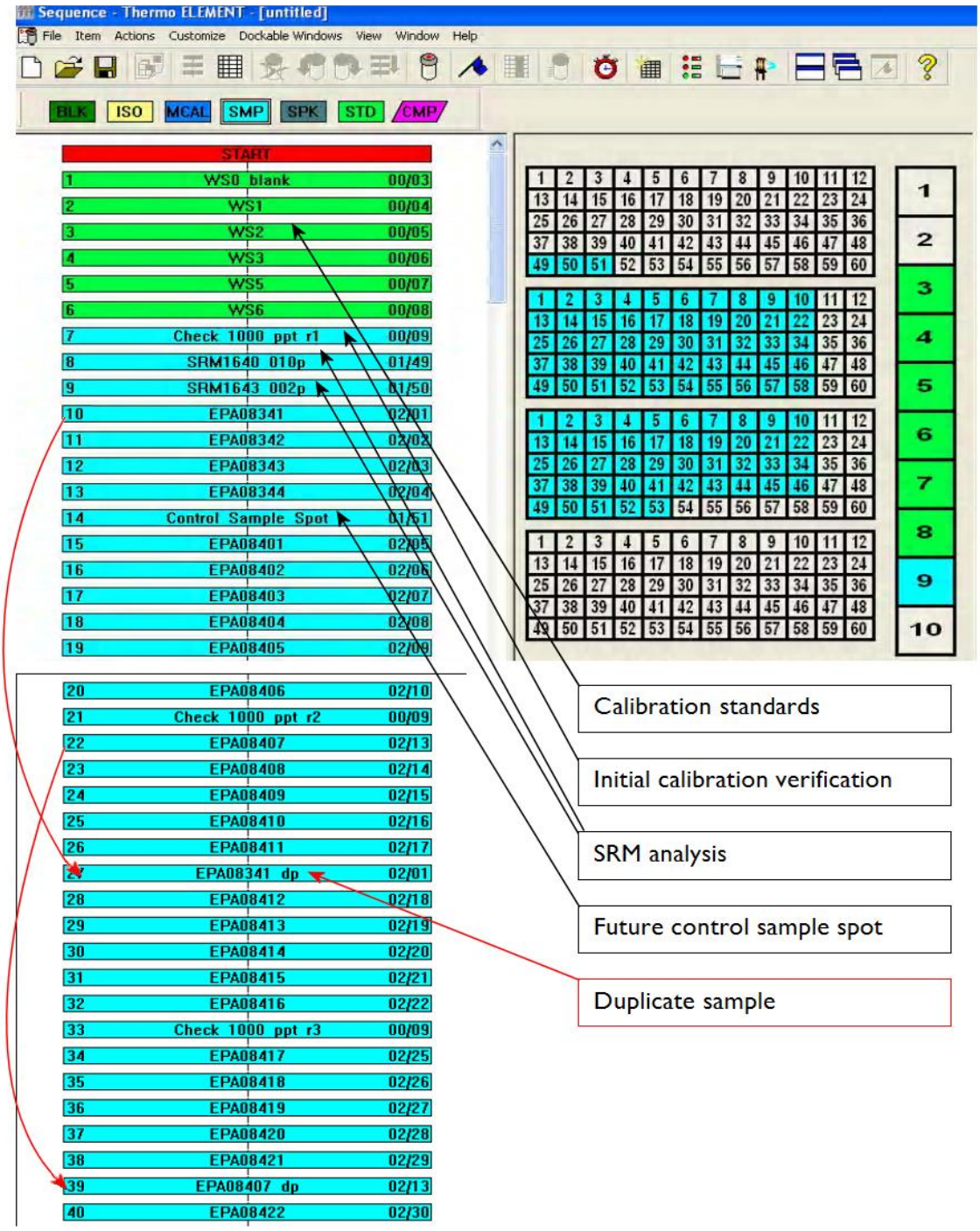
Table 7. Sequence Editor Definitions

Column	Fill-in Instructions	Typical Entries
Rack/Vial	xx/yyy, where xx is rack number and yyy is position number	01/002
Data File	Sample name	DBE00232
Method	Method name	MultiElement_SEAS_Aug2008
Tune Parameter	Today's tune file	pancras_18_ Aug2008
Blank File	None	
Calibration	Today's date	5Aug2008
Report File	*Must use* this custom designed file	Sample_Specific_Report
Standard	Standard file names	WS1
Internal Standard	Internal standard file name	In_Ir_IS_2ppb_Aug2008
Int. Stand. Active	Status	Yes
Dilution Factor	None	
Take-up Time	Time for sample transportation	5
Unit Take-up Time		Min
Wash Time	Varies	30–60 s
Unit Wash		Min
Quantification Type	Must use ext.calib	Quant. (EXT CALIB)
Pump Speed	Optimized rpm	5
Is before bs	None	

5. Figure 11 shows a typical sequence setting with quality control (QC) samples. Wherever "_dp" or "Check_" exists in the sample name, those samples are not to be replaced with a new sample. Those are the spots for QA/QC checks. Carefully follow Figure 11 to properly select/label samples for duplicate analysis. Check samples are placed at position 00/009 of the ASX-510 and are analyzed after every 11 samples, where the sixth sample is a duplicate sample.
6. Click the Start Flag icon to open a Run task window. Enable the "Acquire" and "Evaluate" boxes if not already enabled. Make sure the appropriate sequence file is selected to store the sequence summary of your analytical results. Presently the file "Daily_Sequence_Summary" is in use.

7. Click “Run” to start the sequence.

Figure 11. Sequence Editor with QC samples.



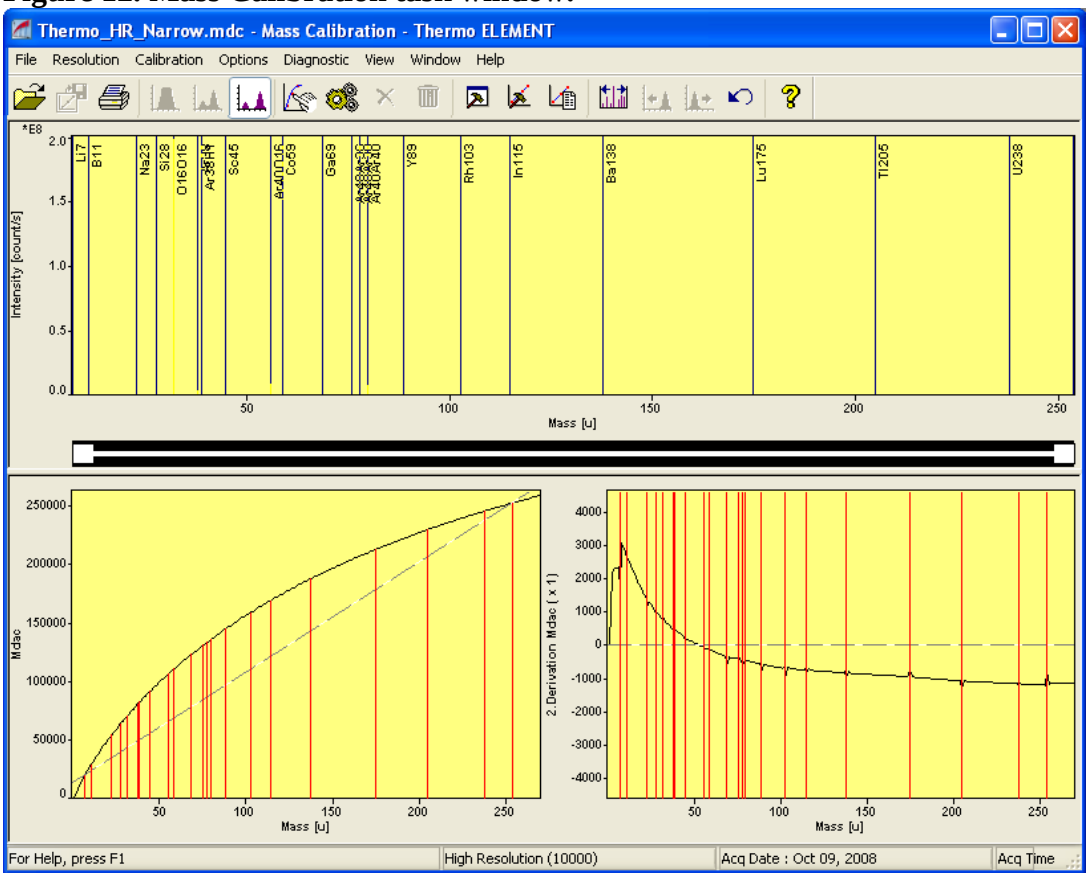
13.0 NEED-BASED PROCEDURES

13.1 *Mass Calibration*

Mass calibration leads to correct identification of mass numbers of the mass peaks of interest. The mass calibration can be executed either manually from the Instrument task window or automatically from the Sequence task window. Only the automatic method is outlined in this SOP.

The Mass Calibration task window (see Figure 12) is used to display the data acquired for the purpose of mass calibration. There are three display panels: mass spectrum (upper), MDAC vs. mass calibration curve (lower left), and second derivative diagnostic curve (lower right). The second derivative curve should be viewed carefully as large spikes in the curve indicate poor calibration. A smooth "L" curve shape with small spikes indicates a good calibration.

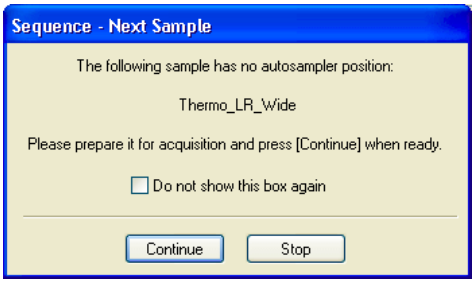
Figure 12. Mass Calibration task window.



Perform the automatic mass calibration from the Sequence Editor after a good tune. Follow the steps below to execute mass calibration. Tune solution should be aspirated during a mass calibration procedure. This procedure assumes that the E2 instrument has not been shut down lately.

1. Open the Sequence Task Editor.

2. Load the calibration sequence file Thermo_Automasscal.seq (located by default in directory C:\Element\SEAS\data\). This sequence contains six samples, each with a unique method to cover all three resolutions with two different mass window settings (wide and narrow).
3. The six samples in this sequence are named LR_Wide, LR_Narrow, MR_Wide, MR_Narrow, HR_Wide, and HR_Narrow and are designated as MCAL samples. Click the Reset icon in the tool bar and select "All" from the dialog box.
4. Choose the current tune file for all six analyses.
5. Click the Start icon in the tool bar to check and start the sequence. This action will open the acquisition window. Because no reports are required from the auto mass calibration sequence, all output check boxes should be cleared. Make sure the boxes "Acquire" and "Evaluate" are checked!
6. Click the Run button in the acquisition window. After an automatic check, the following message appears: "Following sample has no autosampler position." Click Continue in the pop-up screen. ***Note:*** The check box "Do not show this box again" in the pop-up window remains unchecked so that an analysis can be verified for accuracy once it is completed.



7. After LR_Wide is completed, click Continue in the "Sequence – Next Sample" task window. Once LR_Narrow (LR with narrow peak window) is completed, click Stop in the "Sequence – Next Sample" task window.
8. Open the Mass Calibration task window. After verifying the information in Section 13.1.1 (diagnostics of LR calibration), press the Gears button, click "yes" to "delete current calibration" and then Files>Save and return to the Sequence Editor.
9. Continue the sequence until MR Wide and Narrow samples are analyzed. Then click Stop and go to the Mass Calibration task window. After verifying the information in Section 13.1.1, save the file and return to the Sequence Editor.
10. Continue HR_Wide and Narrow. Save.
11. The mass calibration is now completed. Go to Section 13.1.2.

13.1.1 *Diagnostics of a Mass Calibration*

1. In the tool bar of the Mass Calibration task window, click on the "Open Analysis" icon to show the browsing dialog.
2. Open subdirectory Thermo_Automasscal. Find the acquired file for the latest (just completed) analyses.

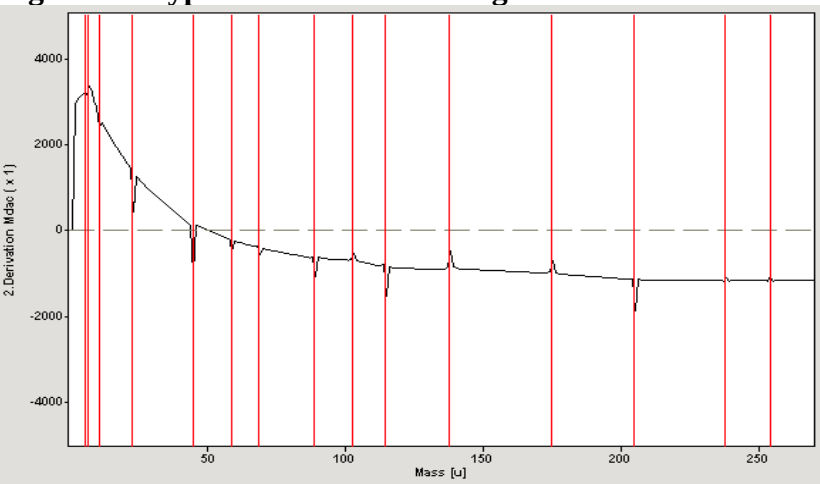
Diagnostic Curve for LR Calibration

Figure 13 shows a typical LR 2nd deriv. diagnostic curve. Notice the following features in the figure:

- There are two spikes at low mass: the Li isotopes ^{6}Li and ^{7}Li seem to be overlapping in the LR plot, which is normal.
- There is a large gap between *m/z* 23 and 45: this causes spiking for both isotopes, which is normal and can be ignored.
- The large spike at *m/z* 45 is normal: this is caused by the inaccuracy at *m/z* 45 due to polyatomic ions (for example $^{12}C^{16}O_2H$ or $^{14}N_2$ ^{16}OH) at the same nominal mass as Sc.

If you see any incorrect identification of peaks go to section 13.1.2 to fix it.

Figure 13. Typical LR 2nd deriv diagnostic curve.



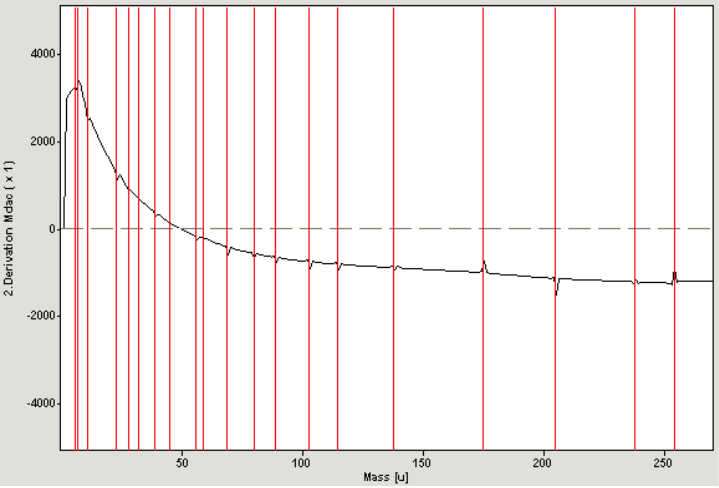
Diagnostic Curve for MR Calibration

Figure 14 shows a typical MR diagnostic curve. The following should be noted from this figure:

- There are two spikes at low mass: the Li isotopes ^{6}Li and ^{7}Li seem to be overlapping in the plot, which is normal.
- There are more points at lower masses: more calibration points are added in the low mass range to ensure mass accuracy.
- The biggest spike is $^{238}U^{16}O$ at *m/z* 254: the counts for $^{238}U^{16}O$ are low in MR so its spectral peak is not well defined. This leads to a higher deviation than for other masses, which is normal.

If you see any incorrect identification of peaks go to Section 13.1.2 to fix it.

Figure 14. Diagnostic curve for MR.



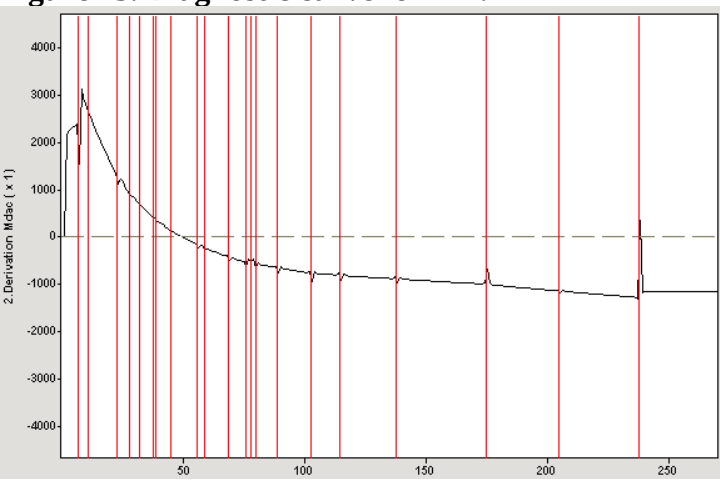
Diagnostic Curve for HR Calibration

Figure 15 shows a typical HR diagnostic curve. In this figure, notice the following:

- There is a large spike at low mass: only ^{7}Li is calibrated in HR as the ^{6}Li count rate is low.
- The spike between ^{7}Li and 11B is normal in HR.
- There is some spiking around *m/z* 80: to ensure an accurate calibration for As and Se, the Ar dimer background interferences at *m/z* 76, 78, and 80 are used as mass calibration points in HR. As these masses are closely spaced, some small spikes may be seen, which is normal.

If you see any incorrect identification of peaks go to Section 13.1.2 to fix it.

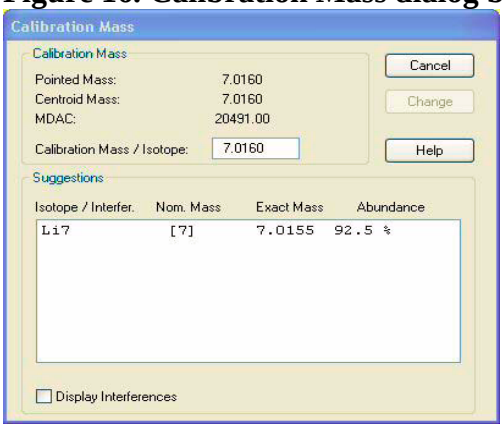
Figure 15. Diagnostic curve for HR.



13.1.2 Check All Masses

1. Click the Manual Calibration icon in the Mass Calibration task window. It will display a list of calibrated masses.
2. Select the first entry in the list. Double click it to zoom to it in the spectrum field. The mass range around the calibrated mass is displayed. The mass marker should be centered on the peak. If the mass is correctly calibrated, move on to the next entry.
3. If the mass marker is not correctly assigned (not centered on the peak), delete this reference point by clicking on the Delete icon.
4. To assign a new reference point, center the mouse over the peak in the spectrum and double click it. A Calibration Mass dialog appears as shown in Figure 16.

Figure 16. Calibration Mass dialog box.



5. Select the correct isotope/interference from the Suggestions list. (***Note:*** Only elemental peaks are initially displayed. To display the interferences, the option box at the bottom of the dialog must be activated.)
6. Click on Add to add the new reference point to the calibration, and click Save to save the new calibration.
7. Check all masses in the list and remember to save the file at the end.

The most commonly miscalibrated masses are between 6 and 70 amu. Comparing isotope ratios or widening the display task window will aid in the identification of problem isotopes such as the following:

- $^{6}Li/^{7}Li$: Use the natural abundance Li isotope pattern, 7.5/92.5, to identify the Li isotopes.
- ^{28}Si: The ^{28}Si peak is to the left of the two interfering polyatomic species.
- $^{16}O_2$: The count rate for $^{16}O_2$ at *m/z* 32 is high, often close to the detector maximum of $5.0*10^{9}$ cps.
- ^{45}Sc: The ^{45}Sc peak is to the left of the interfering polyatomic species.
- ^{69}Ga: The ^{69}Ga peak is to the left of the interfering Ba^{2++} species. A high Ba^{2++} signal indicates non-optimum tuning.

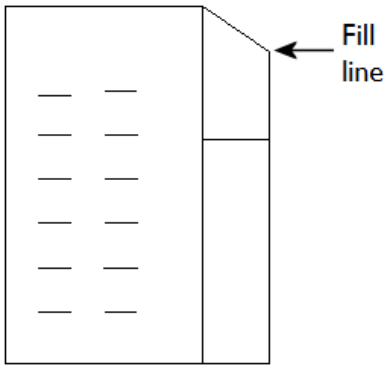
Reinitialization of mass calibration may be required if E2 was shut down lately. This process starts using a default mass calibration, saved in the host computer, and eventually builds a new valid calibration. E2 users should consult the laboratory manager if such a need arises.
Perform the following steps to reestablish mass calibration:

1. Open the default masscal file, and run the LR_Wide analysis.
2. Delete the old calibration, and save the new one.
3. Reset MR and HR calibrations.
4. Start the MR samples.
5. Fix mass peaks as needed.
6. Save and proceed to HR.
7. When done with the masscal, save the HR_Narrow as your new default file.

13.2 *Change Water in Recirculating Chiller*

Note: Use RO water only; *do not* use DI water. It is recommended that you take RO water from D461-B (acid clean lab). A kit with all needed tools is placed on top of the chiller unit.

1. Turn off the chiller.
2. Disconnect the top hose from the chiller with a screwdriver. Clamp the hose to the drain pan and cap the valve on the back of the chiller.
3. Open the reservoir on top of the chiller, unscrew the cap, and place a funnel into the container.
4. Turn on the chiller.
   - Should start to recirculate water to the pan.
   - Start filling water through funnel to flush the system.
   - Chiller may get loud periodically.
5. Turn off the chiller.
6. Pour the drain pan contents into the sink.
7. Reconnect the recirculating hose.
8. Fill reservoir to fill line as shown.
9. Turn on the chiller and ensure there are no leaks.



13.3 *Change Entrance Slit Assembly*

Locate the following tools and accessories before beginning the procedure:

- Torque wrench (comes with the E2 instrument)
- Half-cut 1-mL pipette tip to hold screw
- Flat-type long-stem screwdriver
- Lint-free paper towels
- Powder-free vinyl gloves

Follow step by step the document titled "Slit_exchange_info_from_E2_Maintenance_ Course.pdf," published by Thermo Fisher and found in the laboratory manual holder or in the host computer in the My Documents folder. After completion, turn on the E2 and let it pump down overnight. Make sure the high vacuum is in the order of 10^{-7} mbar before using the E2 or activating the High Voltage on the magnet.

Special note: Perform a mass calibration after a new entrance slit assembly is in place.

13.4 *Maintenance of Sample and Skimmer Cones*

The sample and skimmer cones require cleaning and eventually replacement after prolonged usage. It is important not to damage the tips of the cones during the cleaning process because the ion extraction will be degraded. Follow the steps below to clean them:

1. To remove the sample cone, line up the cone removal tool with the cone locking ring and unscrew one full turn. Insert the magnet into the tool and unscrew the locking ring completely (the magnet will capture the sample cone).
2. To remove the skimmer cone, loosen each Allen screw only one turn. Carefully insert the magnet such that it does not touch the tip of the cone, place it on the skimmer and rotate the skimmer until the notches match the screw positions, and then withdraw the skimmer. The skimmer valve plate will be visible in the skimmer mounting orifice.
3. After removing the cones, place them in two separate clean plastic beakers such that the orifices face upwards. Cover the cones with ultrapure water and sonicate for ~10 min. Rinse with ultrapure water.
4. Mount the cleaned skimmer cone onto the magnet tool, taking care that the magnet does not touch the tip of the cone.
5. Insert the skimmer into the mount and turn it until the notches no longer line up with the Allen screws. Retighten the Allen screws.
6. Before placing the sample cone, replace the graphite seal (O-ring).
7. Place the threaded locking ring on the sample cone and insert the magnet. Insert the sample cone into the front plate. Use the tool to screw the locking ring into the front plate. When tight, remove the magnet and hand tighten further.

14.0 TROUBLESHOOTING

Observation	Cause	Fix
TP A, B, C, or D is red	One of the turbomolecular pumps is not operational or is malfunctioning	Rebuild or replace the pump. Refer to hardware manual.
HR intensity of In is less than 0.7% of its LR intensity	The entrance slit assembly is bad	Replace it. Refer to the tutorial file in the host computer.
HV pressure changes while a scan is in progress or when the entrance slit switches between MR to LR or MR to HR	Bad entrance or exit slit. Place a hemostat or hold-down clamp at the exit gas line. If HV does not change when switching from MR to LR, then it is a bad exit slit.	Change exit slit assembly. Not recommended at the user level. Place a service call.

15.0 PREVENTIVE CARE

15.1 *Daily*

The following maintenance procedures need to be addressed daily:

- Check the sample waste container level.
- Inspect the argon tank supply and its pressure to the instrument.
- Inspect the nebulizer for clogs.
- Inspect the torch and aerosol injector tubes.
- Inspect the sample capillary tubing to be sure it is clean and in good condition.
- Check the peristaltic pump tubing before operation.
- Check the spray chamber for large droplets, indicating the unit needs cleaned. Soak well in 10% (v/v) HNO_3 for 2 hours, rinse well with reagent water.

15.2 Weekly (Refer to the Weekly Maintenance Log, Appendix 3.3)

Inspect the fore pump and interface pump oil levels and color weekly.

15.3 *Monthly (Refer to the Monthly Check List, Appendix 3.4)*

Perform the following on a monthly basis:

- Change the RO water in the chiller unit. Refer to Section 13.2.
- Inspect the RF coil for pits or holes.
- Make sure the peristaltic pump rollers are clean, and remove and clean the

pump head as necessary.

15.4 *Annual (Refer to Yearly Check List, Appendix 3.5)*

A preventive maintenance visit is due from Thermo on an annual basis as part of the service contract. The following services will be performed:

- Skimmer valve O-ring change and lubrication
- Fore pump and interface pump oil change
- Interface oil mist chamber cleanup and filter change

All maintenance activities should be documented on the Yearly Check List.

15.5 *Usage Dependent (Refer to the Usage Dependent Log, Appendix 3.6)*

Experienced analysts can perform the following usage-dependent maintenance:

- Entrance slit assembly change
- Guard electrode change
- RF coil change
- Skimmer and sample cone change
- Torch and sample tube cleanup or maintenance

16.0 WASTE MANAGEMENT

16.1 The analyst is responsible for ensuring the safe storage and disposal of all analytical standards and reagents associated with this method.

16.2 The analyst is responsible for notifying the laboratory manager of disposal needs.

16.3 The analyst is responsible for preserving/storing analyzed samples for future verification.

17.0 DOCUMENTATION AND DOCUMENT CONTROL

17.1 All information concerning sample preparation, standard preparation, instrument conditions, etc., must be written in the analyst's notebook. Any unusual problems or conditions must also be noted.

17.2 Record all maintenance performed on the instrument in the maintenance logbook for this particular instrument.

17.3 Record all analyses including QC samples performed by the instrument in the logbook for this particular instrument.

17.4 Back up analysis data on a weekly basis. Copy the following folders and files to a CDr: a) Sequence data folder, b) Sequence file (*.seq), c) Method file (*.mth), and d) Standards files (.std). Place the CD in a labeled jewel case or paper sleeve,

and deliver the data to the laboratory manager. The CD label should include the date, your name, and a very brief description of the data inside.

18.0 QUALITY CONTROL

In accordance with EPA Method 6020, the multi-element determination of samples by ICPMS, the following practices are undertaken to ensure the highest quality analytical data.

18.1 All QC data are stored permanently and are easily available for reference or inspection.

18.2 A standard traceability record book is kept with certificates of analysis of all primary standards in use.

18.3 An Analytical Calibration Traceability Log (see Appendix 3.7) is kept with expiration dates of primary analytical calibration standards and dates of preparation of stock solutions A, B, and S.

18.4 Instrument detection limits are calculated every three months and kept in the instrument logbook.

18.5 Analytical calibrations are deemed valid only when the regression coefficient (R^2) value exceeds 0.999.

18.6 An initial calibration verification standard and a reagent blank sample are run right after a new calibration.

18.7 Accuracy of analytical calibrations is verified by analyzing Standard Reference Materials such as SRM1643 and SRM1640. If measurements exceed ±15% of the certified elements, current calibration is invalidated. New calibration will be established after the cause for QC failure is identified and corrected.

18.8 To obtain data of known quality in all resolution settings, at least one isotope is analyzed in all resolution settings, where applicable.

18.9 Validity of the existing calibration is verified at a frequency of every 10 analyses. The instrument check standards must agree within ±15%.

18.11 Duplicate samples are analyzed: One in every 10 samples in an analytical sequence is reanalyzed as a new sample. A relative percent of difference (RPD) of less than 20% is the tolerance criteria for reanalysis. RPD is calculated as

$$\left(\frac{|C_1 - C_2|}{(C_1 + C_2)/2}\right) \times 100,$$

where C_1 is the first analysis concentration and C_2 is the second analysis (duplicate sample) concentration.

18.12 Intensities of internal standards are monitored in every analysis. When the intensity of an IS in a sample analysis fails to fall between 80% and 120% of its initial (or reference) value, those samples can either be re-analyzed or diluted and reanalyzed.

18.13 Dilution tests are carried out when an analyte lies outside the established calibration region.

The current version of the E2 software does not support all of the QC steps mentioned above on-line. Use the stand-alone VBA application macro, called “ICPMS Data

Flagger," to flag analytical results after a sequence is completed and the sequence report is generated. Appendix 4 describes how to install and use the ICPMS Data Flagger application.

19.0 REFERENCES

All referenced manuals are located in the blue file holder next to the host computer of E2.

- Finnigan ELEMENT2 Operating Manual (p/n 1091281).
- Finnigan ELEMENT2 hardware manual.
- Finnigan Application Notes: Peak Search Algorithm, Equations Used in E2, and Sequence Reevaluation.
- Interface and Turbo Pump Manuals
- EPA Method 6020: *Inductively coupled plasma – mass spectrometry,* USEPA, Sept. 1999, p1-18.
- ASX-500 Model 510 Auto Sampler Operator's Manual, CETAC Technologies, Omaha, NE, Version 1.0, Rev. 4, April, 2002.
- *Inductively Coupled Plasma Mass Spectrometry Handbook,* Simon Nelms (Editor), Thermo Elemental, Cheshire, UK, Blackwell; 1st edition (October 21, 2005)

Appendix 1: Element 2 HR-ICPMS Method Settings

Instrument Settings:	
RF power	1200–1260 W
Gas flow rates:	
Cool	16 lpm
Auxiliary	0.9–1.0 lpm
Sample	0.96–1.20 lpm
Sample update rate	~100 µL/min
Sampler cone (Pt)	1.1-mm orifice diameter
Skimmer cone (Pt)	0.8-mm orifice diameter
Nebulizer	100-µL Teflon microneb
Spray chamber	Air-cooled cyclone
Detector dead time	30 ns
Internal standard solution	2.0 ppb solution of In115 and Ir193

Isotopes

Low resolution (LR)	Li7, Be9, Rb85, Sr88, Y89, Mo95, Rh103, Pd105, Ag107, Cd111, Sn118, Sb121 Cs133, Ba137, La139, Ce140, Nd146, Sm147, Gd157, Dy163, W182, Pt195, Tl205, Pb206, Pb207, Pb208, Bi209, Th232, U238, (In115, Ir193)
Medium resolution (MR)	Na23, Mg24, Al27, Si28, P31, S32, Ca44, Sc45, Ti47, V51, Cr52, Mn55, Fe57 Co59, Ni60, Cu63, Zn66, Sn118, (In115, Ir193)
High resolution (HR)	K39, Ge72, As75, Se77, Sn118, (In115, Ir193)

Acquisition Parameters

Resolution	**Low**	**Medium**	**High**
Mass task window, %	100	125	150
Samples/peak	30	20	15–20
Sample time/ns	10	20–50	100–500
Scan type	E Scan	E Scan	E Scan
Detector mode (analog/counting)	Both	Both	Both
No. replicates (runs)	3	3	3
No. scans per replicate (pass)	2	2	2

Evaluation Parameters

Resolution	**Low**	**Medium**	**High**
Search task window, %	100	100	80–100
Integration task window, %	40	60	60–70
Integration type	Ave	Ave	Ave
Calibration type	Linear	Linear	Linear
Internal standard (In/Ir)	Indium	Indium	Indium

Appendix 2: Concentrations of Individual Elements in Working Calibration Standards

Element	ws0_ppt	ws1_ppb	ws2_ppb	ws3_ppb	CheckStd	ws5_ppb	ws6_ppb
Na	0	0.400	4.000	10.000	20.000	30.000	100.000
Mg	0	0.400	4.000	10.000	20.000	30.000	100.000
K	0	0.400	4.000	10.000	20.000	30.000	100.000
Ca	0	0.400	4.000	10.000	20.000	30.000	100.000
Si	0	0.400	4.000	10.000	20.000	30.000	100.000
Al	0	0.120	1.200	3.000	6.000	9.000	30.000
Fe	0	0.120	1.200	3.000	6.000	9.000	30.000
Zn	0	0.120	1.200	3.000	6.000	9.000	30.000
Ba	0	0.120	1.200	3.000	6.000	9.000	30.000
Ag	0	0.020	0.200	0.500	1.000	1.500	5.000
As	0	0.020	0.200	0.500	1.000	1.500	5.000
Be	0	0.020	0.200	0.500	1.000	1.500	5.000
Bi	0	0.020	0.200	0.500	1.000	1.500	5.000
Cd	0	0.020	0.200	0.500	1.000	1.500	5.000
Ce	0	0.020	0.200	0.500	1.000	1.500	5.000
Co	0	0.020	0.200	0.500	1.000	1.500	5.000
Cr	0	0.020	0.200	0.500	1.000	1.500	5.000
Cs	0	0.020	0.200	0.500	1.000	1.500	5.000
Cu	0	0.020	0.200	0.500	1.000	1.500	5.000
Dy	0	0.020	0.200	0.500	1.000	1.500	5.000
Gd	0	0.020	0.200	0.500	1.000	1.500	5.000
Ge	0	0.020	0.200	0.500	1.000	1.500	5.000
La	0	0.020	0.200	0.500	1.000	1.500	5.000
Li	0	0.020	0.200	0.500	1.000	1.500	5.000
Mn	0	0.020	0.200	0.500	1.000	1.500	5.000
Mo	0	0.020	0.200	0.500	1.000	1.500	5.000
Nb	0	0.020	0.200	0.500	1.000	1.500	5.000
Ni	0	0.020	0.200	0.500	1.000	1.500	5.000
P	0	0.020	0.200	0.500	1.000	1.500	5.000
Pb	0	0.020	0.200	0.500	1.000	1.500	5.000
Pd	0	0.020	0.200	0.500	1.000	1.500	5.000
Pt	0	0.020	0.200	0.500	1.000	1.500	5.000
Rb	0	0.020	0.200	0.500	1.000	1.500	5.000
Rh	0	0.020	0.200	0.500	1.000	1.500	5.000
Sc	0	0.020	0.200	0.500	1.000	1.500	5.000
Sb	0	0.020	0.200	0.500	1.000	1.500	5.000
Se	0	0.020	0.200	0.500	1.000	1.500	5.000

Element	ws0_ppt	ws1_ppb	ws2_ppb	ws3_ppb	CheckStd	ws5_ppb	ws6_ppb
Sm	0	0.020	0.200	0.500	1.000	1.500	5.000
Sn	0	0.020	0.200	0.500	1.000	1.500	5.000
Sr	0	0.020	0.200	0.500	1.000	1.500	5.000
Tb	0	0.020	0.200	0.500	1.000	1.500	5.000
Ti	0	0.020	0.200	0.500	1.000	1.500	5.000
Th	0	0.020	0.200	0.500	1.000	1.500	5.000
Tl	0	0.020	0.200	0.500	1.000	1.500	5.000
U	0	0.020	0.200	0.500	1.000	1.500	5.000
V	0	0.020	0.200	0.500	1.000	1.500	5.000
W	0	0.020	0.200	0.500	1.000	1.500	5.000
Y	0	0.020	0.200	0.500	1.000	1.500	5.000
S	0	4.000	40.000	100.000	200.000	300.000	1000.000

Appendix 3: Laboratory Forms and Log Sheets

Appendix 3.1. Daily Startup Log

Date/User	Backup Power	Chiller Temp	Ar Gas	Skimmer Cones	Torchbox Exhaust	PeriPump Tubing Condition	Plasma Start Time	High Vacuum, Skimmer Valve Closed	after plasma on, valve open and sample aspiring		Plasma End Time

Appendix 3.2. Daily Setting and Performance Log

Date								
Torch								
x								
y								
z								
Gas Flows								
C								
A								
S								
Power (watts)								
Lenses (v)								
E								
F								
x-D								
y-D								
Sh								
LR Lenses								
Q1R								
Q2R								
Q1F								
MR Lenses								
Q1R								
Q2R								
Q1F								
HR Lenses								
Q1R								
Q2R								
Q1F								
LR Check								
Li (cps)								
In (cps)								
U (cps)								
Oxides Check								
BaO (%)								
Ba++ (%)								
UO (%)								
MR Check								
Co (resolution)								
In (resolution)								

In (cps)								
HR Check								
Ar38 (resolution)								
ArAr (resolution)								
In (resolution)								
In (cps)								
SEM Voltage								
Application								
User								

Appendix 3.3. Usage Dependent Log

Date	Item Replaced	Cause of Change	Operator Initials

Appendix 3.4. Analytical Calibration Traceability Log

Stock A / Stock B:
Date prepared:
Prepared by:

Standard	Concentration	Lot No.	Exp. Date	Final Conc.	Comments
Li					
Be					
Na					
Mg					
Al					
P					
S					
K					
Ca					
Sc					
Ti					
V					
Cr					
Mn					
Fe					
Co					
Ni					
Cu					
Zn					
As					
Se					
Rb					
Sr					
Mo					
Ag					
Cd					
In					
Sn					
Sb					
Cs					
Ba					
La					
Ce					
Nd					
Sm					
Gd					
Dy					
W					

Tl					
Pb					
Bi					
Th					
U					
Si					
Ge					
Rh					
Pd					
Pt					

Appendix 4: ICPMS Data Flagger

To view the analytical results easily, the data in the sequence summary file created by the current software version needs major rearrangement. In addition, the sequence output does not have data quality indicators (flags) for the end user to evaluate an analytical result. Therefore, we developed a VBA application macro that runs in MS Excel for post-processing a sequence summary file. This application does the following:

- Reads the sequence summary file from a drop-down menu
- Reads appropriate instrument detection limit (IDL) values file from a drop-down menu
- Formats species name and rearranges data such that each row contains one sample with all associated analysis information in columns
- Evaluates and introduces flags for below instrument detection concentrations, check sample analysis, SRM sample analysis, and duplicate sample analysis.
- Outputs analytical concentration data, intensity data, and QC sample data separately.

Installation

This application has already been installed in E2's host computer. To install it in another computer, copy the "ICPMS Data Flagger.EXE" file from the E2 host computer (C:\Pancras\..) onto removable media and transfer it to the new computer. Double click the EXE icon to initiate installation, and follow on-screen instructions. This installation creates folder C:\Flag_Program\ and copies the needed folders and files to run the application. The following folders are typically installed:

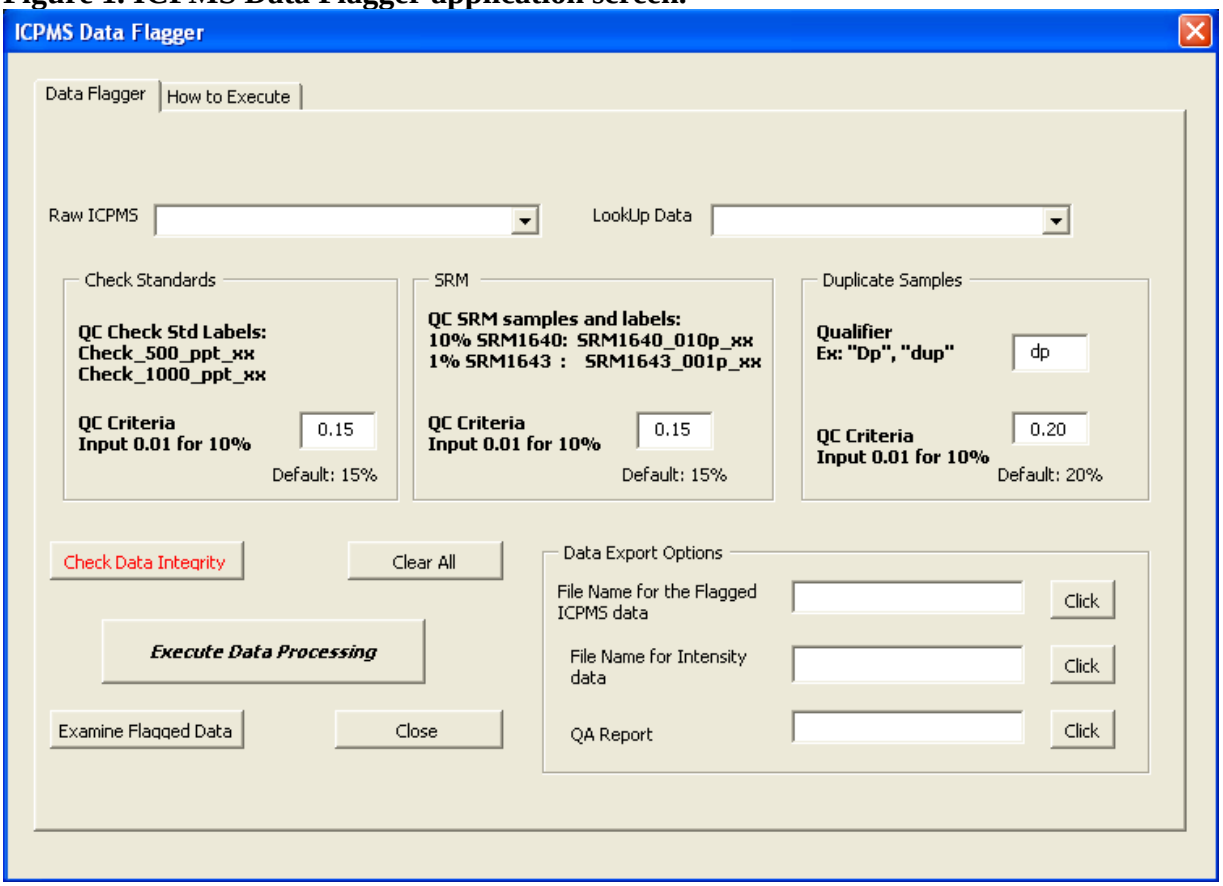
C:\Flag_Program\In\
C:\Flag_Program\Out\

Installation also creates a shortcut icon on the desktop to run the application. It is the user's responsibility to update IDL information in the lookup file, "LookUp_IDL.xls", in the C:\Flag_Program\Out\ folder.

Application

1. Find the .CSV sequence report file in the sample sequence folder. The sequence report file is created and saved by its sequence name (but with a .CSV extension). Copy and move it to folder C:\Flag_program\In\. (***Note:*** This step may not be required in the future version of ICPMS Data Flagger.)
2. Find the ICPMS_VBA application macro on the desktop or in the folder C:\flag_progam\ and double click. This action will open the ICPMS Data Flagger application (see Figure 1). The tab "How to Execute" has step-by-step instructions for first-time users.

Figure 1. ICPMS Data Flagger application screen.



3. Select the sequence report file that you want to process from the "Raw ICPMS" drop-down menu.
4. Select the "LookUp Data" file the same way. The LookUp file has the IDL and necessary QC reference data to evaluate and flag analytical results.
5. Set QC criteria. Default values are entered automatically. Change if needed.
6. Press "Execute Data Processing," and wait until it is done. Warning messages may pop up if the flagger does not find duplicate samples. Follow on-screen instructions.
7. Enter file names in the data export options and press "Click" to export.
8. Click "Examine Flagged Data" to view the processed data. All data used for processing are present in the Excel file that opens up. Sheets are named to reflect its contents. Click "Return to ICPMS Data Flagger" button (top left) to return to the flagger application menu. Do not close the Excel file directly.
9. Use "Clear All" to start the next raw data processing. ***Note:*** Save reports (from Data Export options) before clearing all and starting new processing.
10. Use the "Close" button to exit the flagger application.

Flags

The following flags are used to indicate analytical data quality:

Analysis flags:

- V0: Determined concentration is above the IDL
- V1: Determined concentration is at or below the IDL
- M2: Missing or unavailable data
- SEC: Estimated concentration for a species when the detector was saturated

Quality control flags:

- Cp: Check standard pass
- Cf: Check standard fail
- Cu: Check standard unavailable
- Rp: SRM sample pass
- Rf: SRM sample fail
- Ru: SRM sample unavailable
- Dp: Duplicate sample pass
- Df: Duplicate sample fail

Identification of QC samples (QCflag):

- 1: Check samples
- 2: Duplicate pairs
- 22: Repeat samples

Appendix 5: Alternate Calibration Standard Preparation from Custom Multi-Element Certified Stock Solutions (High Purity Standards, Charleston, SC, USA).

Stock Name	Element	Concentration	Matrix
Stock S	S	10 ppm	Water
Stock A	Al, B, Fe, Zn	300 ppb	2% Nitric Acid
	Ca, Mg, K, Si, Na	1000 ppb	
Stock B, Soln A	As, Be, Bi, Cd, Ce Cs, Cr, Co, Cu, Dy Ga, La, Pb, Li, Mn Ne, Ni, P, Ru, Sa Sc, Se, Sr, Tb, Tl, Th, U, V, Y	100 ppb	2% Nitric Acid
Stock B, Soln B	Sb, Ge, Mo, Ag Sn, Ti, W	100 ppb	2% Nitric Acid + Tr HF
Stock B, Soln C	Pd, Pt, Rh	100 ppb	2% HCl

Table 8: Working Standard (WS) Preparation. Stock solutions are added volumetrically (mL), final addition of reagent blank is added gravimetrically.

Stock	WSO	WS1	WS2	WS3	WS4	WS5	WS6
Stock S		0.04	0.40	1.00	2.00	3.00	10.00
Stock A		0.04	0.40	1.00	2.00	3.00	10.00
Stock B-A		0.02	0.20	0.50	1.00	1.50	5.00
Stock B-B		0.02	0.20	0.50	1.00	1.50	5.00
Stock B-C		0.02	0.20	0.50	1.00	1.50	5.00
Reagent Blank[a]	100.00	99.86	98.60	96.50	93.00	89.50	65.00
Final Mass Solution (g)	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a]Reagent Blank is 0.2% HNO_3 and 0.1% HCl

Appendix 6: Hydraulic Fracturing Analysis Update. Calibration Tables, Analytical Method, and QA Guidelines.

Note: All solutions prepared will have a final 2% HNO_3 and 0.5% HCl (v/v) concentration to match the sample matrix.

Multi-element calibration standards purchased from High Purity Standards (Charleston, SC).

Stock Name	Element	Concentration	Matrix
Stock S	S	10 ppm	Water
Stock A	Al, B, Fe, Zn	300 ppb	2% Nitric Acid
	Ca, Mg, K, Si, Na	1000 ppb	
Stock B, Soln A	As, Be, Bi, Cd, Ce Cs, Cr, Co, Cu, Dy Ga, La, Pb, Li, Mn Ne, Ni, P, Ru, Sa Sc, Se, Sr, Tb, Tl, Th, U, V, Y	100 ppb	2% Nitric Acid
Stock B, Soln B	Sb, Ge, Mo, Ag Sn, Ti, W	100 ppb	2% Nitric Acid + Tr HF
Stock B, Soln C	Pd, Pt, Rh	100 ppb	2% HCl

Table 9: Working Standard Calibration Preparation.

	Stock A (mL)	Stock S (mL)	StockB-A (mL)	StockB-B (mL)	StockB-C (mL)
WS1	0.04	0.04	0.02	0.02	0.02
WS2	0.4	0.4	0.2	0.2	0.2
WS3	1	1	0.5	0.5	0.5
WS4	2	2	1	1	1
WS5	3	3	1.5	1.5	1.5
WS6	10	10	5	5	5
WS7	20	20	10	10	10
WS9			5*		
WS10			10		
WS11		10			
WS12	10				

WS1-WS7 made up to 100.00g final mass with Reagent Blank (2% HNO_3, 0.5% HCl)
WS9 is made by 5 mL StockB-A + 5 mL reagent blank
WS10-12 are 10 mL pours of the stock solution

Table 10: Working Standard Calibration Concentrations.

	WS0	WS1	WS2	WS3	WS5	WS6	WS7	WS9	WS10	WS11	WS12
K	0	0.4	4	10	30	100	200				1000
Si	0	0.4	4	10	30	100	200				1000
Al	0	0.12	1.2	3	9	30	60				300
Fe	0	0.12	1.2	3	9	30	60				300
Zn	0	0.12	1.2	3	9	30	60				300
Ba	0	0.12	1.2	3	9	30	60				300
S	0	4	40	100	300	1000				10000	
Ag	0	0.02	0.2	0.5	1.5	5					
As	0	0.02	0.2	0.5	1.5	5	10				
Be	0	0.02	0.2	0.5	1.5	5					
Bi	0	0.02	0.2	0.5	1.5	~~5~~					
Cd	0	0.02	0.2	0.5	1.5	5					
Ce	0	0.02	0.2	0.5	1.5	5	10				
Co	0	0.02	0.2	0.5	1.5	5	10	50			
Cr	0	0.02	0.2	0.5	1.5	5	10				
Cs	0	0.02	0.2	0.5	1.5						
Cu	0	0.02	0.2	0.5	1.5	5	10	50	100		
Dy	0	0.02	0.2	0.5	1.5						
Gd	0	0.02	0.2	0.5	1.5						
Ge	0	0.02	0.2	0.5	1.5	5					
La	0	0.02	0.2	0.5	1.5	5					
Li	0	0.02	0.2	0.5	1.5	5		50			
Mn	0	0.02	0.2	0.5	1.5	5	10	50	100		
Mo	0	0.02	0.2	0.5	1.5						
Nd	0	0.02	0.2	0.5	1.5						
Ni	0	0.02	0.2	0.5	1.5	5	10	50	100		
P	0		0.2	0.5	1.5	5	10	50	100		
Pb	0	0.02	0.2	0.5	1.5	5					
Pd	0	0.02	0.2	0.5	1.5						
Pt	0	0.02	0.2	0.5	1.5						
Rb	0	0.02	0.2	0.5	1.5	5	10	50			
Rh	0	0.02	0.2	0.5	1.5						
Sb	0	0.02	0.2	0.5	1.5	5					
Se	0		0.2	0.5	1.5	5	10				
Sm	0	0.02	0.2	0.5	1.5						
Sn	0	0.02	0.2	0.5	1.5	5	10				
Sr	0			0.5	1.5	5	10	50	100		
Tb	0	0.02	0.2	0.5	1.5						
Ti	0	0.02	0.2	0.5	1.5	5	10				
Th	0	0.02	0.2	0.5	1.5						
Tl	0	0.02	0.2	0.5	1.5						
U	0	0.02	0.2	0.5	1.5						
V	0	0.02	0.2	0.5	1.5	5	10	50			
W	0	0.02	0.2	0.5	1.5						
Y	0	0.02	0.2	0.5	1.5	5	10				

Table 11: QA/QC Guidelines

Item	Frequency	Criteria	Action
Calibration	Once per sequence	R2 > 0.995	Examine fitting parameters; rerun standards; remake standards
Initial Calibration Verification (Check_xxxx_ppt_r00	After calibration	±15% of target value	Examine calibration; rerun sample, remake sample
IBC (Initial Blank Check)	After calibration	< lowest reportable limit	Examine calibration; rerun sample, remake sample
Check Sample (s) Check_1000_ppt_r## Check_1500_ppt_r## Check_10_ppb_r##	After every 10 unknown samples. High level check (10 ppb) followed by low level check (1000ppt or 1500ppt)	±15% of target value	Examine calibration; rerun sample, remake sample. Examine sequence of unknowns to determine repeats.
Duplicate (_dp)	Every 10 unknown samples; half-way between the Check Sample.	±20% Relative Percent Difference	Remake sample. Examine sequence of unknowns to determine repeats.
SRM (NIST Standard Reference Materials)	Once per sequence (1643e and 1640a each at 2 different dilutions)	±15% of target value	Examine calibration; rerun sample, remake sample. Observe trends of multiple sequences.
QCS (Quality Control Sample)	Analyst Suggestion to verify calibration accuracy	±15% of target value	Examine calibration.

Extraction of Microbes and DNA Genomes from Samples Collected from Artificial Turf Athletic Fields (D-SED-EFAB-011-SOP-01) – June 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Extraction of Microbes and DNA Genomes from Samples Collected from Artificial Turf Athletic Fields

Number: D-SED-EFAB-011-SOP-01	Effective Date: June 9, 2016

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EFAB-011-01

SOP Steward

Name: Nichole Brinkman, Environmental Futures Analysis Branch

Approval

Name: Valerie Garcia
Title: Chief, Environmental Futures Analysis Branch

Concurrence

Name: Brittany Stuart
Title: SED Quality Assurance Manager

STANDARD OPERATION PROCEDURES FOR EXTRACTION OF MICROBES AND DNA GENOMES FROM SAMPLES COLLECTED FROM ARTIFICIAL TURF ATHLETIC FIELDS – JUNE 9, 2016

TABLE OF CONTENTS

1.0 PURPOSE

This standard operating procedure (SOP) describes the extraction of microbes from tire crumb rubber collected from artificial turf athletic field and the procedure for extracting the genomic material from the extracted microbes.

2.0 SCOPE AND APPLICABILITY

This SOP covers laboratory processing steps to extract microbes from particles in the sample, and extract and quantify genomic material, such as deoxyribose nucleic acid (DNA). This SOP covers processing steps unique to tire crumb rubber samples taken from artificial turf athletic fields. This SOP has been developed for the Tire Crumb Project. The method reporting limit is dictated by the Qubit Fluorimeter which is 0.5ng/mL of DNA.

3.0 SUMMARY OF METHOD

Extraction of microbes from sample
The purpose of this method is to concentrate the sample and extract the genomic DNA for downstream genetic analysis such as polymerase chain reaction (PCR) and metagenomic sequencing. Once samples arrive in the lab, a solution containing surfactants is added to the crumb rubber sample and then the sample is vortexed vigorously to dislodge any microbes from the sample into solution. The solution is then filtered through a 0.45um filter to capture and retain microbes on the filter. The sides of the filter apparatus are washed twice with sterile phosphate buffered saline (PBS) to remove potential inhibitors and to help remove any microbes attached to the sides of the filter apparatus. The filter is then stored -20˚C.

Extraction of DNA genomes and determination of yield
Nucleic acids are extracted from the microbes captured on filters using the PowerWater DNA Isolation Kit (MoBio Laboratories) and the DNA yield is quantified using the Qubit Fluorometer 2.0 (Invitrogen).

4.0 DEFINITIONS

DNA – deoxyribose nucleic acid
PBS – phosphate buffered saline
SOP – standard operating procedure
PCR – polymerase chain reaction
PPE – personal protective equipment
HASP – health and safety plan
BARB – biohazard assessment research branch
EMMD – exposure materials & methods division
MEB – microbial exposure branch
BSC – biological safety cabinet

NERL – National Exposure Research Laboratory
TCR – tire crumb rubber
TSA – tryptic soy agar TSB – tryptic soy broth

5.0 HEALTH AND SAFETY WARNINGS

Standard laboratory personal protective equipment (PPE) (i.e., laboratory coat, safety glasses/goggles, and gloves) is required. DNA extractions must take place in a biological safety cabinet (BSC). In addition, any chemical-specific protective gear required is described in the Health and Safety Plan (HASP) in place for each functional laboratory that will be used.

All laboratory procedures in this SOP are covered HASP 2015-086.

6.0 CAUTIONS/INTERFERENCES

All equipment that will come into contact with the sample must be sterilized by autoclaving at 121˚C for at least 15min, or via soaking in a 10% bleach solution for at least 20 min. If bleach is used as the disinfectant, the bleach should be neutralized using sodium thiosulfate. If equipment is not sterilized, it can contaminate the sample.

The holding time for bacterial analysis of samples is generally 6 hours at 4˚C. However, in certain circumstances where the sample collection site is not in geographical proximity to the analytical lab, the holding time can be extended to 24 hours from sample collection. Caution should be used when interpreting results if the sample is held longer than 6 hours as some microbial species may die off or proliferate during an extended holding time.

Once the sample is processed and filtered through a 0.45 um filter, the filter must be stored at -20˚C to prevent degradation of the DNA in the sample. Once DNA has been extracted from the filter, the DNA should be stored at -80˚C to prevent degradation of the DNA.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The techniques of first-time analysts must be reviewed by an experienced analyst prior to completing this SOP alone. During this review, the new analyst must demonstrate his/her ability to complete this procedure alone which must be approved and documented by an experienced analyst/supervisor in the analyst's laboratory notebook.

8.0 EQUIPMENT AND SUPPLIES

Extraction of microbes from TCR sample

- Tween-80 (Sigma-Aldrich P4780)
- Sodium polyphosphate (Sigma-Aldrich 305553) Antifoam Y-30 emulsion (Sigma-Aldrich A6457)

- Reagent grade (MilliQ) water pH meter
- Filter Unit, 1000mL, 0.2 µm, 90mm diameter (Nalgene 567-0020)
- Dulbecco's Phosphate buffered saline (PBS), pH 7.0-7.5 (Sigma-Aldrich D5652) 50mL polypropylene conical (sterile) (Corning 352098)
- Vortex Genie
- Vortex Genie adapter for 50mL tubes (MoBio Laboratories 13000-V1-50) 25mL serological pipette (sterile, Fisher Scientific 170357)
- Membrane filter, 0.45um, 47mm nitrocellulose (sterile, PALL 66278) Forceps (sterile)
- Bunsen burner Ethanol, absolute
- 0.5 - 1L side-arm flask Autoclave
- Sentino Magnetic Filter Funnel or equivalent filtration unit (47mm diameter, sterile) 4˚C refrigerator
- -80˚C freezer Laboratory tubing Vacuum apparatus
- Analytical balance (capable of reading to 0.01g)
- *E. coli* (CN13, ATCC 700609)
- Tryptic soy broth (Becton Dickenson 211825)
- Agar (BD Bacto 214050)
- Nalidixic acid (Sigma-Aldrich N4382)

DNA extraction

- BSC/hood
- PowerWater DNA Isolation Kit (MoBio Laboratories 14900-50-NF) Water bath (55˚C)
- Thermometer Timer Vortex Genie
- Vortex Genie adapter for 2mL tubes (MoBio Laboratories 13000-V1-24) Stopwatch/timer
- Bunsen burner Ethanol, absolute
- 200µL - 1000mL micropipettes and tips

DNA yield quantification

- Qubit Fluorometer 2.0
- Qubit dsDNA HS Assay Kit (Invitrogen Q32851)
- 0.5mL, clear Qubit-compatible tubes (Invitrogen Q32856) 10µL - 1000mL micropipettes and tips

9.0 PROCEDURE

A. Preparation of *E. coli* culture stock for positive controls for microbe extraction and DNA genomic extractions

1. Streak a TSA plate supplemented with 0.1 mg/ml nalidixic acid with *E. coli* stock for isolation and incubate overnight at 37°C.
2. Aseptically inoculate 5 ml of TSB with 0.1 mg/ml nalidixic acid with an isolated *E. coli* colony prepared from the TSA plate above. Incubate overnight at 37°C.
3. Prepare a mid-log phase of *E. coli* by adding 0.75 ml of the overnight culture

prepared above to 30 ml of TSB with 0.1 mg/ml nalidixic acid. Incubate for 4 hours at 37°C.
4. Swirl the culture and prepare 50 aliquots of 0.3 ml of the culture into sterile 1.5 ml tubes.
5. Store cultures at -80°C.

B. Preparation of *E. coli* genomic DNA for positive controls for Qubit DNA yield determination

1. Streak a TSA plate supplemented with 0.1 mg/ml nalidixic acid with *E. coli* (CN13, ATCC 700609) for isolation and incubate overnight at 37°C.
2. Aseptically inoculate 5 ml of TSB with 0.1 mg/ml nalidixic acid with an isolated *E. coli* colony prepared from the TSA plate above. Incubate overnight at 37°C.
3. Prepare a mid-log phase of *E. coli* by adding 0.75 ml of the overnight culture prepared above to 30 ml of TSB with 0.1 mg/ml nalidixic acid. Incubate for 4 hours at 37°C.
4. Remove 3-0.2 ml volumes and extract the DNA with the MoBio PowerWater Kit as instructed in Section F.
5. Prepare fifty (50) 0.005 ml aliquots in 0.6 ml tubes. Store DNA at -80°C.

C. Prepare Elution Solution (20 ml/sample)

1. Add the following to 1L reagent grade water:
   a. 0.05g sodium polyphosphate
   b. 50 µL Tween-80 (Sigma-Aldrich cat #P4780-500ml)
   c. 5 µL Y-30 antifoam (Sigma-Aldrich cat #A6457-100 ml)
2. Filter sterilize using 0.2µm filter units. Adjust pH to 7.0-7.5 using 1N NaOH or HCl. Store at room temperature, use within 1 week.

D. Prepare PBS solution

1. Add 9.6g of Dulbecco's 1X PBS powder to 1L MilliQ water
2. Adjust pH to 7.0 – 7.5 using 1N NaOH or HCl, if necessary
3. Filter sterilize PBS solution using 0.2 µm filter units or autoclave at 121˚C for 15min.
4. Store PBS at 4˚C for up to 1 year.

E. Extraction of microbes from tire crumb rubber

1. Wipe surface of laboratory bench with 10% bleach
2. Upon receipt of samples in the lab, weigh 5g tire crumb per sample and transfer to 50 ml polypropylene conical tube. Record the verification of balance calibration information in the analysts' laboratory notebook.
3. Pipette 20mL of filter-sterilized elution solution into 50 mL conical containing tire crumb rubber sample and seal tube.
4. Attach 50mL tube adapter to Vortex Genie
5. Attach 50mL conical containing sample to Vortex Genie adapter and vortex sample at max speed for 2 min
6. Setup sterilized filtration cone adapter and vacuum system
7. Use aseptic technique to place the 0.45µm membrane filter on the adapter

8. Use a sterile 25 mL pipette to transfer all of the supernatant onto the 0.45 um nitrocellulose membrane water filter.
- ***Note:*** to remove all of the supernatant, place the pipette tip into the tire crumb rubber particles
9. Vacuum entire sample through membrane filter
10. Once sample filtration is complete, wash the inner sides of the filter cone apparatus with 2 separate washings of 15 mL of sterilized PBS.
11. Turn off vacuum and use sterile forceps to remove filter from apparatus and place in a MoBio PowerWater bead tube. Label tube with date, sampleID, analysts initials and store at -20˚C
12. For each batch of samples, prepare a negative TCR extraction control by adding 20 ml of elution solution to an empty tube. Process as described for TCR samples.
13. For each batch of samples, prepare a positive TCR extraction control by adding 0.2 ml of E. coli CN13 positive control culture. Process as described for TCR samples.

F. Extraction DNA genomes and assessment of DNA yield (adapted from manufacturer's protocols (Addendum 1 and 2):
1. Wipe BSC with 10% bleach and turn on UV light in hood for at least 15 min
2. Obtain samples (filters in bead tubes) from -20˚C freezer and allow to thaw at room temperature.
3. Turn on water bath; set temperature to 55°C.
4. Warm solution PW1at 55°C for 5-10 minutes. This solution should be used warm.
5. Check solution PW3 for precipitates. Incubate at 55°C if necessary to dissolve precipitates. This solution can be used warm.
6. Add **1 ml of Solution PW1** to the PowerWater® Bead Tube. **Note:** Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
7. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5.
8. Vortex at maximum speed for 5 minutes.
9. Centrifuge the tubes ≤ 4000 x *g* for 1 minute at room temperature. The speed will depend on the capability of your centrifuge.
10. Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads. **Note:** Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.
11. Centrifuge at 13,000 x *g* for 1 minute.
12. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
13. Add **200 µl of Solution PW2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

14. Centrifuge the tubes at 13,000 x *g* for 1 minute.
15. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
16. Add **650 µl of Solution PW3** and vortex briefly to mix. **Note:** Check Solution PW3 for precipitation prior to use. Warm if necessary. Solution PW3 can be used while still warm.
17. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter. **Note:** A total of two loads for each sample processed are required.
18. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
19. Shake to mix Solution PW4 before use. Add **650 µl of Solution PW4** and centrifuge at 13,000 x *g* for 1 minute.
20. Discard the flow through and add **650 µl of Solution PW5** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
21. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided), labeled with "date, PowerWater extract, sampleID, initials."
22. Add **100 µl of Solution PW6 (**Solution PW6 contains no EDTA) to the center of the white filter membrane. Incubate for 5 minutes at room temperature.
23. Centrifuge at 13,000 x *g* for 1 minute.
24. Discard the Spin Filter basket.
25. Hold DNA on ice or on a cold block.
26. Obtain the *E. coli* genomic DNA Qubit positive control from the -80°C freezer.
27. Set up the number of 0.5 ml Qubit Assay tubes required and label lids.
28. Prepare Qubit working solution by making a cocktail that contains 199 µl of Qubit dsDNA HS buffer and 1 µl Qubit dsDNA HS reagent for each sample.
29. Prepare the 2 standards by adding 10 µl of standard and 190 µl of Qubit working solution to the appropriately labeled tubes.
30. Prepare a Qubit negative control by adding 5 µl of PW6 and 195 µl of Qubit working solution to the appropriately labeled tube.
31. Prepare a Qubit positive control by adding 5 µl of *E.coli* genomic DNA and 195 µl of Qubit working solution to the appropriately labeled tube.
32. Prepare each sample by adding 5 µl of extracted sample DNA and 195 µl of Qubit working solution to the appropriately labeled tubes.
33. Allow all tubes to incubate at room temperature for 2 minutes.
34. On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **dsDNA High Sensitivity** as the assay type.
35. On the Standards Screen, press **Yes** to run a new calibration.
36. Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
37. Remove Standard #1.
38. Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
39. Remove Standard #2.
40. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press

Read.

41. Upon completion of the measurement, the result will be displayed on the screen. **Note:** The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube.
42. Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.
43. Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.
44. To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicate by an adjacent red dash) opens.
45. Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up. The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed. **Note:** The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).
46. To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.
47. Repeat sample readings until all samples have been read.
48. Record DNA yield for each sample in the analyst's notebook.
49. Store DNA extracts at -80°C.
50. TCR and DNA extraction controls will be evaluated by ddPCR as described in D-SED-EFAB-014-SOP-01: Droplet Digital PCR (ddPCR) of gene targets.

10.0 DATA AND RECORDS MANAGEMENT

Data Calculations

To manually convert Qubit reading of ng/mL to DNA yield in ng/µl in the sample, use C1V1 = C2V2 formula:

$$CC2 = \frac{CC1 * VV1}{VV2}$$

Where:
C1 = Qubit DNA yield reading (converted from ng/mL to ng/µl) V1 = volume of Qubit reaction (200µl)
C2 = DNA yield in DNA extract (ng/µl)
V2 = volume of DNA extract added to Qubit reaction (1-20 µl)

Data Management
At the completion of this protocol, the DNA yield resulting from processing each sample is determined. This yield will be used to prepare dilutions of samples for PCR analysis. The DNA yield will be recorded in the analyst's notebook. Likewise, the results of the quality control samples used to assess the performance of the Qubit Fluorimeter will also be recorded in the analyst's notebook. The quality control samples used to assess the performance of TCR and DNA extraction have also been prepared, but must be assessed by ddPCR, which is outside the scope of this SOP. Use D-SED-EFAB-014-SOP-01: Droplet Digital PCR (ddPCR) of gene targets to quantify genomes in these quality control samples.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

Quality control (QC) procedures will include a positive and negative control for each procedure outlined (extraction of microbes from particles in sample, extraction of genomic DNA from captured microbes, determination of DNA yield) to evaluate the performance of each process. This section describes how controls will be prepared and assessed.

1. Controls for extracting microbes from field samples
    a. TCR extraction negative control – 20 ml elution solution added to an empty 50mL conical tubes
        i. QC acceptance criteria: Qubit reading must be below the LOD of 0.5ng/mL
        ii. Frequency – 1 control with every batch of 1-7 samples
        iii. Action to be taken if criteria is not met – If Qubit analysis shows values above the LOQ, the elution solution is contaminated and the batch of samples will be marked as compromised. Microbe extraction negative control will be analyzed with downstream procedures and results of unknown samples will be interpreted in the context of control results.
        iv. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be highlighted and the Technical Lead will be notified.
    b. TCR extraction positive control – 20 elution solution and a known quantity of *E. coli* added to an empty 50mL conical
        i. QC acceptance criteria – Qubit reading of batch mean, ± 30%.
        ii. Frequency – 1 control with every batch of 1-7 samples
        iii. Action to be taken if criteria is not met – A Qubit analysis result outside of the acceptance criteria for this control indicates that the extraction procedure was ineffective and batch of samples will be marked as compromised. Microbe extraction positive control will be analyzed with downstream procedures and results of unknown samples will be interpreted in the context of control results.
        iv. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be

highlighted and Technical Lead will be notified.

2. Controls for genomic DNA extraction
    a. Genomic DNA extraction negative control – a blank filter
        i. QC acceptance criteria: Qubit reading must be below the LOD of 0.5ng/mL
        ii. Frequency – 1 control with every batch of 10-24 samples
        iii. Action to be taken if criteria is not met - If Qubit analysis shows values above the LOQ, the extraction kit reagents are contaminated and the batch of samples will be marked as compromised. Genomic DNA extraction negative control will be analyzed with downstream procedures and results of unknown samples will be interpreted in the context of control results.
        iv. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be highlighted and the Technical Lead will be notified.
    b. Genomic DNA extraction positive control – a known quantity of *E. coli* diluted with elution solution
        i. QC acceptance criteria: Qubit reading of culture batch mean ± 30%.
        ii. Frequency – 1 control with every batch of 10-24 samples
        iii. Action to be taken if criteria is not met - A Qubit analysis result outside of the acceptance criteria for this control indicates that the extraction procedure was ineffective and the batch of samples will be marked as compromised. Genomic DNA extraction positive control will be analyzed with downstream procedures and results of unknown samples will be interpreted in the context of control results.
        iv. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be highlighted and the Technical Lead will be notified.

3. Qubit fluorimeter controls:
    a. Qubit negative control – 5 µl of PW6 (solution used to elute DNA from extraction column)
        i. QC acceptance criteria: Qubit reading must be below the LOD of 0.5ng/mL
        ii. Frequency – 1 control with every batch of genomic DNA samples (10-24 samples). Control will be run prior to running samples.
        iii. Action to be taken if criteria is not met – Samples will not be analyzed. Qubit will be restarted and re-calibrated. A new aliquot of elution solution will be re-run and acceptance criteria met before analyzing samples.
        iv. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be

highlighted and the Technical Lead will be notified.

b. Qubit positive control – an aliquot of E. coli genomic DNA prepared as a positive control
    i. QC acceptance criteria: Qubit reading of extraction batch mean ± 30%. Frequency – 1 control with every batch of genomic DNA samples (10-24 samples). Control will be run prior to running samples.
    ii. Action to be taken if criteria is not met – Samples will not be analyzed. Qubit will be restarted and re-calibrated. A new aliquot of *E. coli* genomic DNA positive control will be re-run and acceptance criteria met before analyzing samples.
    iii. Procedures for reporting QC documentation – Qubit results for each batch of samples, including controls, will be recorded in analysts' notebook. Any violations of acceptance criteria for controls will be highlighted and the Technical Lead will be notified.

12.0 REFERENCES

- D-SED-EFAB-014-SOP-01: Droplet digital PCR (ddPCR) of genomic targets
- MoBio Laboratories, Inc. PowerWater DNA Isolation Kit Instruction Manual (Addendum 1)
- Invitrogen Qubit dsDNA HS Assay Kit Instruction Manual (Addendum 2)

13.0 DOCUMENT HISTORY

Date of Revision	Revision #	Description of Changes
6/9/2016	0	First version

14.0 ADDENDUM 1

PowerWater® DNA Isolation Kit

(For isolation of genomic DNA from membrane filtered water samples)

Catalog No.	Quantity	Filters
14900-50-NF	50 Preps	No filters
14900-100-NF	100 Preps	No filters

Instruction Manual

Table of Contents

Introduction

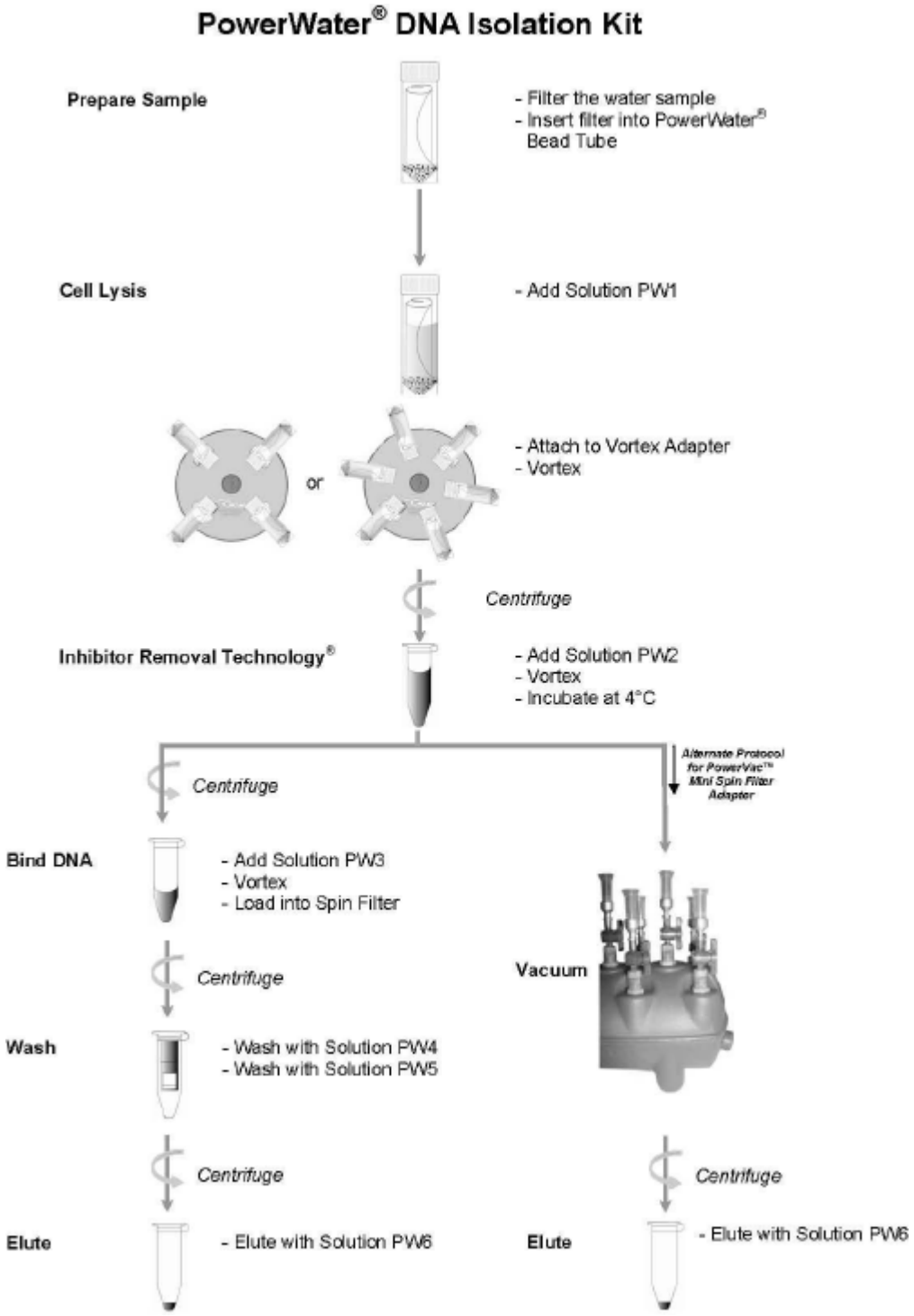
The PowerWater® DNA Isolation Kit can isolate genomic DNA from a variety of filtered water samples. Utilizing our patented Inhibitor Removal Technology® (IRT), even water containing heavy amounts of contaminants can be processed to provide DNA of high quality and yield. The kit can isolate DNA equally as well from any commonly used filter membrane type. This kit differs from our UltraClean® Water DNA Isolation Kit by the addition of a novel bead tube with an optimized bead mix, a reformulated lysis buffer, IRT technology, and the reduction in sample volume so that nearly all processing occurs in a microcentrifuge. Purified DNA is ready to use in a final 100 µl elution volume.

Protocol Overview

The PowerWater® DNA Isolation Kit starts with the filtration of a water sample onto a filter membrane. Filter membranes can be purchased separately from MO BIO or can be user supplied. MO BIO filter membranes are sterile, disposable, and easy to use. The membrane is then added to our special 5 ml bead beating tube containing a unique bead mix. Rapid and thorough lysis occurs through vortex mixing in a reformulated lysis buffer that enhances the isolation of microorganisms from filter membranes. After the protein and inhibitor removal steps, total genomic DNA is captured on the MO BIO Laboratories silica spin column. High-quality DNA is then washed and eluted from the spin column membrane for use in downstream applications including PCR and qPCR.

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
Vortex Adapter for Vortex Genie® 2	13000-V1-15 13000-V1-5	Holds 4 (5 ml or 15 ml) Tubes Holds 6 (5 ml) Tubes
Water Filter Adapter	14800-10-WFA	1
Water Filter (0.45 µm)	14800-10-WF 14800-25-WF 14800-50-WF 14800-100-WF	10 units 25 units 50 units 100 units
Water Filter (0.22 µm)	14880-10-WF 14880-25-WF 14880-50-WF 14880-100-WF	10 units 25 units 50 units 100 units
Vortex Genie® 2 Vortex	13111-V-220 13111-V	1 unit (220V) 1 unit (120V)
PCR Water (Certified DNA-free)	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
RapidWater® DNA Isolation Kit	14810-50-NF	50 preps (No filters)
PowerVac™ Manifold	11991	1 manifold
PowerVac™ Mini System	11992	1 unit + 20 adapters
PowerVac™ Mini Spin Filter Adapters	11992-10 11992-20	10 adapters 20 adapters



Equipment Required

- Centrifuge for 15 ml tubes (≤4000 x *g*)
- Disposable/reusable filter funnels
- Filter membranes (if using a reusable filter funnel) Microcentrifuge (13,000 x *g*)
- Pipettors Vortex
- Vortex Adapter
- Vacuum Filtration System

Kit Contents

	Kit Catalog# 14900-50-NF	Kit Catalog# 14900-100-NF
Component	Amount	Amount
PowerWater® Bead Tubes	50 tubes	100 tubes
Solution PW1	55 ml	110 ml
Solution PW2	11 ml	22 ml
Solution PW3	2 x 18 ml	3 x 24 ml
Solution PW4	2 x 18 ml	3 x 24 ml
Solution PW5	2 x 18 ml	3 x 24 ml
Solution PW6	5.5 ml	11 ml
Spin Filters	50	100
2 ml Collection Tubes	250	500

Kit Storage
Store all reagents and kit components at room temperature (15-30°C).

Precautions
Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solutions PW3, PW4 and PW5 contain alcohol. They are flammable.

Important Notes Before Starting:
Solution PW1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to use.
Solution PW1 should be used while still warm.
Solution PW3 may precipitate over time. If precipitation occurs, warm at 55°C for 5-10 minutes. Solution PW3 can be used while still warm.
Shake to mix Solution PW4 before use.

Experienced User Protocol
- Please wear gloves at all times
- Warm Solution PW1 prior to use at 55°C for 5-10 minutes. Use Solution PW1 while still warm.
- Check Solution PW3 and warm at 55°C for 5-10 minutes if necessary. Solution PW3 can be used while still warm.

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source. Disposable filter funnels, containing 0.22 µm or 0.45 µm filter membranes, can be ordered from MO BIO Laboratories (see page 3). The volume of water filtered will depend on the microbial load and turbidity of the water sample. **(Please see Types of Water Samples in the Hints and Troubleshooting Guide section of the Instruction Manual).**
2. If using a reusable filter funnel, remove the upper portion of the apparatus. If using a MO BIO Laboratories filter funnel, remove the 100 ml upper portion of the filter cup from the catch reservoir by snapping it off.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.
   Note: Do not tightly roll or fold the filter membrane. To see a video of this technique, please visit the PowerWater® DNA Isolation Kit product page on www.mobio.com.
4. Insert the filter into the 5 ml PowerWater® Bead Tube.
5. Add **1 ml of Solution PW1** to the PowerWater® Bead Tube.
   Note: Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm. For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be included. See **Alternate Lysis Method in the Hints and Troubleshooting Guide.**
6. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5.
7. Vortex at maximum speed for 5 minutes.
8. Centrifuge the tubes ≤ 4000 x *g* for 1 minute at room temperature. The speed will depend on the capability of your centrifuge. **(This step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).**
9. Transfer all the supernatant to a clean 2 ml collection tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.
   Note: Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.
10. Centrifuge at 13,000 x *g* for 1 minute.
11. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
12. Add **200 µl of Solution PW2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
13. Centrifuge the tubes at 13,000 x *g* for 1 minute.
14. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
15. Add **650 µl of Solution PW3** and vortex briefly to mix.
    Note: Check Solution PW3 for precipitation prior to use. Warm if necessary. Solution PW3 can be used while still warm.
16. Load 650 µl of supernatant onto a spin filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the spin filter.
    Note: A total of two loads for each sample processed are required.
17. Place the spin filter basket into a clean 2 ml collection tube (provided).
18. Shake to mix Solution PW4 before use. Add **650 µl of Solution PW4** and centrifuge at 13,000 x *g* for 1 minute.

19. Discard the flow through and add **650 µl of Solution PW5** and centrifuge at 13,000 x *g* for 1 minute.
20. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
21. Place the spin filter basket into a clean 2 ml collection tube (provided).
22. Add **100 µl of Solution PW6** to the center of the white filter membrane.
23. Centrifuge at 13,000 x *g* for 1 minute.
24. Discard the spin filter basket. The DNA is now ready for any downstream application. No further steps are required.
25. We recommend storing the DNA frozen (-20°C to -80°C). Solution PW6 contains no EDTA. To concentrate the DNA, see the Hints and Troubleshooting Guide.

Detailed Protocol (Describes what is happening at each step)

- Please wear gloves at all times
- Warm Solution PW1 prior to use at 55°C for 5-10 minutes. Use Solution PW1 while still warm.
- Check Solution PW3 and warm at 55°C for 5-10 minutes if necessary. Solution PW3 can be used while still warm.

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source. Disposable filter funnels, containing 0.22 µm or 0.45 µm filter membranes, can be ordered from MO BIO Laboratories (see page 3). The volume of water filtered will depend on the microbial load and turbidity of the water sample. **(Please see Types of Water Samples in the "Hints and Troubleshooting Guide" section of Instruction Manual).**
   What's happening: A reusable or disposable filter funnel is attached to a vacuum filtration system. Microorganisms are trapped on top of and within the filter membrane.
2. If using a reusable filter funnel, remove the upper portion of the apparatus. If using a MO BIO Laboratories filter funnel, remove the 100 ml upper portion of the filter cup from the catch reservoir by snapping it off.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.
   Note: Do not tightly roll or fold the filter membrane. To see a video of this technique, please visit the PowerWater® DNA Isolation Kit product page on www.mobio.com.
4. Insert the filter into the 5 ml PowerWater® Bead Tube.
   What's happening: Loosely rolling and inserting the filter membrane into the PowerWater® Bead Tube allows for efficient bead beating and homogenization in proceeding steps.
5. Add **1 ml of Solution PW1** to the PowerWater® Bead Tube.
   Note: Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm. For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be included. See **Alternate Lysis Method in the "Hints and Troubleshooting Guide".**
   What's happening: Solution PW1 is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material. It is also part of the patented Inhibitor Removal Technology® (IRT). When cold, this solution will

form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution PW1 should be used while it is still warm.

6. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5.
7. Vortex at maximum speed for 5 minutes
   What's happening: The mechanical action of bead beating will break apart the surface of the filter membrane that contains trapped cells and aids in cell lysis. Use of the vortex adapter will maximize homogenization by holding the tubes equal distance and angle from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.
8. Centrifuge the tubes ≤ 4000 x *g* for 1 minute at room temperature. The speed will depend on the capability of your centrifuge. **(This step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).**
9. Transfer the supernatant to a clean 2 ml collection tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.
   Note: Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.
   What's happening: The supernatant is separated and removed from the filter membrane and beads at this step.
10. Centrifuge at 13,000 x *g* for 1 minute.
    What's happening: Any remaining beads, proteins, and cell debris are removed at this step. This step is important for removal of any remaining contaminating non-DNA organic and inorganic matter that may reduce the DNA purity and inhibit downstream DNA applications.
11. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
12. Add **200 µl of Solution PW2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
    What's happening: Solution PW2 is another part of the patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
13. Centrifuge the tubes at 13,000 x *g* for 1 minute.
14. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
    What's happening: The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yields and quality, avoid transferring any of the pellet.
15. Add **650 µl of Solution PW3** and vortex briefly to mix.
    Note: Check Solution PW3 for precipitation prior to use. Warm if necessary. Solution PW3 can be used while still warm.
16. *What's happening: Solution PW3 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of the DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin filter.*
17. Load 650 µl of supernatant onto a spin filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the spin

filter.
Note: A total of two loads for each sample processed are required. *What's happening: The DNA is selectively bound to the silica membrane in the spin filter basket and the flow through containing non- DNA components is discarded.*

18. Place the Spin Filter basket into a clean 2 ml collection tube (provided).
What's happening: Due to the high concentration of salt in solution PW3, it is important to place the spin filter basket into a clean 2 ml collection tube to aid in the subsequent wash steps and improve the DNA purity and yield.
19. Shake to mix Solution PW4 before use. Add **650 µl of Solution PW4** and centrifuge at 13,000 x *g* for 1 minute.
What's happening: Solution PW4 is an alcohol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the spin filter. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica membrane.
20. Discard the flow through and add **650 µl of Solution PW5** and centrifuge at 13,000 x *g* for 1 minute.
What's happening: Solution PW5 ensures complete removal of Solution PW4 which will result in higher DNA purity and yield.
21. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
What's happening: The second spin removes residual Solution PW5. It is critical to remove all traces of wash solution because the ethanol in Solution PW5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.
22. Place the spin filter basket into a clean 2 ml collection tube (provided).
23. Add **100 µl of Solution PW6** to the center of the white filter membrane.
What's happening: Placing Solution PW6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica spin filter membrane. As Solution PW6 passes through the silica membrane, the DNA that was bound in the presence of high salt is selectively released by Solution PW6 (10 mM Tris) which lacks salt.
Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica spin filter membrane at this step. Solution PW6 contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of PW6 for elution of DNA from the Spin Filter.
24. Centrifuge at 13,000 x *g* for 1 minute.
25. Discard the spin filter basket. The DNA is now ready for any downstream application. No further steps are required.
26. We recommend storing the DNA frozen (-20°C to -80°C). Solution PW6 contains no EDTA. To concentrate the DNA, see the Hints and Troubleshooting Guide.

Thank you for choosing the PowerWater® DNA Isolation Kit!

Vacuum Protocol using the PowerVac™ Manifold

Please wear gloves at all times

For each sample lysate, use one spin filter column. Keep the spin filter in the attached 2 ml collection tube and continue using the collection tube as a spin filter holder until needed for the Vacuum Manifold Protocol. Label each collection tube top and spin filter column to maintain sample identity. If the spin filter becomes clogged during the vacuum procedure, you can switch to the procedure for purification of the DNA by centrifugation. You will need to provide 100% ethanol for step 4 of this protocol.

1. For each prep, attach one aluminum **PowerVac™ Mini Spin Filter Adapter** (MO BIO Catalog# 11992-10 or 11992-20) into the Luer-Lok® fitting of one port in the manifold. Gently press a spin filter column into the PowerVac™ Mini Spin Filter Adapter until snugly in place. Ensure that all unused ports of the vacuum manifold are closed.
   Note: Aluminum PowerVac™ Mini Spin Filter Adapters are reusable.
2. Transfer 650 **Spin Filter column** 1 of prepared sample lysate (from Step 15).
3. Turn on the vacuum source and open the stopcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. Allow the lysate to pass through the **Spin Filter column**. After the lysate has passed through the column completely, load again with the next 650 **Spin Filter** 1 of lysate. Continue until all of column. Close the one-way Luer-Lok® stopcock of that port.
   Note: If spin filter columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.
4. Add 800 n. Open 1 of 100% ethanol into the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.
5. Shake to mix Solution PW4. Add 650 **Solution PW4** 1 of to each Spin Filter. Open the Luer- Lok® stopcock and apply a vacuum until **Solution PW4** has passed through the spin filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
6. Add 650 **Solution PW5** 1 of to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until Solution PW5 has passed through the spin filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
7. Remove the **Spin Filter column** and place in the original labeled **2 ml Collection Tube**
8. Place into the centrifuge and spin at 13,000 × g for 2 minutes to completely dry the membrane.
9. Transfer the **Spin Filter column** to a new **2 ml Collection Tube** and add 100 **Solution** 1 of **PW6** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica spin filter membrane at this step (MO BIO Catalog# 17000-10).
10. Centrifuge at room temperature for 1 minute at 13,000 x g.
11. Discard the **Spin Filter column**. The DNA in the tube is now ready for any downstream application. No further steps are required.

Hints and Troubleshooting Guide

Types of Water Samples

A. Clear Water Samples: Water samples may vary from clear to highly turbid. Larger volumes of clear water can be processed because there is less chance of filter clogging. Potable drinking water will generally allow for very high volumes depending on the quality and particulate count. In most cases, 100 ml to 10 liters can be processed. Some users report processing even higher volumes.

B. Turbid Water Samples: Turbid samples with high levels of suspended solids or sediments will tend to clog filters with a smaller pore size (0.22 micron). Use of 0.45-micron filters is recommended for these types of samples. MO BIO Laboratories offers disposable filter funnels containing membranes of either 0.22 micron or 0.45-micron pore sizes (See page 3 for ordering information). Prior to filtering, samples can be stored in a container to allow suspended solids to settle out. For samples where settling does not occur or is not desired, a method involving stacking filters with larger pore sizes on top of the filter membrane of the desired pore size is recommended. A common set up is to stack a sterile 1-micron filter; this layering will filter out large debris and allow the smaller micron filter to trap microorganisms. The layered filter system can be washed with sterile water or sterile phosphate buffer to knock down some of the trapped microorganisms on the larger pore size filters. Although this is not 100% efficient, it will increase the overall yield of microbial DNA

Filter Membrane Selection
MO BIO Laboratories offers disposable filter funnels containing filter membranes commonly used for water research and testing. The 0.22-micron filter membrane consists of polyethersulfone (Pall Supor®), while the 0.45-micron filter membrane consists of cellulose acetate. Some filter membranes may bind and concentrate inhibitors. To reduce the likelihood of this occurring, filter membrane types may need to be evaluated prior to use.

Forgetting to Warm Solution PW1
If PW1 is not warmed prior to use, continue with the protocol. You will still obtain DNA, but the yields may not be optimal.

Alternate Lysis Method
Heating can aid in lysis of some organisms (fungi and algae) and lead to increased yields. At Step 5, heat the PowerWater® Bead Tube at 65°C for 10 minutes then continue with the protocol at Step 6.

If a Centrifuge for 15 ml Tubes is not Available for use with the 5 ml Tubes in Step 8
Centrifugation at this step helps to separate the supernatant from the filter membrane so that as much of the solution as possible is recovered. If a centrifuge is not available, this step can be skipped with some minor loss of supernatant.

Expected DNA Yields

DNA yields will vary depending on the type of water, sample location, and time of year. Examples of expected yields are provided as a reference. Due to diversity of water sample types, yields may fall outside of the examples provided.

Type of Water Sample	Sample Volume	DNA Yield (ng/µl)
Saltwater Bay	100 ml	40 - 72
Freshwater Lake	100 ml	15 - 25
Lagoon	20-100 ml	3 - 38
Ocean (coastal)	100 ml	3 - 11
Sewage influent	50 ml	95
Treated effluent	50 ml	18

Low A260/230 Ratios are Obtained

A260/230 readings are one measure of DNA purity. For samples with low biomass, which would lead to low DNA yields (<20 ng/µl), this ratio may fall below 1.5. This ratio is not an indicator of amplification ability or DNA integrity. Ethanol precipitation with resuspension into a smaller volume to concentrate the DNA may help to improve the A260/230 ratio**.**

DNA Floats Out of Well When Loaded on a Gel

Residual PW5 Wash Buffer may be in the final sample. To ensure complete drying of the membrane after PW5, centrifuge the spin filter in a clean 2 ml collection tube for an additional minute.

- Ethanol precipitation is the best way to remove residual Solution PW5 (See "Concentrating the DNA" below).
- If you live in a humid climate, you may experience increased difficulty with drying of the membrane in the centrifuge. Increase the centrifugation time at Step 20 by another minute or until no visible moisture remains on the membrane.

Concentrating the DNA (no new tubes are required for this process)

Your final volume will be 100 µl. If this is too diluted for your purposes, add 5 µl of 3M Sodium Acetate and mix. Then add 2 volumes of 100% cold ethanol. Mix, and incubate at -70°C for 15 minutes or -20°C for 2 hours to overnight. Centrifuge at 10,000 x *g* for 10-15 minutes at 4°C. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution PW6).

Storing DNA

DNA is eluted in 10 mM Tris (Solution PW6) and should be used immediately or stored at -20°C or -80°C to avoid degradation. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.

Cleaning of the PowerVac™ Mini Spin Filter Adapters

It is recommended to rinse the PowerVac™ Mini Spin Filter Adapters promptly after use to avoid salt build up. To clean the PowerVac™ Mini Spin Filter Adapters, rinse each adapter with DI water followed by 70% ethanol and flush into the manifold base. Alternatively, remove the adapters and wash in laboratory detergent and DI water. PowerVac™ Mini Spin Filter Adapters may be autoclaved.

Do not use bleach to clean the PowerVac™ Mini Spin Filter Adapters while attached to the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine and should not be used to clean the PowerVac™ Manifold. For more information on cleaning the PowerVac™ Manifold, please refer to the PowerVac™ Manifold manual.

Contact Information

Technical Support:
Phone: MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

Ordering Information:
Direct: Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: orders@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

For the distributor nearest you, visit our web site at www.mobio.com/distributors

15.0 ADDENDUM 2

Qubit™ dsDNA HS Assay Kits
For use with the QubitR 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ dsDNA HS Reagent (Component A)	250 µL or 1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ dsDNA HS Buffer (Component B)	50 mL or 250 mL	NA	• Room temperature	
Qubit™ dsDNA HS Standard #1 (Component C)	1 mL or 5 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit™ dsDNA HS Standard #2 (Component D)	1 mL or 5 mL	10 ng/µL in TE buffer		

NA = Not applicable.

Introduction

The Qubit™ dsDNA HS Assay Kits for use with the Qubit® 2.0 Fluorometer make DNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ dsDNA HS Assay Kits described here, we offer other kits for assaying RNA, protein, and dsDNA at a higher concentration range (*Appendix*, Table 3).

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ dsDNA HS Assay Kits

The Qubit™ dsDNA HS reagent and buffer are designed for room temperature storage. The Qubit™ dsDNA HS reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the DNA standards at 4°C.

Critical Assay Parameters

Assay Temperature

The Qubit™ dsDNA HS assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22-28˚C). The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ dsDNA HS reagent and the Qubit™ dsDNA HS buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, as the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

In order to allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). (The temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer
For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal
We must caution that no data are available addressing the mutagenicity or toxicity of the Qubit™ dsDNA HS reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO.

Treat the Qubit™ dsDNA HS reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol
The protocol below assumes you will be preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you will need fewer tubes (Step 1.1) and less working solution (Step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to Steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument.

1.1 Set up the number of 0.5 mL tubes you will need for standards and samples. The Qubit™
1.2 dsDNA HS assay requires 2 standards. **Note:** Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).
1.3 Label the tube lids.
1.4 Make the Qubit™ working solution by diluting the Qubit™ dsDNA HS reagent 1:200 in Qubit™ dsDNA HS buffer. Use a clean plastic tube each time you make Qubit™ working solution. Do not mix the working solution in a glass container. **Note:** The final volume in each tube must be 200 µL. Each standard tube will require 190 µL of Qubit™ working solution, and each sample tube will require anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.5 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.6 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles. **Note:** Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ dsDNA HS standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.7 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL. **Note:** Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.8 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.9 Allow all tubes to incubate at room temperature for 2 minutes.

1.10 On the home screen of the Qubit® 2.0 Fluorometer, press DNA, and then select dsDNA High Sensitivity as the assay type. The Standards Screen is automatically displayed. Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.11 On the Standards Screen, press Yes to run a new calibration or press No to use the last calibration.

1.12 If you pressed No on the Standards Screen, proceed to Step 1.12. If you selected Yes to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press Read. The reading will take approximately 3 seconds.

Remove Standard #1.

Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press Read. Remove Standard #2.

1.13 If you pressed No on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press Read.

1.14 Upon the completion of the measurement, the result will be displayed on the screen. **Note:** The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.15 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the

next sample, and press Read Next Sample.

1.16 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ dsDNA HS assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

Concentration of your sample = QF value × (200)x

Where:
QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation will be in ng/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up. The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed. **Note:** The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved as a .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or **Read Next Sample**. **Note:** When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

PCR, Library Preparation and MiSeq Sequencing of Samples for 16S Microbiome Analysis (D-SED-EFAB-012-SOP-01) – August 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: PCR, Library Preparation and MiSeq Sequencing of Samples for 16S Microbiome Analysis

Number: D-SED-EFAB-012-SOP-01	Effective Date: August 7, 2016

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EFAB-012-01

SOP Steward

Name: Nichole Brinkman, NERL/SED/EFAB

Approval

Name: Valerie Garcia
Title: Chief, NERL/SED/EFAB

Concurrence

Name: Brittany Stuart
Title: SED Quality Assurance Manager

STANDARD OPERATING PROCEDURES FOR 16S PCR, LIBRARY PREPARATION AND MISEQ SEQUENCING OF SAMPLES FROM NATURAL AND ARTIFICIAL TURF ATHLETIC FIELDS FOR MICROBIOME ANALYSIS – AUGUST 7, 2016

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of this SOP is to outline the steps necessary to perform 16S PCR, then prepare and sequence 16S libraries generated from samples for analysis of the microbiome.

2.0 SCOPE AND APPLICABILITY

The tasks described in this SOP are applicable to a variety of matrices, including, but not limited to, water, soil, mouse gut tissue, mouse feces, zebrafish tissue, and cat feces. Implementation of this SOP assumes that high quality DNA genomes have been prepared from samples.

The SOP outlined here has been adapted from a published method from researchers at the University of Michigan (Kozich et al. 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental Microbiology* 79(17):5112 DOI:10.1128/AEM.01043-13).

3.0 SUMMARY OF METHOD

This SOP describes the steps to amplify and barcode specific variable regions of the 16S rRNA genes extracted from samples followed by purification of amplicons and a procedure to normalize amplicon concentration across samples. Finally, normalized amplicon libraries are sequenced to assess the composition of the 16S microbiome. To accomplish these tasks, several pieces of equipment and software are needed. To amplify and barcode 16S genes in samples, a thermal cycler is needed. Amplification is verified by gel electrophoresis, which requires gel boxes and power supply. Once libraries are purified and normalized, they are quantified with the Qubit Fluorimeter or through qPCR via a real-time PCR instrument (i.e., Life Technologies StepOne Plus) or ddPCR via QX200 ddPCR system. Additionally, the average fragment size of amplicon libraries is assessed with an Agilent Bioanalyzer 2100. Finally, libraries are sequenced using an Illumina MiSeq. Analysis of data produced by the MiSeq is covered in SOP#: D-SED- EFAB-013-SOP-01 *16S rRNA Gene Sequence Analysis*. The procedures described herein involve processing 384 samples (including controls) through 12 plates of PCR reactions, 4 purification and normalization events to make 4 16S libraries that are eventually pooled and sequenced in 1 MiSeq run. The number of samples examined in a MiSeq run can be scaled up or down, depending on the desired coverage per sample.

4.0 DEFINITIONS

BSC – biological safety cabinet
BSL – biosafety level
PCR – polymerase chain reaction

PPE – personal protective equipment
Microbiome – the microorganisms that reside in a particular habitat/niche

5.0 HEALTH AND SAFETY WARNINGS

Standard laboratory PPE for Biosafety Level (BSL) 1 microorganisms is required. This PPE consists of lab coat, safety glasses and gloves. HASP# 2015-046 describes the health and safety considerations for conducting PCR. Gel electrophoresis is covered under HASP# 2016-017.

6.0 CAUTIONS/INTERFERENCES

All bacteria and archaea contain 16S rRNA genes and bacteria are everywhere, including on laboratory surfaces, equipment, and analysts. Furthermore, PCR and subsequent sequencing are sensitive tools for detecting bacteria and archaea in samples. To reduce the risk of potential contamination during the processing of samples, the analyst should wear appropriate PPE and never handle samples or reagents with bare hands. Prior to PCR, samples should only be opened in a BSC that has been sanitized with 10% bleach and UV prior to use. Where applicable, equipment (i.e., micropipettes) should be wiped with 10% bleach. After samples have been amplified by PCR, a BSC is not required for sample manipulation. However, 10% bleach should be used to disinfect benchtop surfaces before and after use to degrade residual amplicons left on lab surfaces. Also, a unidirectional workflow will be implemented to reduce prevent cross-contamination of amplicons between labs. Negative PCR controls will be used to monitor potential contamination events during these operational procedures.

PCR and sequencing are enzymatic reactions that can be disrupted if interfering substances are co-extracted and purified during sample processing. High-quality DNA must be used in these procedures.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The techniques of first-time analysts must be reviewed by an experienced analyst prior to completing this SOP alone. During this review, the new analyst must demonstrate his/her ability to complete this procedure alone which must be approved and documented by an experienced analyst/supervisor in the analyst's laboratory notebook.

8.0 EQUIPMENT AND SUPPLIES

General supplies needed for all steps

- Lab coat Safety goggles
- Laboratory gloves (nitrile or latex) Micropipettes and tips
- Low retention polypropylene microfuge tubes 10 mM Tris-HCl, pH 8.5
- Nuclease-free water

Preparation of mock microbiome positive control

- Genomic DNA of 10 bacterial species (see Table 1) Qubit HS DNA assay kit and tubes
- 10 mM Tris-HCl, pH 8.5
- Qubit Fluorimeter 2.0

Barcode assignment to samples

- Microsoft Excel
- List of barcodes with appropriate 16S V-region primer Illumina's Experiment Manager (IEM) software program

16S rRNA PCR and gel electrophoresis

- 16S Index Primers
- Roche FastStart High Fidelity PCR System, dNTP pack (cat# 4738292001) 96-well PCR plate
- Foil plate seal thermal cycler
- E-Gel 48 Agarose Gels, 2% (Invitrogen #G800802) E-Gel Mother E-Base device (Invitrogen #EBM03), TrackIt 100 bp DNA ladder (Invitrogen #10488058)
- Kodak system with UV transilluminator for visualizing DNA amplicons stained with ethidium bromide and capturing images or equivalent system

Amplicon Purification and Normalization

- SequalPrep Normalization Plate Kit (Invitrogen #A10510-01)
- 96-well storage plates, round well, 0.8ml, MIDI plate (Fisher#AB-0859) 96-well PCR plate
- Sterile reagent reservoirs 8-tube strip of PCR tubes
- Qubit HS DNA assay and tubes

Determination of Library Concentration

- Agilent High Sensitivity DNA Kit (Agilent 5067-4626) Agilent 2100 Bioanalyzer
- KAPA Library Quantification Kits (KAPABiosystems #KK4824)
- StepOne Plus Real-time PCR instrument or equivalent KAPA primers

MiSeq Sequencing

- 1N NaOH
- 16S Sequencing primers
- PhiX Control Kit v3 (Illumina #FC-110-3001)
- MiSeq Reagent Kit v3, 2x300 cycle (Illumina #MS-102-3003) Illumina MiSeq

and MiSeq Control software

9.0 PROCEDURE

Preparation of mock microbiome positive PCR and sequencing control

1. Buy lyophilized genomic DNA of 10 bacterial species from American Type Culture Collection (ATCC, Table1). Store at -80°C upon receipt.
2. Bleach BSC with 10% bleach and turn on UV lamp for at least 15 minutes.
3. Obtain DNAs (Table 1) from storage in -80°C freezer.
4. Obtain unopened bottle nuclease-free water.
5. Centrifuge each tube of lyophilized DNA at 5000 x g for 15 seconds to collect the contents at the bottom of the tube.
6. Add 1 ml (1000 µl) of nuclease-free water to each tube of DNA. Vortex well.
7. Incubate resuspended DNA at 37°C for 1 hour.
8. Vortex resuspended DNA well then centrifuge at 5000 x g for 15 seconds to collect contents.
9. Determine DNA concentration using 10 µl of resuspended DNA and the Qubit (as instructed below in "Qubit analysis of samples"). If concentrations are outside the range of the assay (0.2 – 100 ng), dilute the DNA 10-fold and redo Qubit analysis.
10. Calculate the volume of each bacterial species' resuspended genomic DNA needed to make a dilution to 0.2 ng/µl (or appropriate concentration, see Equation 2).
11. Add the volume of resuspended genomic DNA calculated above to a microfuge tube (see Equation 3). Repeat for each bacterial species.
12. Bring the volume of the pooled bacterial species DNA to the appropriate volume with 10 mM Tris-HCl, pH 8.5.
13. Check concentration via Qubit. The concentration should be within 30% CV of 0.2 ng/µl (0.12-0.26 ng/µl). If concentration is within the desired range, proceed to next step. If the concentration is not within the desired range, prepare a new pool of genomic DNA and use Qubit to determine concentration.
14. Prepare 25 aliquots of mock microbiome genomic DNA by transferring 20 µl of pooled DNA into sterile 1.5 ml tubes labeled "Mock Microbiome, 0.2 ng/µl, date, initials."
15. Store one-time use aliquots in -80°C freezer.

Qubit analysis of samples

16. Prepare the Qubit working solution by adding 1 µl of Qubit dsDNA HS Reagent and 199 µl of Qubit dsDNA HS buffer for each sample to be analyzed. Make a volume that will accommodate all samples plus 2 standards.
17. Label 0.5 ml tubes for each sample and standard to be analyzed.
18. Add 190 µl of Qubit working solution into each of the tubes used for standards.
19. Add 10 µl of each Qubit standard (provided with the kit) to the appropriate tube and mix by vortexing 2-3 seconds, being careful not to create bubbles.
20. Add the appropriate sample volume to a 0.5 ml tube. The sample can be anywhere between 1 and 20 µl.

21. Add the appropriate volume of Qubit working solution to each tube containing sample such that the final volume is 200 µl.
22. Mix by vortexing 2-3 seconds.
23. Allow reactions to incubate at room temperature for 2 minutes.
24. On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **dsDNA High Sensitivity** as the assay type. The Standards Screen is automatically displayed
25. On the Standards Screen, press **Yes** to run a new calibration.
26. Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds. Remove Standard #1.
27. Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. Remove Standard #2.
28. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. Upon the completion of the measurement, the result will be displayed on the screen. **Note:** The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see Equation 1 in Data and Records Management, below) or the Qubit® 2.0 Fluorometer performs this calculation for you by following steps 29 to 33.
29. Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.
30. Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.
31. To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.
32. Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up. The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed. **Note:** The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).
33. Record this value.
34. To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or **Read Next Sample**.
35. To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.
36. Repeat sample readings until all samples have been read.

Barcode assignment to samples

37. Make a spreadsheet that lists the 90 samples along with negative and positive PCR controls to create a library of 96 samples for sequencing
38. Fill in sample identifiers for each sample.
39. Add a column of underscores between each sample column of identifiers.
40. Create another column and concatenate sample info to create a string that includes all sample info. Use "…, TEXT(cell, "yyyy, mm, dd")," to keep the date format. The name should look like: Tire_264_KY123_2016-04-01_S1.
41. Open IEM software and choose to make a new plate.
42. List the sample IDs for a library of 96 samples (note – we currently prepare 4 libraries for each 16S sequencing run on the MiSeq).
43. Add i7 and i5 barcodes that will be assigned to each sample.
44. Save prepared plate as a .plt file for making a sample sheet.
45. Use barcode assignments for PCR reactions below.
46. Use IEM to create a sample sheet using the .plt files for all libraries to be sequenced. Transfer the sample sheet to the Lablan for upload to the instrument.

PCR of 16S rRNA gene and analysis of amplification by gel electrophoresis

47. In a PCR clean room, bleach PCR hood and turn on UV lamp for at least 15 minutes.
48. Wipe the barrels of all micropipettes to be used with 10% bleach
49. Reconstitute indexed primers to 100 µM (see Kozich et al. Supplemental Material for primer design).
50. Dilute indexed primer stocks to 10 µM working stocks.
51. Prepare PCR master mix as shown in Table 2.
52. Move to DNA lab. Bleach BSC with 10% bleach and turn on UV lamp for at least 15 minutes.
53. Wipe the barrels of all micropipettes to be used with 10% bleach.
54. Obtain the barcoded primers diluted to 10uM from the -20°C freezer and thaw on ice.
55. Obtain a tube of prepared mock microbiome DNA from -80°C freezer and thaw on ice.
56. Obtain the DNA extracts of samples from -80°C freezer and thaw on ice.
57. Prepare dilutions of sample extracts in 10 mM Tris-HCl, pH 8.5 to 0.2 ng/µl (see Equation 2 and Equation 3).
58. Add 5 l of the sample extract to the appropriate wells of the 96-well reaction plate. Samples will be run in triplicate. Table 3 is an example of the 96-well PCR plate set up.
59. Add 5 l of 10 mM Tris-HCl, pH 8.5 to the negative control wells (see Table 3).
60. Add 5 l of mock microbiome DNA to the positive control wells (see Table 3).
61. Add 41 µl of the PCR master mix to the corresponding wells of the plate (see Table 3).
62. Add 2 ul of the appropriate forward and reverse primers to the wells of the

plate (see Table 3).

63. Cover plate with foil adhesive. Mix and centrifuge plate.
64. Run reactions through PCR cycling in the MJ 200-PTC with the following default conditions: 95°C for 2 minutes, then 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds; and 72°C for 1 minute. Followed by a final extension step of 72°C for 10 minutes then a 4°C soak.
65. Plug the E-Gel Mother Base into an electrical outlet using the adaptor plug on the base.
66. Set the run time to 20 minutes.
67. Remove the 2% agarose gel from the package. Record the lot number and expiration date of the gel and DNA ladder.
68. Remove the plastic comb and insert the gel into the base such that the two copper electrodes on the right side of the gel cassette come in contact with the two electrode connections on the base. When the gel is properly inserted into the base, the fan in the base turns on and a red light illuminates at the left corner of the base.
69. Load 10 µl of each PCR reaction into the wells of the gel.
70. Load 5 µl of the 100 bp ladder into the "M" wells of the gel. Add 8 µl of nuclease-free water to the same well.
71. Press and release the pwr/prg button to begin electrophoresis. The red light changes to green and the display counts down the time remaining on the run. At the end of the run, the current automatically shuts off and the power base signals the end of the run with a flashing light and rapid beeping.
72. Press and release the pwr/prg button to stop the beeping. Remove the gel cassette.
73. Use the Kodak 1D system and transilluminator to visualize the results.
74. Verify QC of PCR by evaluating positive and negative PCR controls (see Section 11.0).
75. Save the gel images as .jpeg files.
76. Re-cover top of PCR plate with another piece of adhesive foil. Use a Sharpie and label plate with name, date and project and PCR number. Store plate of amplicons in -20°C freezer.

Amplicon Purification and Normalization

77. Obtain 3 plates of 16S PCR reactions from the -20°C freezer.
78. Obtain a MIDI plate. Transfer the replicate 16S PCR reactions of samples by pooling into 1 well of the MIDI plate. Label the MIDI plate "Date, project, pooled PCR amplicons, library name, initials."
79. Mix pooled reactions well by pipetting up and down 3-5 times. Transfer 25 µl of pooled PCR product from the MIDI plate into the wells of a SequalPrep™ Normalization plate.
80. Add an equivalent volume of SequalPrep™ Normalization Binding Buffer, mix completely by pipetting up and down.
81. Incubate the plate for 1 hour at room temperature to allow binding of DNA to the plate surface. Mixing is not necessary at this stage.
82. Transfer the amplicon/Binding Buffer mixture to another, fresh

SequalPrep™ Normalization plate (labeled #2) to sequentially bind more DNA.

83. Allow DNA to bind to sides of the plate #2 at room temperature for 1 hour.
84. To plate #1, add 50 µl SequalPrep™ Normalization Wash Buffer to the wells using 200 µl tips. With the same tips, mix by pipetting up and down twice to improve removal of contaminants, then completely aspirate the buffer from wells and discard. Be sure not to scrape the well sides during aspiration.
85. Using 10 µl tips, remove remaining liquid from wells. A small amount of residual wash buffer (1–3 µl) is typical and does not affect the subsequent elution or downstream applications.
86. Add 20 µl SequalPrep™ Normalization Elution Buffer to each well of the plate #1 using the 10 µl tips.
87. Mix by pipetting up and down 5 times. Ensure that the buffer contacts the entire plate coating (up to 20 µl level).
88. Incubate at room temperature for 5 minutes. Hold eluate of the plate #1 until the plate #2 needs to be eluted. Use the 20 µl eluate from plate #1 to elute the second plate.
89. After the 1-hour incubation of the plate #2, aspirate and discard the liquid from wells using the 200 µl tips. Be sure not to scrape the well sides during aspiration.
90. Repeat washing steps (84-85) for second plate. Use the 20 µl eluate from plate #1 to elute the DNA from plate #2 (instead of adding fresh SequalPrep™ Normalization Elution Buffer).
91. After elution of the plate #2, transfer the purified and normalized amplicons to a clean 96-well plate. Label the plate "Date, project, SequalPrep Products, library name, initials."
92. To prepare a library, use the multichannel pipette and 10 µl tips, remove 5 µl from each well and transfer into an 8-tube strip. Then combine the contents of each tube in the 8-tube strip to a sterile, RNase-, DNase-, DNA-free 1.5 ml tube, labeled "date, project, library, initials."
93. Vortex pooled samples and transfer 5 µl to a Qubit tube for determination of DNA yield.
94. Seal the 96 well plate of purified, normalized amplicons with foil and store at -20°C freezer.
95. Store DNA library at -20°C freezer.
96. Seal midi plate of pooled 16S amplicons (those not purified with SequalPrep) with foil and store at -20°C freezer.
97. Determine the concentration of library using the Qubit HS DNA assay.

KAPA Q-PCR Library Quantification

98. In PCR clean room, add 1 ml Primer Premix (10X) to the 5 ml bottle of KAPA SYBR FAST Q-PCR Master Mix (2X) and mix by vortexing for 10 seconds. Record the date of Primer Premix addition on the KAPA SYBR FAST Q-PCR Master Mix bottle.
99. Prepare the following dilutions of each library in 10 mM Tris-HCl, pH 8.5:

a. 1:1
b. 1:10
c. 1:1000
d. 1:2000
e. 1:4000

100. Dispense 6 µl of master mix into the wells of a 96 well qPCR plate.
101. Add 4 µl of each library dilution and standards (6) to the appropriate wells of the PCR plate. Record lot numbers of standards used in lab notebook. All samples should be run in triplicate.
102. Cover plate with optical adhesive plate seal.
103. Vortex and centrifuge plate.
104. Set up a run in the StepOne Plus instrument.
105. Use the following values for standards concentration:
    a. Standard 1 = 20 pM
    b. Standard 2 = 2 pM
    c. Standard 3 = 0.2 pM
    d. Standard 4 = 0.02 pM
    e. Standard 5 = 0.002 pM
    f. Standard 6 = 0.0002 pM
106. Run plate in StepOne Plus instrument using the following conditions: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds and 60°C for 45 seconds. Add a melt curve analysis at the end of the run to check for primer/adaptor dimer.
107. Find the DNA concentration determined from the standard curve for each dilution of the library as determined by the StepOne Plus software. The standard curve must demonstrate an amplification efficiency between 90-110% and the R2 value must be ≥ 0.99.
108. Determine the library concentration by taking the average of the triplicate data points corresponding to the most concentrated library DNA dilution that falls within the dynamic range of the DNA Standards.

Agilent Library Fragment Size Analysis

109. Obtain libraries from -20°C freezer.
110. Obtain Agilent HS DNA Kit from 4°C fridge and allow to equilibrate to room temperature for 30 minutes.
111. Prepare 1:1 and 1:10 dilutions of libraries in 10 mM Tris-HCl, pH 8.5.
112. Add 15 µl of High Sensitivity DNA Dye concentrate (blue cap) to a High Sensitivity DNA gel matrix vial (red cap).
113. Vortex solution well and spin down. Transfer to spin filter provided with kit.
114. Centrifuge at 2240 x g for 10 minutes. Protect solution from light. Use prepared gel-dye mix within 6 weeks of preparation.
115. Put a new High Sensitivity DNA chip on the chip priming station.
116. Pipet 9 µl of the gel dye mix in the well, marked “G” with black background.
117. Make sure that the plunger is positioned at 1 ml and then close the chip priming station.

118. Press plunger until it is held by the clip.
119. Wait for exactly 60 seconds using a timer, then release clip.
120. Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.
121. Open the chip priming station and pipette 9 µl of gel-dye mix into the wells marked with a "G" with a gray background.
122. Pipette 5 µl of the marker (green cap) in all sample and ladder wells. Do not leave any wells empty.
123. Pipette 1 µl of High Sensitivity DNA ladder (yellow cap) in the well marked with a ladder.
124. In each of the 11 sample wells, pipette 1 µl of sample or 1 µl of marker.
125. Put the chip horizontally in the adapter and vortex for 1 minute at the indicated setting (2400 rpm).
126. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 minutes.
127. Export electropherograms and report.
128. Determine the average peak size by evaluating the fragment sizes assessed from the Bioanlayzer for each library.

Create final library pool for sequencing

129. Calculate library concentration using equation 4 or equation 5 (see Section 10.0 below).
130. If sequencing more than 1 library, dilute the libraries to the concentration of the least concentrated library using 10 mM Tris-HCl, pH 8.5.
131. Create a single final 16S library by adding 15 µl of each normalized library to a single 1.5 ml microfuge tube. Label with date, project, pooled 16S libraries, library concentration, and initials.
132. Store final library at -20°C until ready to sequence.

MiSeq sequencing of libraries

133. Prepare Sample Sheet using IEM and .plt files previously prepared.
134. Obtain an aliquot of the sequencing primers (Read 1, index, and Read 2 primers) at 100 µM and PhiX v3 control library from the -20°C freezer.
135. Bleach counter space. Wipe the barrels of all micropipettors to be used with 10% bleach.
136. Obtain the final 16S library pool from the -20°C freezer.
137. Remove a 2x300 cycle reagent cartridge from the -20°C freezer. Place in room temperature water bath for 1 hour. Place HT1 buffer tube in 4°C fridge to thaw.
138. When the reagent cartridge has thawed, dry bottom with paper towel. Invert the cartridge repeatedly (20 times) to check each well is thawed. This also serves to mix the reagents. Place on ice.
139. Place 3 µl of the 100 µM Read 1 Sequencing Primer(s) into a clean PCR tube. Repeat in separate tubes for the Index Primer(s) and Read 2 Sequencing Primer(s).
140. Using a 1000 µl pipette tip, break the foil over wells 12, 13, and 14 of the reagent cartridge.

141. Use a sterile 1 ml pipette to remove some liquid, approximately 0.5 ml from well 12 and transfer to the tube with the Read 1 sequencing primer. Vortex this solution then transfer the entire contents back to well 12.
142. Use a sterile 1 ml pipette to remove some liquid from well 13 and transfer to the tube with the Index primer. Vortex this solution well then transfer the entire contents back to well 13.
143. Use a sterile 1 ml pipette to remove some liquid from well 14 and transfer to the tube with the Read 2 sequencing primer. Vortex this solution well then transfer the entire contents back to well 14.
144. Prepare a fresh dilution of 0.2N NaOH.
145. To a sterile 1.5ml tube add 10 µl of 16S DNA library, and 10 µl of 0.2N NaOH.
146. To a separate tube add 2 µl PhiX library (10 nM), 3 µl PCR grade water, and 5 µl of 0.2N NaOH.
147. Vortex both tubes to mix and spin for 1 minute at 400rcf. **Note: NaOH concentration on the flow cell must remain under 0.001N.** Adjusting the concentration of the NaOH used to denature the DNA to 0.1N may be necessary if library concentration is 1 nM or below.
148. Allow the libraries to incubate at room temperature for 5 minutes. Immediately add 980 µl of HT1 to the 16S DNA library, and 990 µl HT1 to the PhiX library and place on ice.
149. Use HT1 to further dilute both libraries (16S and PhiX) to 8 pM. Keep libraries.
150. Combine the libraries such that it contains 30% PhiX library to increase overall diversity of the library - combine 700 µl of 8.0pM 16S Library and 300 µl 8 pM PhiX library in a final tube. Vortex.
151. Use a 1000 µ tip and break the foil over well 17 of the reagent cartridge.
152. Load 600 µl of the 8 pM 16S DNA library:PhiX library solution into well 17 on the reagent cartridge.
153. Set reagent cartridge aside. Unbox flow cell and PR2 bottle.
154. Thoroughly rinse the flow cell with Milli-Q water. Carefully dry by blotting with lint free wipes. Give special attention to the edges and points of intersection between the glass and plastic.
155. Visually inspect the flow cell to ensure there are no blemishes, particles, or fibers on the glass.
156. If necessary, wet a new wipe with 100% alcohol and wipe the glass on both sides avoiding the rubber intake ports.
157. Open Illumina Control software on the MiSeq instrument. Follow on screen instructions and load the flow cell, reagent cartridge, and PR2 bottle. Empty and replace the waste bottle.
158. Ensure the machine recognizes the correct sample sheet and the run parameters are correct.
159. Wait for the MiSeq to perform its pre-run checks, and press start. Record run in log book.
160. A 2x300 cycle run takes approximately 55 hours to complete. Once the run has finished, record the run metrics (Number of clusters/mm^2, percent of clusters passing the filter, estimated yield of data, percent of reads passing Q30) displayed on the MiSeq instrument.

161. Transfer the contents of the waste bottle to the satellite waste container then replace waste bottle.
162. Perform the post run wash with 0.5% Tween 20 buffer to flush the fluidics lines.
163. Transfer the data from the instrument to the LabLan using Syncovery.
164. Analysis of the data is described in D-SED-EFAB-013-SOP-01.

10.0 DATA AND RECORDS MANAGEMENT

Calculations

Calculate the concentration of the sample measured by Qubit

$$\text{Concentration of sample} = \text{QF value} \times \left(\frac{200}{x}\right) \qquad \text{Equation 1}$$

Where: QF value is the value given by the Qubit 2.0 Fluorometer and *x* is the number of microliters of sample added to the assay.

Calculate the volume of extract needed to make a dilution that contains 0.2 ng/µl in 25µl

$$V1 = \frac{(M2V2}{M1)} \qquad \text{Equation 2}$$

Where: $M1$ is the concentration of DNA in the sample in ng/µl, $V1$ is the volume of extract needed to make a dilution that contains 0.2 ng/µl in 25µl (µl), $M2$ is the total concentration of DNA required in 25 µl (i.e.5 ng) and $V2$ is the total volume of the dilution (25 µl).

Calculate the volume of 10 mM Tris needed to dilute extracts to 0.2 ng/µl in 25µl

$$\text{Volume of 10 mM Tris-HCl, pH 8.5 (µl)} = 25\ \mu l - X \qquad \text{Equation 3}$$

Where: X is the volume of extract required to make a dilution that contains 0.2 ng/µl in 25µl (µl)

Calculate the 16S library concentration

a) if qPCR was used to assess library concentration

$$X = D\left(\frac{452}{F}\right)Y \qquad \text{Equation 4}$$

Where: X is the library concentration in pM, D is the DNA concentration in pM determined from KAPA qPCR, F is the average fragment length from the bioanlayzer and Y is the dilution factor.

b) if Qubit was used to assess library concentration

$$X = DD_{653.9} \times S \qquad \text{Equation 5}$$

$$1000 \qquad \overline{1000}$$

Where: D is the 16S library DNA concentration in ng/µl and S is the length of the amplicon in bp

Data Storage

For each run, the MiSeq prepares the directories containing the data named:

YYMMDD(start of run)_M01250(instrument serial number)_XXXX(instrument run number)_000000000_XXXXX(Flow cell lot number).

Within the directory, the data will be in the form of .fastq.gz (zipped) files. Filenames containing the sequence data will be the sample names used in the sample sheet_L001_R1/R2(read direction)_001. The entire directory of data will be transferred to the LabLan using Syncovery to ensure the integrity of the file copy.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

The quality control procedures required to demonstrate successful performance of PCR and MiSeq sequencing include the use of positive and negative controls. To ensure quality of data collection during PCR, a negative control consisting of the buffer used to dilute samples will be used. This should result in the absence of a band after gel electrophoresis. A positive control for PCR consists of a collection of genomic DNA from 10 bacterial species, referred to as the mock microbiome. This should result in a band of the correct amplicon size after gel electrophoresis. The mock microbiome is also used as a positive control for sequencing and will be used to calculate sequencing error. A library control prepared by Illumina (PhiX control) will also be used to assess performance of the sequencing run.

The procedures for preparing the mock microbiome to control for PCR and sequencing quality are included in this SOP. The PhiX sequencing control is available from Illumina.

PCR positive and negative controls will be run with each plate of samples (we typically run 30 samples in triplicate on each PCR plate). The positive and negative controls from 12 PCR plates eventually get pooled for sequencing. In addition, every sequencing run will contain the prepared PhiX genome library, spiked in at 30% of the total DNA included in the sequencing run.

If the PCR negative controls result in a band or the PCR positive controls result in the absence of a band, the amplicons will be discarded and the PCR will be repeated. If the sequencing error (as determined from the mock microbiome) is high (i.e., higher than 3%, the cutoff for taxonomic classification) or the PhiX analysis is aberrant (i.e., the percentage of reads aligned to the PhiX genome are not reflective of the percent of PhiX spiked into the library pool), the data will be interpreted with caution. However, if funds are available, the sequencing run will be repeated.

The results of the quality control procedures will be documented in the analysts' notebook. All QC failures will be reported to the project lead. A discussion of the overall QC procedures and results will be described in the manuscript.

12.0 REFERENCES

- Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology* 79(17):5112. DOI: 10.1128/AEM.01043-13.

13.0 DOCUMENT HISTORY

Date of Revision	Revision #	Description of Changes
8/7/2016	0	First version

14.0 TABLES

Table 1. Bacterial species and ATCC numbers for mock microbiome

Bacterial species	ATCC Number
Streptococcus pneumoniae	BAA334D-5
Staphylococcus aureus	BAA-1718D-5
Porphyromonas gingivalis	33277D-5
Neisseria meningitides	BAA335D-5
Listeria monocytogenes	BAA-679D-5
Lactobacillus gasseri	33323D-5
Deinococcus radiodurans	1393D-5
Acinetobacter baumanni	17978D-5
Bacillus cereus	10987D-5
Rhodobacter sphaeroides	17023D-5

Table 2. Preparation of 16S PCR Master Mix

Components	Volume per PCR Reaction	Volume per Master Mix 102 total reactions
10X Reaction Buffer	5	510
Nuclease-free water	33.5	3417
DMSO	1	102
10 mM dNTPS	1	102
Enzyme Blend (5U/µl)	0.5	51
Total	41	4182

Table 3. 96-well plate layout of samples and 16S PCR primers with embedded barcodes

	Row→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Col ↓	Primer ↓→	VX-7YY			VX-7YY			VX-7YY			VX-7YY		
A	VX-5ZZ	Sample 1			Sample 2			Sample 3			Sample 4		
B	VX-5ZZ	Sample 5			Sample 6			Sample 7			Sample 8		
C	VX-5ZZ	Sample 9			Sample 10			Sample 11			Sample 12		
D	VX-5ZZ	Sample 13			Sample 14			Sample 15			Sample 16		
E	VX-5ZZ	Sample 17			Sample 18			Sample 19			Sample 20		
F	VX-5ZZ	Sample 21			Sample 22			Sample 23			Sample 24		
G	VX-5ZZ	Sample 25			Sample 26			Sample 27			Sample 28		
H	VX-5ZZ	Sample 29			Sample 30			Mock Microbiome			NTC		

Droplet Digital PCR (ddPCR) Analysis of Genomic Targets (D-SED-EFAB-014-SOP-01) – August 2016

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Droplet Digital PCR (ddPCR) Analysis of Genomic Targets

Number: D-SED-EFAB-014-SOP-01	Effective Date: August 16, 2016

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EFAB-014-01

SOP Steward

Name: Nichole Brinkman, Environmental Futures Analysis Branch

Approval

Name: Valerie Garcia
Title: Chief, Environmental Futures Analysis Branch

Concurrence*

Name: Brittany Stuart
Title: SED Quality Assurance Manager

* Optional Field
NERL-SOP.2(11/2005)

STANDARD OPERATING PROCEDURE FOR DROPLET DIGITAL PCR (DDPCR) ANALYSIS OF GENOMIC TARGETS – AUGUST 16, 2016

TABLE OF CONTENTS

1.0 PURPOSE

This standard operating procedure (SOP) describes the amplification and quantitation of genomic targets using droplet digital PCR (ddPCR).

2.0 SCOPE AND APPLICABILITY

ddPCR can be used to quantify RNA and DNA targets of prokaryotes, eukaryotes, archaea, and viruses in a variety of sample matrices, including, but not limited to, water, air, and rubber surfaces. The use of this procedure is contingent upon sample preparation that releases RNA and/or DNA from cells or viral capsids.

3.0 SUMMARY OF METHOD

The use of quantitative PCR (qPCR) to determine the concentrations of targeted microbes in environmental samples has become a standard tool used in microbiology because it is sensitive and specific for the intended microbial targets. The quantitative nature of qPCR relies on the linear relationship that exists between log10 target sequences in a sample and the number of thermal cycles needed to reach a defined threshold (often referred to as the cycle threshold) where fluorescence is detectable above background but is still increasing exponentially due to PCR amplification. Fluorescence can be produced either by the binding of intercalating dyes or by the cleavage of target sequence specific probes that both occur stoichiometrically with the generation of amplified target sequence fragments. Based on this relationship, standard curves of log10 target sequences vs. cycle threshold measurements can be generated from well characterized source material (standards) using a linear regression model and target sequences in unknown samples can be quantified by interpolation of their cycle threshold measurements on the standard curves. In this way, qPCR provides quantitation of unknown samples relative to known standards.

Despite the utility of qPCR in many environmental applications, limitations inherent in the linear regression approach can lead to inconclusive results such as when target concentrations of unknown samples fall outside of the dynamic range of the standard curve and when PCR inhibitors are present in the sample. In the former scenario, quantities of target sequences in unknown samples become inaccurate or are unable to be determined. While in the latter scenario, PCR inhibition directly affects amplification efficiency resulting in an underestimation of the true target sequence concentration in the sample.

Digital PCR (dPCR) utilizes the components of qPCR, but eliminates the need for a standard curve. Therefore, the disadvantages intrinsic to quantity estimates inferred from a curve are also eliminated. However, quantitation is still feasible and offers precision in quantity determinations beyond the capability of qPCR. In dPCR, samples are portioned into thousands of nanoliter-sized PCR reactions such that each reaction contains zero or one or more DNA templates. After endpoint PCR amplification, each reaction is screened to determine whether amplification occurred. The number of positive and negative

reactions is scored and used to determine the number of DNA molecules (λ) present in the reaction using the Poisson distribution from which the following equation is derived:

$$\lambda\lambda = -\ln 1 - \frac{xx}{nn}$$

Where x is the number of positive PCR reactions and n is the total number of reactions.

Droplet digital PCR (ddPCR) uses an oil-based approach to partition the nanoliter reactions. This SOP describes the steps to perform ddPCR using BioRad's QX200 ddPCR system.

4.0 DEFINITIONS

ADG – automated droplet generator
BSC – biological safety cabinet
BSL – biosafety level
ddPCR – droplet digital
PCR DNA – deoxyribonucleic acid
PCR – polymerase chain reaction
RNA – ribonucleic acid
RT-ddPCR – reverse transcriptase-ddPCR
PPE – personal protective equipment

5.0 HEALTH AND SAFETY WARNINGS

Standard laboratory PPE for Biosafety Level (BSL) 1 microorganisms is required. This PPE consists of lab coat, safety glasses, and gloves.

HASP# 2015-046 describes the health and safety considerations for conducting PCR. Use of the ddPCR reader in the post-PCR lab is covered under HASP# 2016-017.

6.0 CAUTIONS/INTERFERENCES

PCR is a sensitive tool for detecting genomic targets in samples. To reduce the risk of potential contamination during the processing of samples, the analyst should wear appropriate PPE and never handle samples or reagents with bare hands. Prior to PCR, samples should only be opened in a BSC that has been sanitized with 10% bleach and UV prior to use. Where applicable, equipment (i.e., micropipettes) should be wiped with 10% bleach. After samples have been amplified by PCR, a BSC is not required for sample manipulation. However, 10% bleach should be used to disinfect bench top surfaces before and after use to degrade residual amplicons left on lab surfaces. Also, a unidirectional work-flow will be implemented to prevent cross-contamination of amplicons between labs. Negative PCR controls will be used to monitor potential contamination events during these operational procedures. Samples and master mixes containing enzymes should be

kept on ice or in a cold block to maintain integrity/activity during PCR set up.

Care should be taken when generating droplets in the manual mode. Air bubbles can cover the bottom of the well and result in 2,500-7,000 fewer droplets and poor data quality. They are difficult to see. To avoid creating air bubbles, use the pipetting technique described in detail in Section 9 "Manual Droplet Generation," Step 3. These techniques ensure samples wet the bottoms of the wells so they are wicked into the microchannels (necessary for proper droplet generation).

PCR is an enzymatic reaction that can be disrupted if interfering substances are co-extracted and purified during sample processing. For best results, high-quality DNA should be used in these procedures.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The techniques of first-time analysts must be reviewed by an experienced analyst prior to completing this SOP alone. During this review, the new analyst must demonstrate his/her ability to complete this procedure alone which must be approved and documented by an experienced analyst/supervisor in the analyst's laboratory notebook.

8.0 EQUIPMENT AND SUPPLIES

General supplies needed for all steps

- Lab coat
- Safety goggles
- Laboratory gloves (nitrile or latex) Micropipettes and tips
- Low retention polypropylene microfuge tubes 10 mM Tris-HCl, pH 8.5

ddPCR or RT-ddPCR

- Forward primer
- Reverse primer
- Dual-labeled probe (non-fluorescent quencher must be used with BioRad platform)
- ddPCR Droplet Reader Oil (BioRad cat# 1863004)
- Automated Droplet Generation Oil for Probes (BioRad cat# 1864110)
- Droplet Generation Oil for Probes (BioRad cat# 1863005)
- QX200 Droplet Generation Oil for EvaGreen (BioRad cat# 1864006)
- AutoDG Oil for EvaGreen (BioRad cat# 1864112)
- DG8 Cartridges and Gaskets (BioRad cat# 1864007)
- DG32 Automated Droplet Generator Cartridges (BioRad cat# 1864108)
- Pipet Tips for the Auto DG (BioRad cat# 1864120)
- Pipet Tip Waste bins for the AutoDG System (BioRad cat# 1864125)
- ddPCR Supermix for Probes (no dUTP) (BioRad cat# 1863024)
- One-Step RT-ddPCR Advanced Kit for Probes (BioRad cat# 1864021)

- QX200 ddPCR EvaGreen Supermix (BioRad cat#186-4033)
- Eppendorf 96-well twin.tec PCR plates (Eppendorf cat# 951020346)
- Pierceable Foil Heat Seal (BioRad cat# 1814040)
- Microseal-A Film (BioRad cat# MSA5001)
- Rainin Tips LTS 20UL Filter RT-L10FLR (Rainin cat #17007957)
- Rainin Pipet-Lite Multi Pipette L8-50XLS= (Rainin cat #17013804)

The QX200 AutoDG Droplet Digital PCR System consists of several pieces of instrumentation to perform ddPCR analysis. PCR reactions are sequestered into thousands of droplets using either the Droplet Generator or Automated Droplet Generator. PCR plates are sealed using the PX1 PCR Plate Sealer. PCR is performed in a C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module. PCR amplification is assessed with the QX200 Droplet Reader and QuantaSoft Software.

9.0 PROCEDURE

Preparation of Positive (RT-)ddPCR Controls for the FAM and VIC/HEX Channels

1. Identify material to be used as a positive control. This can be a genomic extract of the organism of interest or a synthetic construct, like a minigene containing the targeted genomic sequence.
2. Determine the concentration of the DNA stock to be used as a positive control.
3. Dilute the positive control stock to 3.2×10^4 genomic copies/ul in a volume sufficient to prepare the desired number of aliquots. This quantity equates to approximately 10 copies of the ddPCR target per droplet, assuming an average of 16,000 droplets per reaction.
4. Prepare 25 µl aliquots of the positive control and store at -80°C.

Preparation of Exogenous Internal Amplification Controls

1. Identify material to be used as an exogenous internal amplification control. This can be a genomic extract of a specific organism or a synthetic construct, like a minigene containing the targeted genomic sequence.
2. Determine the concentration of the DNA stock to be used as an exogenous internal amplification control.
3. Dilute the exogenous internal amplification control stock to 1.3×10^4 genomic copies/ul in a volume sufficient to prepare the desired number of aliquots. This quantity equates to approximately 1.6 copies of the ddPCR target per droplet, assuming an average of 16,000 droplets per reaction.
4. Prepare 200 µl aliquots of the exogenous internal amplification control and store at -80°C.

Preparation of (RT-)ddPCR Reactions

1. Bleach PCR hood in clean room. Turn on UV for at least 15 minutes.
2. Wipe the barrels of all micropipettors to be used with 10% bleach.

3. In PCR hood, prepare the ddPCR or RT-ddPCR mixes, examples are shown in Section 14, Tables 1-2 below. Move to DNA lab.
4. Bleach biosafety cabinet in DNA lab. Turn on UV for at least 15 minutes. Wipe the barrels of all micropipettors to be used with 10% bleach.
5. Obtain samples, positive controls and internal amplification control aliquots from the -80°C freezer.
6. Prepare dilutions of samples as indicated in Table 3 with 10 mM Tris-HCl, pH 8.5.
7. Obtain an Eppendorf 96-well twin.tec PCR plate (these are the ONLY plates that fit in the ADG and reader).
8. Dispense 18 µl of the ddPCR/RT-ddPCR master mix into the 5 NTC wells as outlined in Table 3.
9. To the ddPCR/RT-ddPCR negative control (NTC) wells, load 7 µl of 10 mM Tris-HCl, pH 8.5 to the corresponding wells of the plate as indicated in Table 3.
10. To the remaining ddPCR/RT-ddPCR master mix, add 2 µl of the exogenous internal amplification control (1.3×10^4 genomic copies/ul) for the remaining reactions: 2 x (100- 5) = 190µl.
11. Dispense 20 µl of the ddPCR/RT-ddPCR master mix with the exogenous internal amplification control to the remaining reactions in the 96 well plate as indicated in Table 3.
12. Add 5 µl of the appropriate positive control sample to the positive control wells as indicated in Table 3.
13. Add 5 µl of the appropriate sample to the wells as indicated in Table 3.
14. If using the ADG to make droplets, seal the plate with the appropriate foil seals and PX1 PCR Plate Sealer. If using the manual Droplet Generator to make droplets, seal the plate with Microseal-A.
15. Mix plate thoroughly by vortexing briefly, followed by centrifugation at 500 x g for 15 seconds.
16. Allow the reactions to equilibrate to room temperature for about 3 minutes before loading into the droplet generator.

Manual Droplet Generation

1. The QX200 droplet generator prepares droplets for up to eight samples at time. Droplet generation takes ~ 2 minutes for each set of 8 samples (~30 minutes for a 96-well plate).
2. All 8 sample wells in the DG8 droplet generator cartridge must contain sample (or 1X buffer control), and all 8 oil wells must contain droplet generator oil.
3. Do not load sample or oil into the DG8 cartridge unless it is inserted in the holder.
4. Insert the DG8 cartridge into the holder with the notch in the cartridge at the upper left of holder.
    a. Open the cartridge holder by pressing the latches in the middle.
    b. Slide the DG8 cartridge into the right half of the holder, then drop it down.
    c. Press the halves of the holder together to snap it closed.
5. Dispense the 80 µl of droplet generator oil for each reaction in the reagent reservoir.
6. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge **with the considerations listed below**:

   a. Use only 20 µl aerosol-barrier (filtered) Rainin pipet tips; do not use 200 µl tips.
   b. Gently slide the pipet tip down the side of the well at a ~15° angle until it passes over the ridge near the bottom. Holding the angle, ground the pipet tip against the bottom edge of the sample well while slowly dispensing a small portion of the sample; do not pipet directly onto the side (wall) of the well.
   c. After dispensing about half the sample, slowly draw the tip up the wall while dispensing the rest of the sample; do not push the pipet plunger past the first stop.
7. Using a multichannel pipet, fill each oil well (bottom row) with 70 µl of DG oil from the reagent reservoir.
8. Hook the gasket over the cartridge holder using the holes on both sides. The gasket must be securely hooked on both ends of the holder; otherwise, pressure sufficient for droplet generation will not be achieved.
9. Open the QX200 droplet generator by pressing the button on the green top and place the cartridge holder into the instrument. When the holder is in the correct position, both the power (left light) and holder (middle light) indicator lights are green.
10. Press the button on the top again to close the door. This initiates droplet generation: a manifold positions itself over the outlet wells, drawing oil and sample through the microfluidic channels, where droplets are created. Droplets flow to the droplet well, where they accumulate. The droplet indicator light (at right) flashes green after 10 seconds to indicate droplet generation is in progress.
11. When droplet generation is complete, all three indicator lights are solid green. Open the door by pressing the button and remove the holder (with DG8 cartridge still in place) from the unit. Remove the disposable gasket from the holder and discard it. The top wells of the cartridge contain droplets, and the middle and lower wells are nearly empty with a small amount of residual oil.
12. Pipet 40 µl of the contents of the top wells (the droplets) into a single column of a 96-well PCR plate, **using the following pipetting techniques to avoid shearing or coalescing the droplets**:
   a. Use an 8-channel manual L-50 pipet with 200 µl tips (not wide or narrow bore).
   b. Place the cartridge holder on a flat surface that has been sterilized with 10% bleach and position the pipet tips in each of the 8 top wells at a ~30-45° angle, vertical into the junction where the side wall meets the bottom of the well; do not allow the tips to be flat against the bottom of the wells.
   c. Slowly draw 40 µl of droplets into the pipet tip (should take ~5 seconds and ~5 µl air is expected); do not aspirate >40 µl as this causes air to percolate through the droplets. Pipet slowly. Apply a stable resistive force to the plunger to draw and aspirate droplets smoothly into and out of pipet tips.
   d. To dispense droplets into the 96-well plate, position the pipet tip along the side of the well – near, but not at, the bottom of the well – and slowly dispense droplets (~5 seconds).
   e. To prevent evaporation and contamination with particulates, cover the plate as you work. To cover the plate, set the tip box lid or a piece of foil over the plate.
13. Once droplets are removed, press the latches on the DG8 cartridge holder to open it.

Remove the empty DG8 cartridge and discard it.

Automatic Droplet Generation and ddPCR

1. When powering on the Automated Droplet Generator (ADG), you will see a startup screen while the instrument powers on and performs a self-check. Please note that the door will automatically close.
2. If the instrument deck is empty, the indicator lights on the deck of the ADG should be off, indicating that no consumables are present. The corresponding areas of the touch screen will be gray. If consumables were present in the instrument when power was lost or disconnected, an error message will appear prompting you to check and reset the consumables if necessary. The cartridges, gaskets, tips, and plates are all single-use consumables and should be discarded in the biohazard waste after use. If the consumables in the instrument are of questionable use after power loss, please remove and discard.
3. Bring the ADG out of idle mode by touching the screen.
4. Check the indicator lights on the deck of the ADG instrument and the consumable icons on the touch screen.
5. To create a plate of droplets with the ADG:
    a. Touch the Configure Sample Plate button at the bottom center of the screen.
    b. Touch or swipe across the screen to select the columns in which your samples are located on the sample plate. Touching a selected column deselects it. You can touch any orientation of columns.
    c. The plate name and plate notes are optional; touching the files will bring up a keyboard on the screen. Click OK when done.
6. Based on the number of columns selected in the previous step, the consumable icons on the screen will begin to blink yellow to indicate where new consumables need to be loaded into the instrument.
    a. If the blinking yellow icon displays Load on the screen, remove the previously used consumable (if applicable) and load a new consumable into the designated are of the instrument.
    b. If the icon remains gray on the screen, that consumable is not needed to complete the currently configured run.
7. Open the door on the ADG instrument by lifting up on the handle at the front to the instrument. The electronic braking system will assist you in this task and prevent the lid from closing accidentally. Note: the ADG door will close the preserve HEPA-filtered enclosure if left open for longer than 20 minutes while in idle mode. There is an audible click when the door brake releases, and the door closes slowly. Please observe the pinch points of the instrument and keep hands clear.
    a. To avoid contamination, load the consumables from the back to the front of the instrument. Nothing should be placed on the instrument deck outside to the dedicated consumable holders.
8. Load the DG32 ADG Cartridges along the back row of the instrument:
    a. Remove the plastic wrapping from the DG32 cartridges and place, with the green gaskets to the right, into the three plate holders. The holders are keyed for proper orientation of each DG32 cartridge to prevent incorrect loading.

   b. The lights on the DG32 plate holders will change from yellow to green when the DG32 cartridges are inserted correctly. If a light remains yellow, try repositioning the plate in a different orientation.
   c. As the lights turn green on the deck, the corresponding icons on the touch screen will go from blinking yellow to solid green and Ready will be displayed.
9. To load the ADG Pipet tips along the center row of the instrument:
   a. Remove the plastic wrapping and box lids from the tip boxes and place into the plate holders in the middle of the deck. Only full tip boxes should be loaded.
   b. Remove the tip waste bin containing any tips from a previous run and replace with a clean waste bin.
   c. The lights on the tip box holders will changed from yellow to green when the tip boxes are inserted correctly.
   d. As the lights turn green on the deck, the corresponding areas of the touch screen will go from blinking yellow to solid green, and "Ready" will be displayed.
   e. Please note that only ADG Pipet Tips should be used; other tips can damage the instrument.
10. To load the 96-well PCR plate containing your prepared ddPCR reactions into the front row of the instrument:
   a. The sample plated can be sealed with a PX1 Plate Sealer and heat-sealing foil in advance of loading into the ADG instrument. Each well should contain 25 µl of the prepared ddPCR
   b. Load the 96-well PCR plate containing your prepared ddPCR reactions into the front row of the instrument.
   c. Place the plate into the front left plate holder, labeled on the screen as Sample Plate. The holder is keyed for proper orientation and contains plate clips to support sealed plates.
   d. The light on the Sample Plate holder will change from yellow to green when the plate is inserted correctly. If the light remains yellow, try repositioning the plate in a different orientation.
   e. As the light turns green on the deck, the corresponding icon on the touch screen will go from blinking yellow to solid green, and Ready will be displayed.
   f. Note that a sample plate is required for every run, regardless of the number of columns selected.
11. To load the Droplet Plate assembly:
   a. The cooling block should be placed in a -20°C freezer for at least 2 hours before configuring a run on the ADG instrument and inserting into the instrument. The block goes from a pink color at room temperature to a dark purple color when properly cooled.
   b. Remove the cooling block from the freezer and place into the front right plate holder, labeled on the screen as Droplet Plate. The holder is keyed for proper orientation of the cooling block. The block should be a dark purple color, indicating that it is at the proper temperature. If the block is pink, it has warmed up and should not be used.
   c. The light on the instrument will change from yellow to green when the block is

interested correctly. If the light remains yellow, try repositioning the block in a different orientation.

d. As the light turns green on the deck, the corresponding area of the screen will go from blinking yellow to solid green, and Ready will be displayed.
e. Place a clean 96-well PCR plate for droplet collection into the cooling block accessory. The cooling block is also keyed for proper orientation of the plate.
f. A clean droplet plate is required for every run, regardless of the number of columns selected. Once generated, the droplets will be dispensed into the sample plate orientation as the ddPCR reactions were taken from the sample plate.

12. To load the ADG Oil into the instrument:
    a. Remove the cap from the bottle of ADG oil and twist the bottle into the tower of the oil delivery system at the front left corner of the instrument. Turn the bottle until it does not move; the label on the bottle should face out.
    b. Select the type of ADG oil that was loaded into the instrument by touching the droplet for either Probes or EvaGreen oil, depending on the type of assay being used. The droplet you select will turn blue; touch OK to set the oil type.
    c. The oil level icon on the screen will turn blue and display the current oil level of the bottle. The system will display the oil type at the bottom left of the screen as well.
    d. If a bottle of ADG oil was previously loaded, you may be prompted to confirm the type of oil currently loaded into the instrument.
    e. If the last plates were run on the ADG with a different oil type than the one being currently loaded and selected, the instrument will perform a small volume purge of the oil through the delivery system and into the oil waste reservoir. The total run time will be a few minutes longer when this occurs, but droplet generation will not be impacted.
13. Once all of the consumables are loaded and the corresponding lights are green on the deck and touch screen, a blue Start button will appear at the bottom right of the screen. Touching Start will bring up a confirmation window indicating the type of Droplet Generation Oil loaded, the number and orientation of columns selected for droplet generation and any plate name and details entered during configuration of the sample plate.
14. Once you have confirmed the plate setup, touch the Start Run button to being droplet generation. The door will automatically close at the beginning of the run and must remain closed during the run. Opening the door before the Droplets Ready message appears may cause the instrument to terminate the run and samples to be lost.
15. After a brief initialization the ADG will display a countdown timer on the screen with time remaining until the plate of droplets is ready. No additional action is needed until the plate is ready. This initialization can take 1-5 min, depending on whether the oil type has been changed or not.
16. Once the plate of droplets is ready, the screen will display a finalizing window followed by a blue Droplets ready message with a timer showing time elapsed since complete. The droplet plate at the front right corner of the instrument will be illuminated blue. The corresponding icon on the touch screen will also pulse blue; the door on the instrument will unlock and the droplet plate can be removed. Please wait to

remove the oil bottle as noted on the screen; the unused oil is being returned to the bottle.

17. Remove the droplet plate containing ddPCR droplets and seal within 30 minutes of droplet generation completing as described below in "Plate sealing and ddPCR/RT-ddPCR."
18. Remove any consumables from the ADG that have been completely used and discard. DG32 cartridges, tips, and plates are single-use consumables. The ADG will remember the status of the consumables as long as it remains powered on; if a consumable has been only partially used, leave it the ADG for the next run.

Plate sealing and ddPCR/RT-ddPCR

1. Seal the PCR plate with foil (BioRad cat # 181-4040) immediately after transferring droplets to avoid evaporation. Note: the foil seals have to be compatible with the PX1 PCR plate sealer. With the recommended product, the red stripe should be facing up.
2. Set the plate sealer temperature to 180°C and time to 5 seconds (remove support block while sealer is heating).
3. Touch the arrow to open the PX1 tray door. Position the support block on the tray with the 96-well side facing up. Place the 96-well plate onto the support block and ensure that all plate wells are aligned with the support block.
4. Secure the plate on the support block and touch the seal button. The tray will close and heat sealing will initiate.
5. When heat sealing is complete, the PX1 door opens automatically. Remove the plate from the block for thermal cycling. Remove the support block from the PX1.
6. Check that all the wells in the plate are sealed; the depressions of the wells should be visible on the foil. Once sealed, the plate is ready for thermal cycling.
    a. Begin thermal cycling within 30 minutes of sealing the plate, or store the plate at 4°C for up to 4 hours prior to thermal cycling.
7. Run reactions through the PCR/RT-ddPCR cycle in the BioRad C1000 Touch instrument with the appropriate conditions.
8. For ddPCR, the following conditions should be used: 95°C for 5 minutes*, followed by 50 cycles of 95°C for 30 seconds*, and 55°C for 1 minute*, then 98°C for 10 minutes* and a 4°C hold**. (*ramp time should be ~2°C/seconds, **ramp time should be ~1°C/second).
9. For RT-ddPCR, the following conditions should be used: 50°C for 1 hour, 95°C for 10 minutes, followed by 50 cycles of 95°C for 30 seconds, and 55°C for 1 minute*, then 98°C for 10 minutes and a 4°C hold (* adjust ramp rate to 2°C/second).
10. Use a heated lid set to 105°C and set the sample volume to 40 µl.
11. Note: After PCR, the plate should be read within 24 hours. If not run immediately, the plate should be stored at 4°C until reading can occur.

Droplet Reader

1. Power on the QX200 Droplet Reader using the switch at the back. Allow it to warm up for 30 minutes, then switch on the PC and launch QuantaSoft software.
2. Check the indicator lights on the front of the droplet reader. The first two lights at left

should be solid green, indicting power is on, there is sufficient oil in the designated oil reservoir, and there is <700 ml in the waste bottle. If the lights are flashing amber, the run cannot be started. Clean out the waste bottle by placing a hazardous waste label on it and storing it in the satellite waste secondary container or replace the oil (see manual for instructions).

3. Place the 96-well PCR plate into the plate holder:
    a. Place the 96-well PCR plate containing the amplified droplets into the base of the plate holder. Well A1 of the PCR plate must be in the top left position and place the top on the PCR plate.
    b. Move the release tabs of the top of the plate holder in the "up" position and place the top on the PCR plate. Firmly press both release tables down to secure the PCR plate in the holder.
    c. Press the button on the green lid to open the droplet reader. Load the plate holder in to the droplet reader and press the button on the lid again to close the cover. Confirm the first three indicator lights are green.
4. In QuantaSoft software, click SETUP in the left navigation bar to define your experiment.
5. Click TEMPLATE>NEW.
6. Select the wells by double clicking.
7. In the "Name" field of the Sample well editor, enter the SampleID.
8. In the "Experiment" field of the Sample well editor, select ABS.
9. In the "Supermix" field of the Sample well editor, select ddPCR Supermix for Probes (no dUTP) or One-Step RT-ddPCR Kit for Probes.
10. Define Assay 1 (channel 1, the FAM channel) in the Target 1 box:
    a. In the "Name" field of the Target 1 well editor, enter the assay target
    b. In the "Type" field, select Ch1Unknown, Ch1 Positive or NTC.
11. Define Assay 2 (channel 2, the VIC/HEX channel) in the Target 2 box:
    a. In the "Name" field of the Target 2 well editor, enter the exogenous internal amplification control assay target.
    b. In the "Type" field, select Ch2Unknown, Ch2 Positive or NTC.
12. When done, click APPLY or OK to save the information.
13. Save the file using the following format: "YYYY-MM-DD_TCR_PCRX", where YYYY-MM-DD is the date of the ddPCR run and PCRX is the consecutive number of PCR plates run for this sample set.
14. Click RUN. Up to 1 minute later, a green circle appears next to the abort button and flashes periodically to indicate the run is in progress. Active and analyzed wells are also highlighted in green in the plate map.
15. When droplet reading is complete, all four indicator lights are solid green. Open the door and remove the plate holder from the unit. Remove the 96-well PCR plate from the holder and discard it.
16. Save data files to L:\Lab\Lablan\MCEARD_digitalPCR\dPCR\Brinkman.
17. Use SOP#: D-SED-EFAB-015-SOP-01 *Quantitative Analysis of ddPCR Data* to assess positive and negative droplet populations based on droplet fluorescence and to calculate copies of genomic targets and internal amplification control in each sample.

10.0 DATA AND RECORDS MANAGEMENT

The data output from the QuantaSoft software is a directory of files that includes files containing fluorescence data of every droplet detected in the 96-well plate and QuantaSoft analysis of the droplet data. The entire directory will be copied to the LabLan for backup and access for further analysis, as described in SOP#: D-SED-EFAB-015-SOP-01.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

The quality control procedures required to demonstrate successful performance of (RT)ddPCR include the use of positive and negative controls. To ensure quality of data collection during PCR, a negative control consisting of the buffer used to dilute samples will be used. This should result in baseline fluorescence amplitude levels for every droplet detected. A positive control for (RT-)ddPCR consists of DNA containing the targeted amplicon, either from extracts of organisms or synthesized DNA constructs. This control will contain 10^5 copies of the target per reaction, resulting in an average of 10 copies per droplet and amplification in 100% of droplets.

If the (RT-)ddPCR negative controls result in droplets with fluorescence amplitude levels indicating amplification or the PCR positive controls result in the less than 100% positive droplets, the amplicons will be discarded and the (RT-)ddPCR will be repeated. The results of the quality control procedures will be documented in the analysts' notebook. All QC failures will be reported to the project lead. A discussion of the overall QC procedures and results will be described in the manuscript.

12.0 REFERENCES

- This SOP was adapted from BioRad documents for the QX200 Droplet Digital PCR System, which can be found at http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr- system?tab=Documents
- Instruction Manual, QX200 Droplet Generator, Rev C (BioRad # 10031907)
- Instruction Manual, QX200 Automated Droplet Generator (BioRad # 1864101)
- Instruction Manual, QX200 Droplet Reader and QuantaSoft Software, Rev D ((BioRad # 10031906)
- Instruction Manual, PX1 PCR Plate Sealer, Rev A (BioRad # 10023997)
- Instruction Manual, C1000 Touch Thermal Cycler, Rev B (BioRad # 10021377)
- QX200 ddPCR EvaGreen Supermix Product Insert, Rev C (BioRad # 10028376)
- ddPCR Supermix for Probes (No dUTP) Product Insert, Rev D (BioRad # 10026868)
- One-Step RT-ddPCR Advanced Kit for Probes Product Insert, Rev A (BioRad # 10049226)

13.0 DOCUMENT HISTORY

Date of Revision	Revision #	Description of Changes
8/16/2016	0	First version

14.0 TABLES

Table 1. ddPCR Master Mix.

Component	Volume/ Reaction (µl)	Volume/Mix (µl) 100 Reactions	Final Concentration	Lot#, Expiration Date
Nuclease-free water	2.45	245	To 20 µl	
2X Supermix for probes (no dUTP)	12.5	1250	1X	
Forward primer (FAM, 50 µM)	0.45	45	900 nM	
Reverse primer (FAM, 50 µM)	0.45	45	900 nM	
Probe (FAM, 10 µM)	0.625	62.5	250 nM	
IAC Forward primer (VIC/HEX, 50 µM)	0.45	45	900 nM	
IAC Reverse primer (VIC/HEX, 50 µM)	0.45	45	900 nM	
IAC Probe (VIC/HEX, 10 µM)	0.625	62.5	250 nM	
Total Volume	**18**	**1800**	-	

IAC = Internal amplification control

Table 2. RT-ddPCR Master Mix.

Component	Volume/ Reaction (µl)	Volume/Mix (µl) 100 Reactions	Final Concentration	Lot#, Expiration Date
Nuclease-free water	5.7	570	To 20 µl	
4X One-Step Advanced RT-ddPCR Supermix	6.25	625	1X	
Reverse transcriptase	2	200	20 U/µl	
DTT (300 mM)	1	100	15 mM	
Forward primer (FAM, 50 µM)	0.45	45	900 nM	
Reverse primer (FAM, 50 µM)	0.45	45	900 nM	
Probe (FAM, 10 µM)	0.625	62.5	250 nM	
IAC Forward primer (VIC/HEX, 50 µM)	0.45	45	900 nM	
IAC Reverse primer (VIC/HEX, 50 µM)	0.45	45	900 nM	
IAC Probe (VIC/HEX, 10 µM)	0.625	62.5	250 nM	
Total Volume	**18**	**1800**	-	

IAC = Internal amplification control

Table 3. Sample Layout for (RT-)ddPCR in a 96-well Plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample A, undiluted			Sample C, 1:4			Sample F, 1:2			Sample I, undiluted		
B	Sample A, 1:2			Sample D, undiluted			Sample F, 1:4			Sample I, 1:2		
C	Sample A, 1:4			Sample D, 1:2			Sample G, undiluted			Sample I, 1:4		
D	Sample B, undiluted			Sample D, 1:4			Sample G, 1:2			Positive (RT-) ddPCR control, FAM target	Positive (RT-) ddPCR control, VIC/HEX target	Negative (RT-) ddPCR control NTC
E	Sample B 1:2			Sample E, undiluted			Sample G, 1:4					
F	Sample B, 1:4			Sample E, 1:2			Sample H, undiluted					
G	Sample C, undiluted			Sample E, 1:4			Sample H, 1:2					
H	Sample C, 1:2			Sample F, undiluted			Sample H, 1:4					

Analysis of data generated from Droplet Digital PCR (ddPCR) (D-SED-EFAB-015-SOP-01) – June 2017

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division
Research Triangle Park, North Carolina, Headquarters
Athens, Georgia
Cincinnati, Ohio Las
Vegas, Nevada

STANDARD OPERATING PROCEDURE

Title: Analysis of data generated from Droplet Digital PCR (ddPCR)

Number: D-SED-EFAB-015-SOP-01	Effective Date: June 12, 2017

SOP was developed: ☒ In-house ☐ Extramural

Alternative Identification: EFAB-015-01

SOP Steward

Name: Nichole Brinkman

Approval

Name: Lindsay Stanek
Title: Chief, Environmental Futures Analysis Branch

Concurrence*

Name: Brittany Stuart
Title: SED Quality Assurance Manager

* Optional Field/NERL-SOP.2(11/2005)

STANDARD OPERATING PROCEDURE FOR ANALYSIS OF DATA GENERATED FROM DROPLET DIGITAL PCR (DDPCR) – June 12, 2017

TABLE OF CONTENTS

1.0 PURPOSE

This SOP describes the procedure to analyze data generated from the BioRad QX200 Droplet Digital PCR System (ddPCR).

2.0 SCOPE AND APPLICABILITY

ddPCR can be used to quantify RNA and DNA targets of prokaryotes, eukaryotes, archaea, and viruses in a variety of sample matrices, including, but not limited to, water, air, and rubber surfaces. The use of this procedure is contingent upon sample preparation that releases RNA and/or DNA from cells or viral capsids. Proper analysis is contingent upon running proper controls to facilitate fluorescence amplitude cluster analysis and monitoring of potential PCR inhibition.

3.0 SUMMARY OF METHOD

The data produced by the QX200 Droplet Digital PCR system is composed of fluorescence amplitude data from thousands of droplets produced in a ddPCR reaction. Based on the amplitude value, each droplet will be clustered into positive or negative bins. The number of droplets in each bin is counted and the number of total and positive droplets is used to determine the number of target DNA molecules present from a Poisson distribution. The QuantaSoft Software is used to cluster droplets and determine DNA target concentration (see reference).

On occasion, the software is not able to cluster the droplets and, in this case, an alternative method is used to assess the fluorescence amplitude threshold to cluster droplets. Here, the mean and standard deviation of the fluorescence amplitude data from the droplets of the no template control reactions are used to determine a negative threshold value. The value is applied manually in the QuantaSoft software and DNA target concentrations are calculated. Finally, the total number of DNA target molecules per unit volume of sample are calculated by accounting for sample processing steps.

4.0 DEFINITIONS

ddPCR – droplet digital PCR

5.0 HEALTH AND SAFETY WARNINGS

The ddPCR analysis is performed at an office workstation and not in a laboratory; therefore, there are no necessary health and safety precautions to perform this procedure.

6.0 CAUTIONS/INTERFERENCES

ddPCR amplification of DNA targets in environmental samples often produce “rain,” or droplets with a wide range of fluorescent amplitude values. This can make droplet clustering difficult. Proper controls should be included to seed clustering efforts as well as monitoring for PCR inhibition.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

The techniques of first-time analysts must be reviewed by an experienced analyst prior to completing this SOP alone. During this review, the new analyst must demonstrate his/her ability to complete this procedure alone which must be approved and documented by an experienced analyst/supervisor in the analyst's laboratory notebook.

8.0 EQUIPMENT AND SUPPLIES

QuantaSoft Version 1.7.4.0917
QuantaSoft Analysis Pro Software v1.0.596
Data file (.qlp) from LabLan directory
Project's OneNote notebook

9.0 PROCEDURE

9.0 Open QuantaSoft Analysis Pro version 1.0.596

9.1 Click Browse and find the .qlp file located in the associated project folder located at: L:\Lab\Lablan\MCEARD_digitalPCR\dPCR\Brinkman\. Select file and click OK.

9.2 Select all wells of the plate and click Auto Analyze. In the window that pops up, select
Combined Wells and click OK.

a. If AutoAnalyze successfully clustered the droplets, continue onto step 9.1.4.
b. If AutoAnalyze failed to cluster the droplets, a pop-up window will appear indicating the failure. If this occurs, go to the section "Manually determine and apply threshold" to determine and manually apply the threshold before continuing onto step 4.

9.3 Go to the 1D Amplitude Menu.

a. Click on the "+" icon in the top right corner of the 1D Channel 1 Amplitude plot to expand the plot.
b. Click on the menu icon in the top right corner of the 1D Channel 1 Amplitude plot and select Copy. Paste the image in the OneNote notebook page and annotate appropriately.
c. Minimize the 1D Channel 1 Amplitude plot.
d. Click on the "+" icon in the top right corner of the 1D Channel 2 Amplitude plot to expand the plot.
e. Click on the menu icon in the top right corner of the 1D Channel 2 Amplitude plot and select Copy. Paste the image in the OneNote notebook page and annotate appropriately.
f. Minimize the 1D Channel 1 Amplitude plot.

9.4 Select all wells and save the file locally as .ddpcr in a local network drive using the file naming convention established for the project.

9.5 Select the 1D amplitude menu. In the upper right corner of the Well Data Table, select the menu icon. Click Export the data to Excel and save the data as .xlsx in

a local network drive.

9.6 Drag .ddPCR and .xlsx files to the OneNote notebook page for the experiment.

9.7 Open the .xlsx file from the OneNote notebook and label the tab containing the raw data "Raw."

9.8 Copy the raw data into a new tab for data manipulation and label the tab "Processed."

9.9 In the copied tab, remove all columns except Sample, Target, Conc(copies/ul), Accepted Droplets, Positives and Negatives.

9.10 Sort data by Sample.

9.11 Move all data associated with the positive and negative ddPCR controls to a new tab. Label tab "Controls."

a. Assess the results of the positive, negative, and internal amplification controls according to the established QC criteria specific to the project.
b. Record summary of QC samples in the OneNote notebook by notating "PASS" for samples that meet acceptance criteria or "FAIL" for samples not meeting acceptance criteria.

9.12 Navigate back to the "Processed" tab with pruned data and sort by target.

9.13 Transfer all the data and column headings associated with Channel 2 to a new tab labeled with the DNA target name (e.g., 16S, IAC, NoVGII, etc.)

a. Move the conc(copies/ul) data to the right-most column by cutting and pasting.
b. Add a column titled "Copies/25 ul Reaction."
c. For each sample, enter a formula to multiply the value in "Conc(copies/ul)" by the volume used in the ddPCR reaction in ul (typically this is 25 ul but may vary. If a different volume is used the column header for 9.14.b should be revised to reflect the appropriate volume).
d. Add a column titled "Dilution Factor"
    i. Enter the whole number of the dilution was used (e.g., enter 100 for 100-fold dilution).
e. Add a column titled "Adjusted copies/5ul Sample Extract"
    i. For each sample, enter a formula to multiply the value in "Copies/25 ul Reaction" by the value in "Dilution Factor"
        - This result will return the number of copies in 5 ul of sample extract that went into each ddPCR reaction.
f. Add a column titled "Copies/total extract volume"
    i. For each sample, enter a formula to multiply the value in "Adjusted copies/5ul Sample Extract" by (100/5)
        - This result will return the number of copies in the entire volume of extract.
        - The factor (100/5) results from using 5 ul of a 100 ul final extract volume in ddPCR.
g. If applicable, add a column titled "Copies/unit volume"
    i. For each sample, enter a formula to divide the value in "Copies/total extract volume " by X, where X is the factor of

the desired unit volume from the whole volume.

- This result will return the number of copies in the desired unit volume of sample. For example, in a sample where 50 ml of water was filtered, the value in "Copies/total extract volume" represents the entire 50 ml sample. If interested in copies/ml, X=50.

Manually determine and apply threshold

9.14 Open the .qlp file in QuantaSoft Version 1.7.4.0917.
9.15 Highlight the NTC (no template control) reactions.
9.16 In the Setup window, click on Options
9.17 Click on Export Amplitude and Cluster Data
9.18 Save .csv files (1 from each well) in a local network drive.
9.19 Copy all data for Ch1 Amplitude (column A) and Ch2 Amplitude (column B) into a new spreadsheet titled Threshold Determination_target_PlateX.xlsx. This data is the fluorescence values from every droplet in the NTC reaction. Append the Ch1 and Ch2 amplitude values from all other NTC reactions on the plate.
9.20 For Ch1 and Ch2, calculate and record the following:
    a. Mean amplitude of droplets from all NTC reactions
    b. Standard deviation of the amplitude of droplets from all NTC reactions
    c. 10 x standard deviation = Multiply the standard deviation by 10.
    d. Threshold = Add 10X stdev to the mean amplitude value.
9.21 Open the .qlp file in QuantaSoft Analysis Pro version 1.0.596.
9.22 Go to the 1D Amplitude Menu.
9.23 Select the "threshold multiple wells" icon.
9.24 Manually set the threshold for all wells using the mean + 10 stdev determined from the NTC replicate reactions.
9.25 Save file as .ddPCR and continue analysis as indicated above (go to Step 9.4).

10.0 DATA AND RECORDS MANAGEMENT

Analysis of samples performed in QuantaSoft Analysis Pro will be saved as a .ddpcr file and stored locally on the analyst's network drive. Additionally, the file itself or the path and filename will be recorded in the project's OneNote Notebook. The DNA target concentration data will be exported from QuantaSoft Analysis Pro as an Excel file for further analysis. The .xlsx file will be stored locally on the analyst's network drive and linked to the project's OneNote Notebook.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

Appropriate controls for ddPCR include positive controls that result in ddPCR reactions where approximately 99.9% and 80% of the droplets are positive. The positive controls with 99.9% of positive droplets are used for clustering and those with 80% positive droplets are used to assess ddPCR performance according to established quality control (QC) criteria. In addition, negative controls are run without template and are used for

clustering and to assess potential background contamination issues. Finally, an internal amplification control is used and spiked into every reaction. This control will return in a concentration and will be compared to established QC criteria to assess whether amplification has been impeded.

If the ddPCR negative controls result in droplets with fluorescence amplitude levels indicating amplification or the PCR positive controls result in the less than the targeted percentage of positive droplets or established QC criteria values, the results will be flagged and the ddPCR will be repeated.

The results of the quality control procedures will be documented in the project's OneNote notebook according to step 9.12. All QC failures will be reported to the project lead. A discussion of the overall QC procedures and results will be described in the manuscript.

12.0 REFERENCES

http://www.bio-rad.com/en-us/applications-technologies/absolute-quanitifaction-pcr-targets-with-droplet-digital-pcr-system

13.0 DOCUMENT HISTORY

Date of Revision	Version #	Description of Changes
6/7/2017	-01	First version.

16S rRNA Gene Sequence Analysis (D-SED-EIB-SOP-1907-0) – January 2018

U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Systems Exposure Division
Ecosystem Integrity Branch

STANDARD OPERATING PROCEDURE

SOP Title: 16S rRNA Gene Sequence Analysis

SOP ID: D-SED-EIB-SOP-1907-0	Effective Date: January 30, 2018

SOP was developed: ☒ In-house ☐ Extramural: N/A

SOP Discipline*: Molecular Biology

Alternative Identification: D-SED-EFAB-013-SOP-01

SOP Contact Signature

Name: Scott Keely

Signature/Date:

Management Signature

Name: Brent Johnson

Title: Chief, Ecosystem Integrity Branch

Signature/Date:

QA Signature

Name: Brittany Stuart

Signature/Date:

STANDARD OPERATING PROCEDURE FOR 16S RRNA GENE SEQUENCE ANALYSIS – January 30, 2018

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the process used to process 16SrRNA gene sequences generated using Illumina's MiSeq platform using paired end reads. The process used to perform 16S PCR and prepare and sequence 16S libraries can be found in SOP#: D-SED-EFAB-SOP-1012-1 (Alt. ID: D-SED-EFAB-012-SOP-01). These steps must be performed prior to executing this procedure.

2.0 SCOPE AND APPLICABILITY

This procedure applies to paired-end reads generated for the MiSeq platform.

3.0 SUMMARY OF PROCEDURE

The goal of this SOP is to process 16S rRNA gene sequences that are generated using Illumina's MiSeq platform. The SOP uses indexed reads to multiplex a large number of samples (i.e. 384) on a single MiSeq experiment. Mothur software will be used to filter the sequences and determine their taxonomic content.

4.0 DEFINITIONS

4.1 OTU – operational taxonomic unit
4.2 QAPP – quality assurance project plan
4.3 SOP – standard operating procedures
4.4 Read.contig – merged read-pairs from MiSeq
4.5 Fastq – is a sequence file from the MiSeq that contains DNA sequences and base-call scores.
4.6 Silva database – a vast collection of 16S rRNA gene sequences. See https://www.arb-silva.de/

5.0 HEALTH AND SAFETY WARNINGS

This section is not applicable. This procedure does not involve any laboratory analysis or handling of chemicals, reagents, or standards that may pose a health and safety risk.

6.0 CAUTIONS/INTERFERENCES

6.1 The biggest vulnerability when performing this procedure is during the processing of large datasets is maintaining data integrity. However, SyncRecovery is used when transferring data from the instrument to the LabLAN to maintain data quality.
6.2 Another source of error is sequencing errors. Positive controls are used to monitor for these types of errors.
6.3 Contamination of samples may occur and negative controls are used during analysis to monitor for this potential issue.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

Prior to being authorized to perform this procedure, individuals shall be trained by someone familiar with this procedure. Individuals with a computer science and statistics background is desirable.

8.0 EQUIPMENT AND SUPPLIES

8.1 RedHat Enterprise operating system, or Linux equivalent
8.2 Mothur version 1.36.5 or latest, open source software available at: https://www.mothur.org/wiki/Download_mothur

9.0 PROCEDURE

NOTE: This procedure is intended to describe the major steps for preparing MiSeq sequences for taxonomic placement. Modifications or revisions to the steps described here may be necessary to meet specific project objectives and will be documented in a research notebook or the project file.

9.1 Create a stability.files file to link sample names with read 1 and read 2 fastq files. This file is a tab delimited file that includes three columns that correspond to the sample name, read 1 file name, and read 2 file name. Issue the following commands in Redhat Enterprize Linux OS using this bash script (make.stabilityfiles.sh):

```
#!/bin/bash
ls *R1_001.fastq >read1.txt
ls *R2_001.fastq >read2.txt
paste read1.txt read2.txt >bothreads.txt
less read1.txt | sed 's/^\(.*\)\(_.*_.*_.*_.*\.fastq\)/\1/g'| sed 's/-/|/g' >samplenames.txt
paste samplenames.txt bothreads.txt >stability.files
```

9.2 Run this bash script ./make.stabilityfiles.sh
9.3 Create a script for mothur **version 1.39.5** software that contains the following mothur commands (statements beginning with # are comments to explain the steps):

1. #place all gunzip'd fastq files in mothur directory

2. #set.dir(input= *,output= *, tempdefault=*) if needed

3. #make contigs from the R1 and R2 MiSeq files

```
make.contigs(file=stability.files, processors=16)
summary.seqs(fasta=current)
```

4. # Remove sequences that are not between 240-275 nucleotides for V4, modify as needed

```
screen.seqs(fasta=current, group=current, summary=current, maxambig=0,
        maxlength=275, minlength=240, maxhomop=8)
summary.seqs(fasta=current)
```

5. # Find unique sequences

```
unique.seqs(fasta=current)
count.seqs(name=current, group=current)
summary.seqs(count=current)
```

6. # Align Miseq sequences to silva database

```
align.seqs(fasta=current,reference=silva.v4.fasta,flip=T)
summary.seqs(fasta=current,count=current)
screen.seqs(fasta=current, count=current, start=1968, end=11550)
summary.seqs(fasta=current,count=current)
```

7. # Trim overhangs at both ends and within gaps and run unique.seqs for new duplicates should they appear

```
filter.seqs(fasta=current, vertical=T, trump=.)
unique.seqs(fasta=current, count=current)
```

8. # Remove sequencing error by pre-clustering (i.e., merging) MiSeq sequences allowing 2 nucleotide differences (average of 1 per 100 bp of 16S sequence).

```
pre.cluster(fasta=current, count=current, diffs=2)
```

9. # Use vsearch algorithm to remove chimeras

```
chimera.vsearch(fasta=current, count=current, dereplicate=T)
remove.seqs(fasta=current, accnos=current)
summary.seqs(fasta=current,count=current)
```

10. # Classify MiSeq sequences using the silva database. Use 80 cutoff

```
classify.seqs(fasta=current, count=current, reference=trainset16_022016.rdp.fasta,
        taxonomy=trainset16_022016.rdp.tax, cutoff=80)
```

11. # Remove sequences that misclassify as chloroplasts, mitochondria, or eukaryotes

```
remove.lineage(fasta=current, count=current, taxonomy=current,
        taxon=Chloroplast-Mitochondria-unknown-Eukaryota)
summary.tax(taxonomy=current,count=current)
summary.seqs(fasta=current,count=current)
```

12. # Calculate distances between 16S sequences.

    dist.seqs(fasta=current,cutoff=0.03)

13. # Cluster sequences into OTUs by using the dist table from previous step above. The label "column=" specifies the dist files created by the prior step.

    cluster(column=current,count=current,cutoff=0.03)

14. # Create a table with rows = samples and columns = otu counts.

    make.shared(list=current, count=current, label=0.03)

15. # Create taxonomy list for the previous step

    classify.otu(list=current, count=current, taxonomy=current, label=0.03)

10.0 DATA AND RECORDS MANAGEMENT

10.1 The process used to reproduce analysis is described in the Procedure section.
10.2 OneNote will be used to keep records of data analysis.
10.3 The data analysis files will be stored at L:\Lab\Lablan\MiSeq_Analytics

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 A vulnerability when performing **t**he **p**rocedure above is maintaining data integrity; however, SyncRecovery is used when transferring data from the MiSeq instrument to the LabLAN to maintain data quality.
11.2 Another vulnerability is due to human error when labeling sequence files and consequently impacting the creation of mothur stability.files in the Procedure. Secondary data review will be used to mitigate this problem.
11.3 Another source of error is sequencing errors. Positive controls are used to monitor for these types of errors. Acceptability of these controls is determined by a project's quality criteria as described in applicable QAPPs or SOPs.
11.4 Contamination of samples may occur and negative controls are used during analysis to monitor for this potential issue. Acceptability of these controls is determined by a project's quality criteria as described in applicable QAPPs or SOPs.
11.5 PCR chimeras will be detected and removed using mothur command **chimera.vsearch.**
11.6 Some sequences misclassify as chloroplasts, mitochondria, or eukaryotes. These will be removed using mothur command **remove.lineage**.

12.0 REFERENCES

- R Core Team (2014). R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
- Jari Oksanen, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2016). vegan: Community Ecology Package. R package version 2.3-3. http://CRAN.R-project.org/package=vegan.
- Huson et al., MEGAN Community Edition - Interactive exploration and analysis of large-scale microbiome sequencing data, to appear in: *PLoS Computational Biology*, 2016.
- https://www.mothur.org/wiki/MiSeq_SOP
- https://www.mothur.org/wiki/Category:Commands
- http://rmarkdown.rstudio.com/

13.0 DOCUMENT HISTORY

Version Number	Name	Date	Description of Changes
0	Scott Keely	1/24/2018	Original version

Setup and Operation of Small Environmental Emissions Chambers During Testing (MOP 802) – August 2016

Miscellaneous Operating Procedure 802

Setup and Operation of Small Environmental Emissions Chambers During Testing

Authored by Matthew Allen, Jacobs Technology 08/08/2016 Date

Approved by

EPA Team Leader	Xiaoyu Liu	Digitally signed Date: 2016.08.08/12:17:49 -04'00'
EPA QA Representative	Paul Groff	Date: 2016.08.08/14:23:18 -04'00'
Jacobs Work Assignment Leader	Matthew Allen	Date: 2016.08.08
Jacobs QA Officer	Zora Drake-Richman	Date: 2016.08.08

Keywords: small chambers, source, air exchange, relative humidity, temperature

MISCELLANEOUS OPERATING PROCEDURE (802) FOR SETUP AND OPERATION OF SMALL ENVIRONMENTAL EMISSIONS CHAMBERS DURING TESTING – August 8, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Bob Wright
Jacobs	Matt Allen
	Zora Drake-Richman
Laboratory Copy	Maintained by Jacobs Technology Electronic Filing
Electronic Filing	APPCD SharePoint

Revision Record

Revision	Date	Responsible Person	Description of Change
0	Unknown	Unknown	Initial document
1	Aug. 2003	Unknown	Updated after move to new facility
2	Feb. 2014	Roache	Updated with new equipment for RH and temperature and a third incubator
3	May 2016	Allen	Updated MOP references, definitions, and chamber preparation and setup
4	July 2016	Allen	Updated chamber assembly procedures in PROCEDURE section
5	August 2016	Allen	Made revisions based on EPA QA review comments. QLOG No. G-APPCD-0016211, QTRAK No. 11037_18, QA Category III

MISCELLANEOUS OPERATING PROCEDURE NO. 802

TITLE

Setup and Operation of Small Environmental Emissions Chambers during Testing

PURPOSE

To provide written repeatable procedures for implementing small chamber emissions tests from a variety of sources in the Environmental Emissions Small Chamber Laboratory.

SUMMARY OF METHOD

Small environmental chambers are utilized to evaluate emissions from sources in a controlled temperature, relative humidity (RH) and air exchange rate atmosphere over extended time periods. These chambers conform to ASTM Standard Guide D5116-11 — Standard Guide for Small-Scale Environmental Chamber Determinations of Organic Emissions from Indoor Materials/Products.

DEFINITIONS

ACH	Air exchange rate
ASTM	American Society for Testing and Materials (ASTM International)
C	Celsius
cm	centimeter(s)
DAS	Data Acquisition System
DC	Direct Current
°	degree(s)
DNPH	2,4-Dinitrophenylhydrazine
FEP	Fluorinated ethylene propylene
GC/MS	Gas Chromatography/Mass Spectrometry
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography h^{-1} per hour
in	inch(es)
L	liter(s)
min	minute(s)
mL	milliliter(s)
MOP	Miscellaneous Operating Procedure
%	percent
PTFE	Polytetrafluoroethylene
RH	Relative humidity
RTD	Resistance Temperature Detector
sec	second(s)
VOC	Volatile Organic Compound(s)

RELATED DOCUMENTS

- ASTM Standard Guide D5116-11: *Standard Guide for Small-Scale Environmental Chamber Determinations of Organic Emissions from Indoor Materials/Products*, ASTM International, West Conshohocken, PA, 2011.
- MOP 803: *Operation of the Opto Display Software Data Acquisition System (DAS) in the small chamber laboratory.*
- MOP 806: *Operation of the clean air system for the small chamber laboratory.*
- MOP 812: *Collecting and desorbing air samples on DNPH-Silica gel cartridges.*
- SOP-6404: *Collecting air samples from the small environmental testing chambers using Carbopack™ X sorbent tubes.*
- SOP 6405: *Glassware and chamber cleaning procedure.*

All related documents listed herein can be accessed in the Tire Crumb Rubber folder on the NRMRL L: share drive at: *L:\Lab\NRML_Public\Tire Crumb Rubber\QA Documents\MOPs.* Hardcopies of these documents will also be on file in laboratory E378-A.

EQUIPMENT AND SUPPLIES

Note: All equipment, unless otherwise noted, is located in E-378.

Table 1 lists all of the equipment, manufacturer, model number, home office location of the manufacturer, and a short description of its purpose.

Table 1. List of Small Chamber Laboratory Equipment

Component	Manufacturer	Model #	Location	Description
Regulator	Wilkerson	B18-03-FK00	Richland, MI	Regulates incoming house air pressure
Compressed Air Dryer	Hankison	SSRD10-300	Ocala, FL	Dries house air
Carbon Trap	Supelco	24565	Bellefonte, PA	Carbon filter for house air
Moisture Trap	Supelco	23992	Bellefonte, PA	Dries house air
Pure Air Generator	AADCO	737-11A	Cleves, OH	Generates clean air source
Regulator	Norgren	B736-2AK-API-RMG	Littleton, CO	Regulates incoming clean air pressure

Component	Manufacturer	Model #	Location	Description
Mass Flow Controllers (16)	Teledyne	HFC-E-202	Hampton, VA	Control air flow rates to humidity system
1000 mL Round Bottom Flask (9)	Prism Glass	PRG-5795- 03	Raleigh, NC	Water source for chamber RH control
Constant Temperature Bath	Fisher Thermo Scientific	Neslab RTE 7/BlueM stainless	Newington, NH	Controls temperature to round bottom flask for RH system
Midget Impinger Bubbler (8)	Prism Glass	PRG-5030- 23	Raleigh, NC	Generate aerial moisture for RH control
Top Fin® Aquarium Long Air Stone	Petsmart Home Office, Inc.	6741433	Phoenix, AZ	Provide agitation to the water bath
Incubator (2)	So-Low	C-SCN4-52- 8	Cincinnati, OH	Provides controlled environment for chambers
Incubator	Forma Scientific	39900	Marietta, OH	Provides controlled environment for chambers
Inlet RH Probes (8)	Vaisala	HMT333	Helsinki, Finland	Measure RH of inlet air to small chambers
Internal Chamber RH Probes (8)	Vaisala	HMT335	Helsinki, Finland	Measure RH inside center of small chambers
Thermocouples (9)	Pyromation	E-Type	Fort Wayne, IN	Measure temperature inside center of chambers
Pressure Transmitter	Vaisala	PTA 427	Helsinki, Finland	Measure barometric pressure of small chamber laboratory
Opto Control System	Opto 22	B3000	Temecula, CA	Communication hub for MFCs, RH Probes, Thermocouples
Electrical Control Box	Carotek, Inc.	AT-607983	Mathews, NC	Control panel to house Opto hardware components
Opto Operation Computer	Dell	Optiplex 745	Round Rock, TX	Houses Opto software used to monitor, log, and regulate small chamber environments
Vacuum Pump	Welch	2565B-50	Skokie, Il	Generates negative pressure for sampling lines
Mass Flow Controllers (4 per vacuum pump)	Coastal Instruments	FC-260	Burgaw, NC	Regulate air rate of sampling pump lines
Mass Flow Control Box (1 per vacuum pump)	Porter Instrument Co.	CM4	Hatfield, PA	Readout box to control air rate of sampling pump lines
Gilibrator	Sensidyne	800286	Clearwater, FL	Used to determine air rate of sampling pump lines

Table 2 lists other supplies and materials used during operation of the small environmental testing chambers, manufacturer, model number, home office location of the manufacturer, and a short description of its purpose.

Table 2. List of Small Chamber Laboratory Supplies and Materials

Component	Manufacturer	Model #	Location	Description
1½" computer cooling fan	Evercool Thermal LLC	EC-4020M12CA	City of Industry, CA	Mixes air inside the chamber during testing
¼" Swagelok® tube fitting nut	Swagelok® Company	SS-402-1	Wake Forest, NC	Caps outlet manifold at circulation fan wiring entry point
9.5 mm silicone septa	Sigma Aldrich	20652	St. Louis, MO	Seals outlet manifold at circulation fan wiring entry point
20-gauge fan electrical wires	Alpha Wire	6714	Elizabeth, NJ	Supplies electrical power to the chamber circulation fan
7.25" length extension springs	McMaster-Carr	CUSTOM	Elmhurst, IL	Suspends the circulation fan from the chamber inlet and outlet manifolds inside the chamber
1-1/2" diameter HDPE floating spheres	United States Plastic Corporation	3551	Lima, OH	Reduces evaporation of the water in the bath; and minimizes heat transfer from the ambient air to the water bath
Deionized water filter	Dracor Water Systems	RT 844 (or equivalent)	Durham, NC	Provides deionized water for the wet bulb impingers and the wet bulb impinger water bath
Glass sampling manifold	Prism Glass	EPA-MFD-5port	Raleigh, NC	5-port glass sampling port used to introduce sampling media to the chamber effluent airstream
Glass extension tube	Prism Glass	CUSTOM	Raleigh, NC	10-inch-long x ½-inch outer diameter glass tube used to extend exhaust manifold tubing to the outside of the incubator
Chamber lid O-ring	Row, Inc.	Custom (19.210 ID x 0.197 C/S FEP/SIL)	Addison, IL	Used to seal the chamber lid and tub when the lid is in place during testing

The small chamber laboratory has the capability to operate eight 53-L stainless steel chambers.

Figure 1 shows two of the chambers in the Forma Scientific two-chamber incubator, and Figure 2 shows the constant temperature bath for the humidification system.

Figure 1. Picture of two small environmental chambers in the Forma Scientific Model 39900 incubator

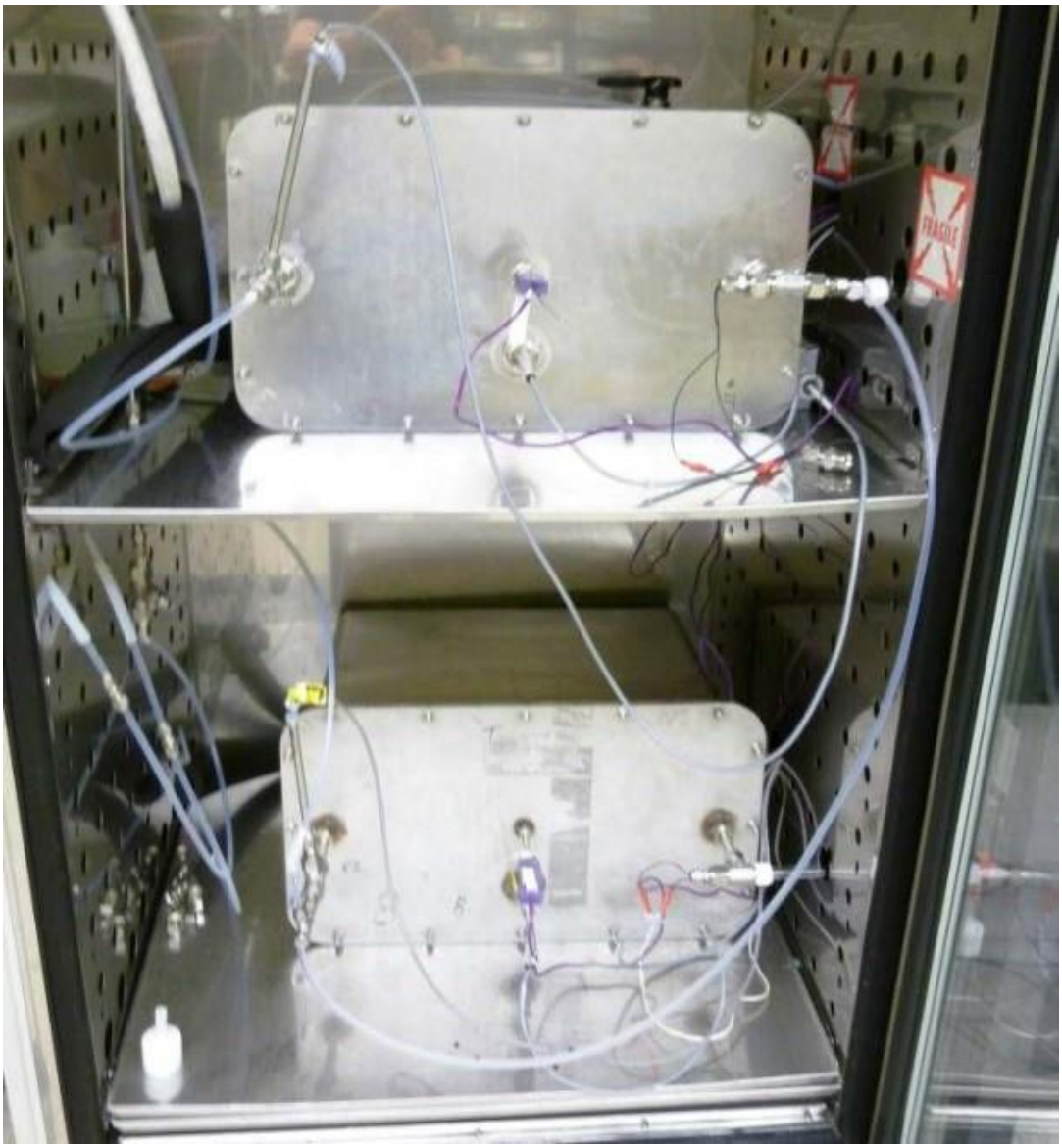
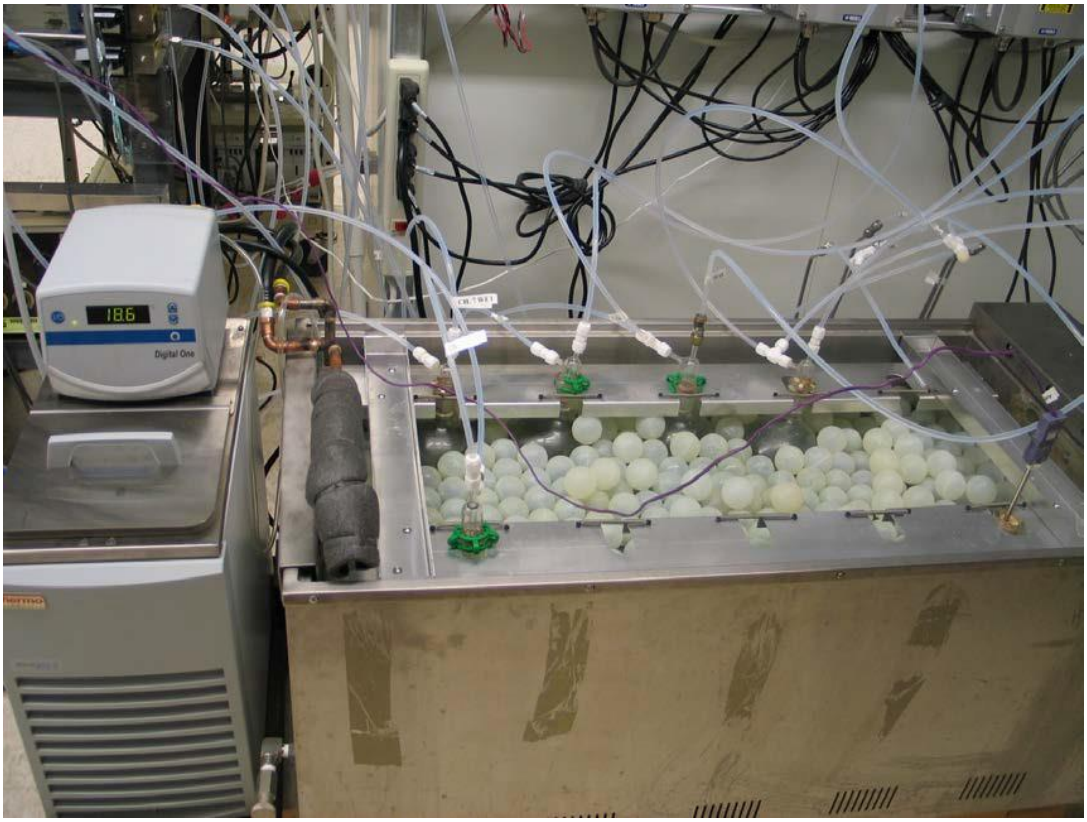


Figure 2. Picture of constant temperature water bath for chamber system



These chambers conform to ASTM Standard Guide D5116-11 — Standard Guide for Small-Scale Environmental Chamber Determinations of Organic Emissions from Indoor Materials/Products [1]. Each stainless-steel chamber has nominal measurements of 51 cm (width) by 25 cm (height) by 41 cm (depth). Clean air, free of volatile organic compounds (VOCs), is supplied to the chambers through a dedicated clean air system, which consists of house-supplied high-pressure oil-free air, a pure air generator (AADCO model 737-11A, AADCO Instruments, Cleves, OH), a dryer (Hankinson model SSRD10-300, Canonsburg, PA), a Supelco activated charcoal canister (Supelco, Bellefonte, PA), a Supelco micro-sieve canister and gross particle filters (Grainger Speedaire, Chicago, IL).

Each chamber is equipped with inlet and outlet manifolds for the air supply, a Pyromation E-type thermocouple (Fort Wayne, IN) for temperature measurement in the chamber, and two resistance temperature detector (RTD) probes, Vaisala model HMT333 (Helsinki, Finland) for measuring the RH of the supplied air and a Vaisala model HMT335 for measuring the RH air inside the chamber.

The relative humidity of the air supplied to the chamber is controlled by blending dry air with air that is humidified by bubbling through an impinger submerged in a temperature-controlled water bath. To humidify the chamber air, a temperature-regulated water bath was constructed from a Blue M stainless steel 75-gallon tub with the copper coils winding across the bottom and a

Thermo Fisher Scientific Neslab RTE 7 chiller. The chiller circulates temperature-controlled deionized water through the coiled copper tubing system spread across the bottom of the tub. A Top Fin long air stone supplied with house compressed air regulated with a needle valve is located on the bottom of the tub. The air stone is used to provide agitation to the water bath. An aluminum frame was constructed to support ten 1-liter round bottom boiling flasks. Each flask is filled with deionized water. In one flask, a thermocouple has been inserted through a rubber stopper to monitor the temperature of the water bath on the OPTO 22 DAS. In the remaining flasks, a fritted glass impinger allows zero grade air to bubble through the deionized water and combine with the dry air to establish the desired relative humidity. The control of the ratio of the dry air to the humidified air determines the relative humidity of the air going to the chamber. The tub is filled to the bottom of the aluminum support frame with deionized water. On top of the water, a double layer of 1-1/2" diameter hollow high-density polyethylene (HDPE) spheres has been added to: (1) reduce evaporation of the water in the bath and (2) minimize heat transfer from the ambient air to the water bath.

All of the tubing and fittings used in the humidified air system are polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP) and/or 316 stainless steel. All connections to the glass flasks use PTFE fittings.

An OPTO 22 data acquisition system (OPTO 22, Temecula, CA) continuously records the temperature and relative humidity of the air, the barometric pressure and temperature in the laboratory, and the outputs of the mass flow controllers. A 1½" (3.8 cm) computer cooling fan (RadioShack, Fort Worth, TX) is placed in the chamber to provide mixing for all of the small chamber tests.

The chambers are housed in one of three temperature-controlled incubators – two So-Low model C-SCN4- 52-8 (Cincinnati, OH) and a Forma Scientific model 39900 (Marietta, OH). Table 1 lists all of the equipment associated with the small chamber laboratory.

All small chamber system components including temperature, relative humidity and mass flow controllers are calibrated once a year by the EPA Metrology Laboratory. Their procedures are documented in:

- FV-0201.1 GENERAL-Molbloc.doc
- TH-0301.0 GENERAL-Hart Dry Well.doc
- TH-0300.0 GENERAL-RH.doc
- PR-0400.0 GENERAL-Mensor.doc

These Metrology Laboratory files are located on the EPA intranet at:
L:\Lab\NRML_Metlab\METLAB MOPs – ACTIVE.

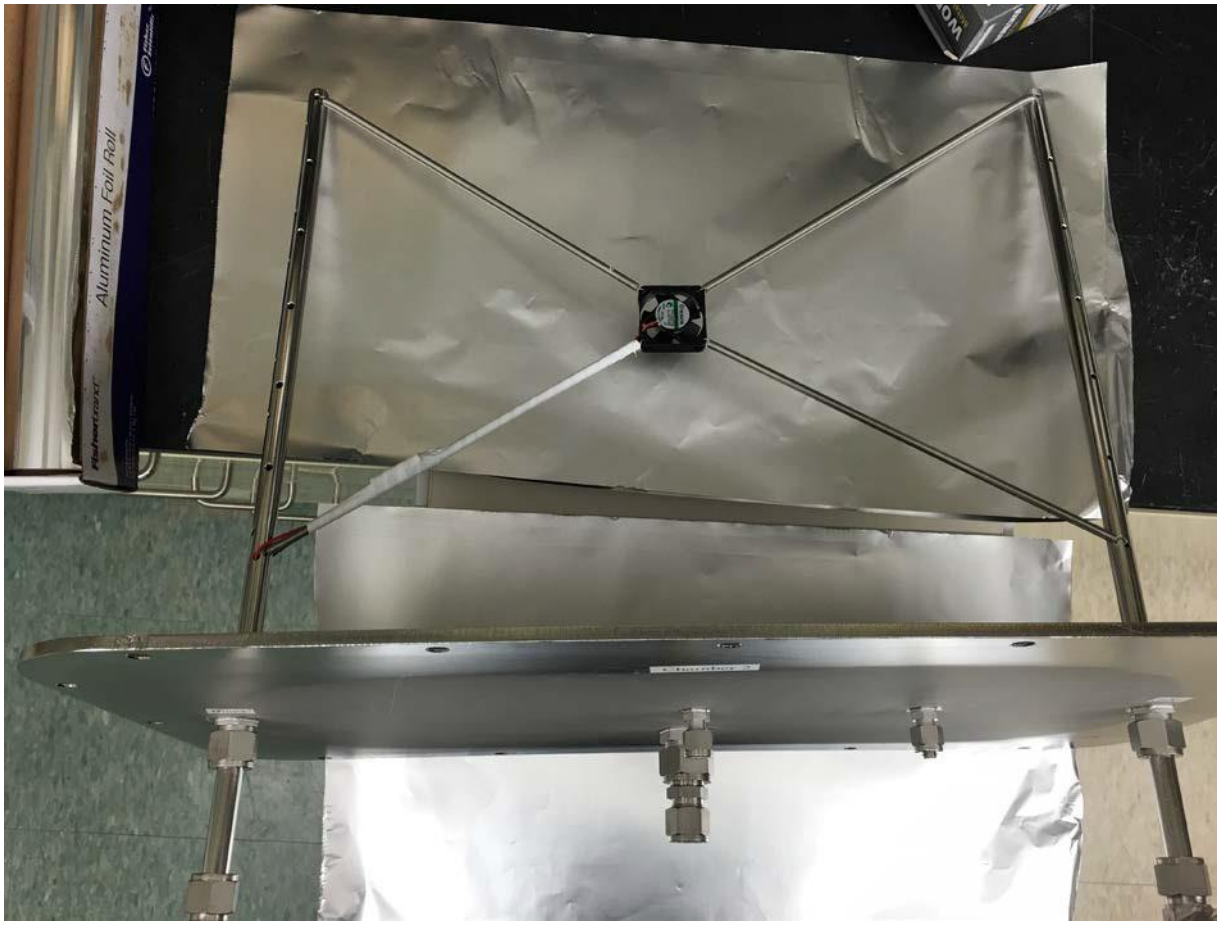
PROCEDURE

Stainless steel chambers (53-L volume) are used most extensively in the Small Chamber Laboratory. Sources are placed in the chambers, and clean humidified air is passed through the chamber. The resulting effluent is then collected for analysis by gas chromatography/ mass

spectrometry (GC/MS), high-performance liquid chromatography (HPLC), or other analytical methods. The chamber environmental conditions (i.e., relative humidity, temperature, and air exchange rate) are specified in individual test plans.

1. Clean and prepare the chamber as described in MOP-6405.
2. Don clean nitrile gloves.
3. Place new, unused foil over assembly area.
4. Insert inlet and outlet diffusers into appropriate faceplate ports, approximately 16 ¼", according to faceplate labels. Diffuser type can be identified by diffusion hole size. Exhaust diffusion holes have a larger diameter of 4 mm; inlet diffusion holes have a smaller diameter of 3 mm. Tighten the diffuser ½" SwageLok® nuts equipped with stainless steel front and back ferrules until finger tight. The diffusion holes should face away from the center of the chamber.
5. If a mixing fan is desired for the chamber test, install a 1½" brushless DC cooling fan by snaking a red and a black wire through the outlet diffuser holes on the inside of the chamber and connecting them to the fan wires with a butt splice. The fan is suspended in the center of the chamber by attaching the four extension springs in an "X" pattern between the inlet and outlet of the diffuser. Hook the other end of each spring to the mixing fan. The fan faces upward so it blows towards the top of the chamber. Final setup is shown in Figure 3, below.

Figure 3. Picture of Mixing Fan setup



6. Thread two wires through the outlet diffuser holes on the inside of the chamber and connect them to the fan wires using two electrical butt splices. Verify that the fan is blowing upwards when plugged in. Tape the exposed fan wires with Teflon from the fan to the outlet diffuser.
7. Connect a ½" stainless-steel Swagelok® union to the end of each diffuser using ½" stainless steel Swagelok® nuts equipped with stainless steel front and back ferrules. Make sure the center fitting of the union is turned toward the outside of the chamber. Thread the two fan wires through the union on the outlet diffuser. Connect a ½" to ¼" stainless steel Swagelok® reducer to the end of the outlet union fitting. Seal the end of the diffuser manifold on the outside of the chamber with a ¼" Swagelok® tube fitting nut with a 9.5 mm pre- conditioned Thermogreen™ LB-2 septa (Supelco part # 20652, or equivalent) placed into the end of the fitting.
8. Connect a male disconnect terminal to the black fan wire and a red female disconnect terminal to the red fan wire approximately 3" from the wires emerging from the ¼" Swagelok® nut with the septa. Connect a female disconnect terminal to the black 12V DC power supply transformer wire and a male disconnect terminal to the red power supply wire. Verify the fan is blowing upward after connection.
9. Outfit the inlet diffuser Swagelok® union center fitting with a ½" stainless-steel nut equipped with 12-mm PTFE front and back ferrules. Connect a ½" to ¼" stainless steel Swagelok® reducer to the end fitting of the inlet diffuser union.
10. Outfit the chamber faceplate ¼" port with a ¼" stainless steel Swagelok® tube fitting nut equipped with ¼" PTFE front and back ferrules.
11. Outfit the chamber faceplate ¾" port with a ¾" female to ½" male (or appropriately sized for the chamber's RH probe) stainless steel Swagelok® reducing union. Install a ½" stainless steel Swagelok® tube fitting nut equipped with ½" PTFE front and back ferrules on the ½" end of the reducing union. This fitting will secure the chamber RH probe once the chamber is in the incubator. It may be necessary to install the PTFE ferrules on the RH probe prior to placing the probe into the port.
12. Install the red chamber O-ring into the groove on the inside of the chamber faceplate. Ensure the O-ring is securely seated in the groove.
13. Lining up the faceplate bolt holes with the bolts on the front flange of the chamber, place the faceplate onto the chamber. The chamber label and the faceplate label will line up when the faceplate is correctly oriented in relation to the chamber. Verify the O-ring has remained seated in the groove. If the O-ring slips out of the groove, remove the faceplate from the chamber, reseat the O-ring, and reinstall the faceplate on the chamber. Use 7/16" brass nuts to secure the faceplate to the chamber. Tighten the two side center nuts first, then the center top and bottom nuts. Working around the faceplate, tighten opposite pairs of the remaining nuts until all nuts are wrench tight. Do not over-tighten the brass nuts, as this could strip the threads of the chamber bolts or even snap the welds of the chamber bolts.
14. Place the chamber inside the incubator and record the date and time in the laboratory notebook. Chamber locations and probes associated with each chamber are listed in Table 3 and shown in Figures 4 and 5, below.

Figure 4. Incubator 1 with Chamber Locations

Figure 5. Incubator 2 with Chamber Locations Table 3. List of Incubator Chamber Locations

Chamber Number	Position	Inlet RH Probe ID	Chamber RH Probe ID	Temperature Probe ID
1	Incubator 1 Position 1	MT-111	MT-112	TE-111
2	Incubator 1 Position 2	MT-121	MT-122	TE-121
3	Incubator 1 Position 3	MT-131	MT-132	TE-131
4	Incubator 1 Position 4	MT-141	MT-142	TE-141
5	Incubator 2 Position 2	MT-151	MT-152	TE-151
6	Incubator 2 Position 1	MT-161	MT-162	TE-161
7	Incubator 2 Position 4	MT-171	MT-172	TE-171
8	Incubator 2 Position 3	MT-181	MT-182	TE-181

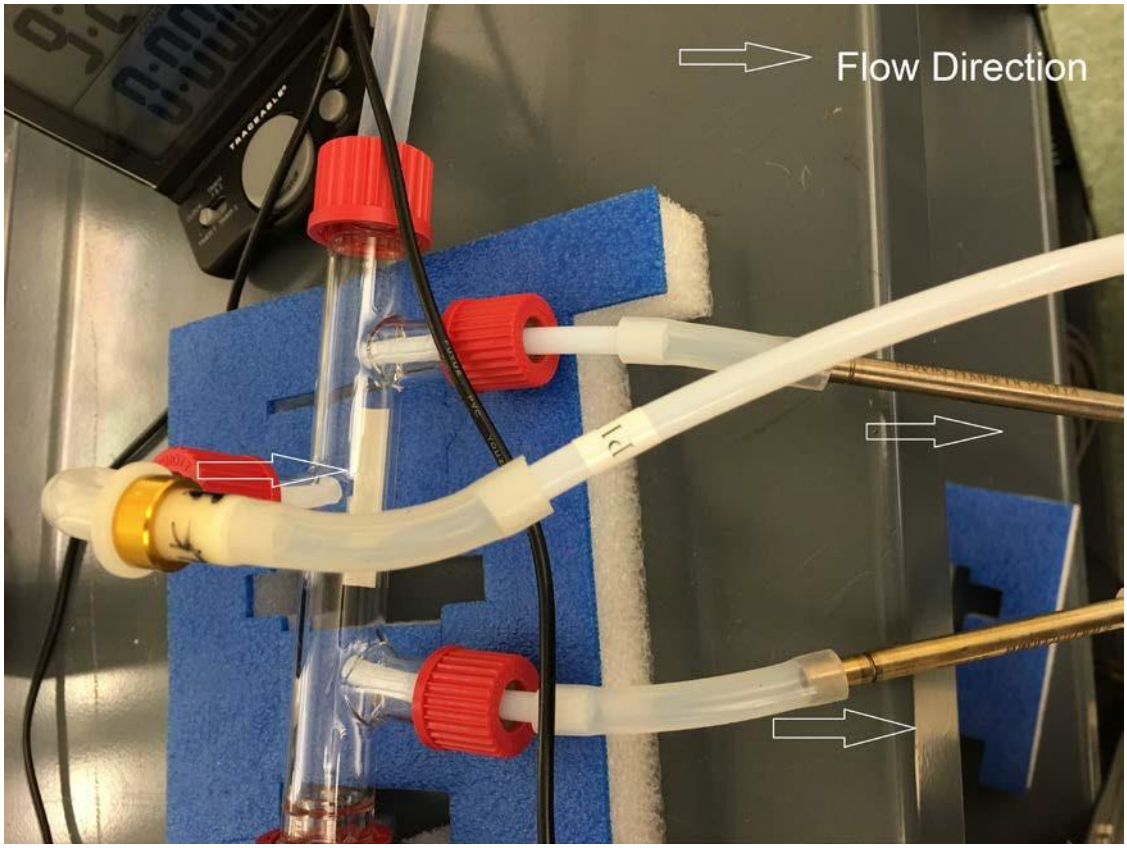
15. Once the chamber is placed inside the incubator in its predefined position, install the inlet RH probe, outlet RH probe, and thermocouple. Remove the frit and install the inlet RH probe in the ½" PTFE front and back ferrules and ½" stainless steel nut. Install the outlet RH probe in the ½" PTFE front and back ferrules and ½" stainless steel nut in the bottom middle of the faceplate. Install the thermocouple in the ¼" front and back ferrules and ¼" stainless steel nut in the top middle of the faceplate. Ensure that each probe is securely fastened and the nuts are tight. Record all times of insertion for each probe.
16. Install the sampling manifold with a 5/8" glass tube to the chamber on the outlet side of the chamber. Ensure all caps are secure on the sampling manifold.
17. Using the OPTO 22 system on the PC in laboratory E378-A, set the environmental parameters for chamber operation, as described in MOP-803 and specified in the relevant QAPP/test plan.

    The following is the typical range of the operating parameters:

    i. Air Exchange Rate: 0.2 to 2.0 h-1 (approximately 900 mL/min)
    ii. Air Velocity: (1 cm above substrate) 5 to 20 cm/sec
    iii. RH (inlet air): 8 to 65%
    iv. Chamber Temperature: 15 to 60°C
    v. Test Period: Test specific, see applicable QAPP/Test Plan
    vi. Loading Factor: Test specific, see applicable QAPP/Test Plan

18. Connect the inlet line to a Gilibrator using a 2" section of silicone tubing. Refer to the manufacturer's operating manual for instructions on Gilibrator operation. Verify that inlet flows match those specified in the QAPP/Test Plan. Record three measurements of the inlet flow with the Gilibrator. Record the time, incubator temperature, and barometric pressure.
19. Connect the inlet line to the inlet diffuser at the ¼" port and record the time.
20. Connect the fan to power and record the time.
21. Using the Gilibrator, verify that the outlet flows match those specified in the project QAPP/Test Plan. Record triplicate outlet flow measurements with the Gilibrator. Record the time, incubator temperature, and barometric pressure.
22. Collect background air samples from the chamber according to MOP-812 and SOP-6404. Refer to the test QAPP/Test Plan for sampling duration and flow rates.
23. If a substrate will be used during chamber testing, prepare, and condition the substrate as specified in the QAPP/Test Plan.
24. Before starting the test, check relative humidity and temperature via the OPTO-22 display to ensure that the set parameters remain steady. Consult the project QAPP/test plan to determine the test's acceptance range for the chamber environmental parameters. Record temperature and relative humidity data in the study log book.
25. Measure the chamber's inlet flow with a Gilibrator primary flow calibrator to confirm set point and air exchange rate. Record flow rate and air exchange rate data in the study log book.
26. After at least 12 hours, or the time period specified in the QAPP/Test Plan, collect the chamber background samples following procedures described in SOP-6404 and MOP-812. See Figure 6 for the sampling manifold setup during sample collection.

Figure 6. Picture of Sampling with DNPH and Carbopack X Cartridges



27. After background sampling is completed, disconnect the inlet flow, disconnect the fan, and remove the glass extension tube and sampling manifold. Record the time each component was disconnected. Remove peripheral nuts from around the face of the chamber using a ratchet with a 7/16" driver or a 7/16" wrench. After all nuts have been removed, carefully remove the chamber lid by holding the air inlet and outlet extensions and pulling the lid back. Once the lid is off, record the time in the laboratory notebook.
28. Place the prepared test material on the center of the bottom of the chamber. Confirm that the mixing fan is on and blowing in the upward direction prior to sealing the chamber lid. Record the time the test material was placed in the chamber.
29. Replace the chamber lid onto the chamber, ensuring that the O-ring remains in place. Record the time the chamber is closed. Once on, replace all of the peripheral nuts and tighten until snug. Once the nuts are secure, measure the inlet flow with the Gilibrator and record triplicate flow rates, incubator temperature, and barometric pressure.
30. Attach the fitting for measuring the exhaust flow. Measure the outlet flow with the Gilibrator. If the exhaust flow is equal to the inlet flow minus 5% of the inlet flow or greater than the inlet flow, proceed to the next step. If the exhaust flow is more than 5% lower than the inlet flow, then recheck each of the chamber lid nuts as well as all fittings attached to the chamber. If the flow remains more than 5% lower than the inlet flow, remove all peripheral nuts, realign the chamber lid gasket, and repeat the above procedure until chamber seals. If chamber does not seal discontinue the test.

31. Attach the glass extension tube and sampling manifold. Connect the exhaust tubing and manifold.
32. Collect air samples from the chamber following the schedule in the QAPP/Test Plan and procedures in SOP-6404 and MOP-812.
33. Continue to monitor the environmental conditions using the display on the chamber's environmental data collecting computer. Record any deviations from the test protocol. If the system fails, discontinue the test.
34. At the completion of the test, collect and record chamber outlet flow readings using the Gilibrator. Record the incubator temperature and barometric pressure. Disconnect the inlet air supply line. Using the Gilibrator collect triplicate inlet flow measurements and record them in the test notebook. Record the incubator temperature and barometric pressure.
35. Open chamber as described in Step 27 to remove test material from the chamber. Store or discard the test material as detailed in the QAPP/Test Plan.

Operation of the OPTO Display Software Data Acquisition System (DAS) in the Small Chamber Laboratory (MOP 803) – August 2016

U.S. EPA Office of Research and Development National Risk Management Research Laboratory
Air Pollution Prevention and Control Division

Miscellaneous Operating Procedure 803
Operation of the OPTO Display Software Data Acquisition System (DAS) in the Small Chamber Laboratory

Authored by	Matt Allen, Jacobs (Work Assignment Leader)	08/08/2016 Date
Approved by		
EPA Team Leader	Xiaoyu Liu	Digitally signed Date: 2016.08.08/12:17:49 -04'00'
EPA QA Representative	Paul Groff	Date: 2016.08.08/14:23:18 -04'00'
Jacobs Work Assignment Leader	Matthew Allen	08/08/2016 Date
Jacobs QA Officer	Zora Drake-Richman	08/08/2016 Date

Keywords: OPTO, Environmental Data, Data Acquisition

MISCELLANEOUS OPERATING PROCEDURE (803) FOR OPERATION OF THE OPTO DISPLAY SOFTWARE DATA ACQUISITION SYSTEM (DAS) IN THE SMALL CHAMBER LABORATORY – August 8, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Bob Wright
Jacobs	Matt Allen
	Zora Drake-Richman
	Gary Folk
Laboratory Copy	Maintained by X. Liu
Electronic Filing	Scientific Data Share L:\LAB\NRML_Public\SVOCs2013\Tris Phosphate FR\QA Documents\Approved MOPs

Revision Record

Revision	Date	Responsible Person	Description of Change
0	February 2003		Initial release
1	August 2003		Updated scope of MOP to cover operation of OPTO system only
2	May 2015	Matt Allen	Updated MOP to outline navigation of OPTO system in chamber setup. Added screenshots from OPTO system. Included data download into Excel files.
3	January 2016	Matt Allen	Made minor formatting revisions based on EPA QA review. QLOG No. A-16211, QTRAK No. 11037.
4	June 2016	Matt Allen	Added Updating Calibration Factors section
5	August 2016	Matt Allen	Made revisions based on EPA QA review comments. QLOG No. G-APPCD- 0016211, QTRAK No. 11037_16, QA Category B.

TITLE

Operation of the OPTO Display Software Data Acquisition System (DAS) in the Small Chamber Laboratory

PURPOSE

To have a written and repeatable procedure for the operation of the OPTO Display DAS program in Small Chamber Laboratory (E-378A).

SUMMARY OF METHOD

The OptoDisplay Runtime (OPTO) data acquisition system controls, monitors, collects, and records environmental conditions within the 53-L small chamber testing system through the management of a series of mass flow controllers. During chamber setup, the OPTO is used in conjunction with the clean supply air system and incubators to establish environmental conditions (air exchange and humidity) within test chambers as specified by project test plans. Chamber airflows and humidity levels are controlled by the OPTO system, while the chamber temperatures are controlled using the incubator temperature setpoints. During testing, the OPTO is used to monitor, maintain, and record environmental conditions within test chambers.

DEFINITIONS

ACH	air exchanges per hour
APPCD	Air Pollution Prevention and Control Division
°C	degree(s) Celsius
DAS	data acquisition system
EPA	U.S. Environmental Protection Agency
ft	foot (feet)
HMI	human-machine interface
L	liter(s)
m3	cubic meter(s)
MFC	mass flow controller
mBar	millibar(s)
min	minute(s)
mL	milliliter(s)
MOP	Miscellaneous Operating Procedure
OPTO	OptoDisplay Runtime system
OS	Operating System
PC	personal computer
RH	relative humidity
Temp	temperature
UPS	uninterruptable power supply
V	chamber volume

RELATED DOCUMENTS

MOP 801: *Preparation of small (53-L) chambers for testing*
MOP 802: *Operation of environmental small chambers during testing*
MOP 804: *Operation of the OPTO Display DAS using the touchscreen panel*
MOP 806: *Operation of the clean air system for the small chamber laboratory*

EQUIPMENT

Table 1. List of Small Chamber Laboratory Equipment

Component	Manufacturer	Model #	Location	Description
Inlet RH Probes (8)	Vaisala	HMT333	Vantaa, Finland	Measure RH of inlet air to small chambers
Internal RH Probes (8)	Vaisala	HMT335	Vantaa, Finland	Measure RH inside center of small chambers
Thermocouples (12)	Pyromation	E-Type	Fort Wayne, IN	Measure temperature inside center of chambers
Pressure Transmitter	Vaisala	PTA 427	Vantaa, Finland	Measure barometric pressure of small chamber laboratory
OPTO Control System	Opto 22	B3000	Temecula, CA	Communication hub for mass flow controllers (MFCs), RH probes, thermocouples
OPTO Display Software	Opto 22	OPTO Display Runtime R4.1a	Temecula, CA	Software that controls the OPTO system from the laboratory computer
Electrical Control Box	Carotek, Inc	AT-607983	Mathews, NC	Control panel to house OPTO Hardware Components
OPTO Operation Computer with Windows XP OS	Dell	Optiplex 745	Round Rock, Tx	Houses OPTO Display software used to monitor, log, and regulate small chamber environments (RH, air changes per hour (ACH), Temp.)
Mass Flow Controllers (16)	Teledyne Hastings Instruments (15) / Millipore Tylan® (1)	HFC-E-202 FC-260	Hampton, VA Billerica, MA	Regulates airflows to chamber system
Surgitron ® Joslyn Alternating Current Secondary Surge Arrester	Thomas & Betts Power Solutions	1250-32-E	Richmond, VA	Helps prevent damage to electronic equipment from transient voltage "spikes" and, while charged, maintains power to equipment in the event of a power outage

PROCEDURE

General Information
The OPTO software program is part of the Data Acquisition System (DAS) in the Small Chamber Laboratory. OPTO is a software package used to create human-machine interfaces (HMIs) or operator interfaces for monitoring control systems. OPTO Display is used to create an HMI that monitors all configured parameters, providing real-time and historical data to the operator.

For more specific and detailed information relating to the operation of this instrument, refer to the OPTO Display User's Guide. This guide can be found in the dropdown menu of the HELP tab found on all of the OPTO's display screens.

DAS Setup

The hardware of the OPTO is a large (6 ft x 4 ft x 1 ft) gray metal panel box located next to the two-chamber incubator system in the E378-A laboratory. This box is coupled to a personal computer (PC) for software, data acquisition and storage.

To ensure that the DAS control panel is operating, open the gray control panel box by lifting and sliding the metal clips on the side of the door. There is an internal power switch located inside the gray panel box on the top circuit row just to the left of the Surgitron ® Joslyn Alternating Current Secondary Surge Arrester. This switch should be in the ON position. The switch on the front of the control panel should be in the ON position and the power light should be lit.

The front panel of the gray control box contains a touch screen interface that can be used to operate the DAS. This MOP will not cover the use of the touch screen interface. See MOP-804 for procedures covering the operation of the DAS through the touch screen on the front panel. If the DAS is supplied power through an uninterruptible power supply (UPS), the operation of the DAS will continue during a power outage as long as the UPS remains charged.

Uploading OPTO DAS Sensor Calibration Data

Mass flow controllers, temperature, moisture, and pressure transmitters being controlled by the OPTO system will undergo periodic calibration. The APPCD Metrology Lab will collect all necessary calibration according to approved MOPs. Their procedures are documented in:

- FV-0201.1 GENERAL-Molbloc.doc
- TH-0301.0 GENERAL-Hart Dry Well.doc
- TH-0300.0 GENERAL-RH.doc
- PR-0400.0 GENERAL-Mensor.doc

These Metrology Laboratory files are located on the EPA intranet at:
L:\Lab\NRML_Metlab\METLAB MOPs – ACTIVE. Calibration reports are kept on file in laboratory E378-A and electronically on the NRMRL L: drive.

After analysis and reduction of the raw calibration data, the OPTO DAS will be updated with new calibration curves for each data transmitter. As transmitter response plots are typically linear, the new calibration updates will simply be an updated plot slope, hereafter referred to as the scale (slope of a probe's linear response plot), and a y-intercept, hereafter referred to as the offset (the y-intercept of a probe's linear response plot).

Scales and offsets are uploaded into the OPTO system via initialization (init) text files. The init files are stored in the C: drive of the PC in laboratory E378-A on which the OPTO DAS software is installed. The location of these files is: C:\SCADA files\OptoControl. To upload new calibration data, follow the following procedures:

To use the PC operating the OPTO DAS in laboratory E378-A, go to C:\SCADA files\OptoControl.

1. Open the OptoControl folder and locate the file named init.txt.
2. Rename the init.txt file to differentiate the old calibration data file from the new calibration data file when the new file is uploaded.
3. Copy the new init.txt file, which was generated by the Metrology Laboratory, into the OptoControl folder. The new init.txt file should be named init.txt.
4. Close the OPTO Runtime software.
5. From the Start Menu in the lower left side of the screen, open OPTO Control.
6. In the Mode dropdown menu, select Configure.
7. In the Configuration box, edit a parameter line and select OK.
8. Open the same configuration parameter box and edit the parameter back to its original value to enable the system to recognize a change to the software configuration.
9. In the Mode dropdown menu, select Debug.
10. The Save Strategy box will open. Select Yes.
11. The Download Warning box will open. Select Yes. The OPTO software will display the progress of the configuration download. When the download is complete, proceed with Step 13.
12. In the Controller dropdown menu, select Inspect and then Run. Click Close.
13. In the Controller dropdown menu, select Stop and then Close.
14. In the Controller dropdown menu, select Save Strategy to send the newly uploaded strategy configuration to the OPTO control unit from the PC.
15. In the Debug dropdown menu, select Run.
16. Exit from the OPTO Control software.
17. Open the OPTORuntime software. The newly uploaded calibration scales and offsets will be displayed in the Sensor Cal screen.

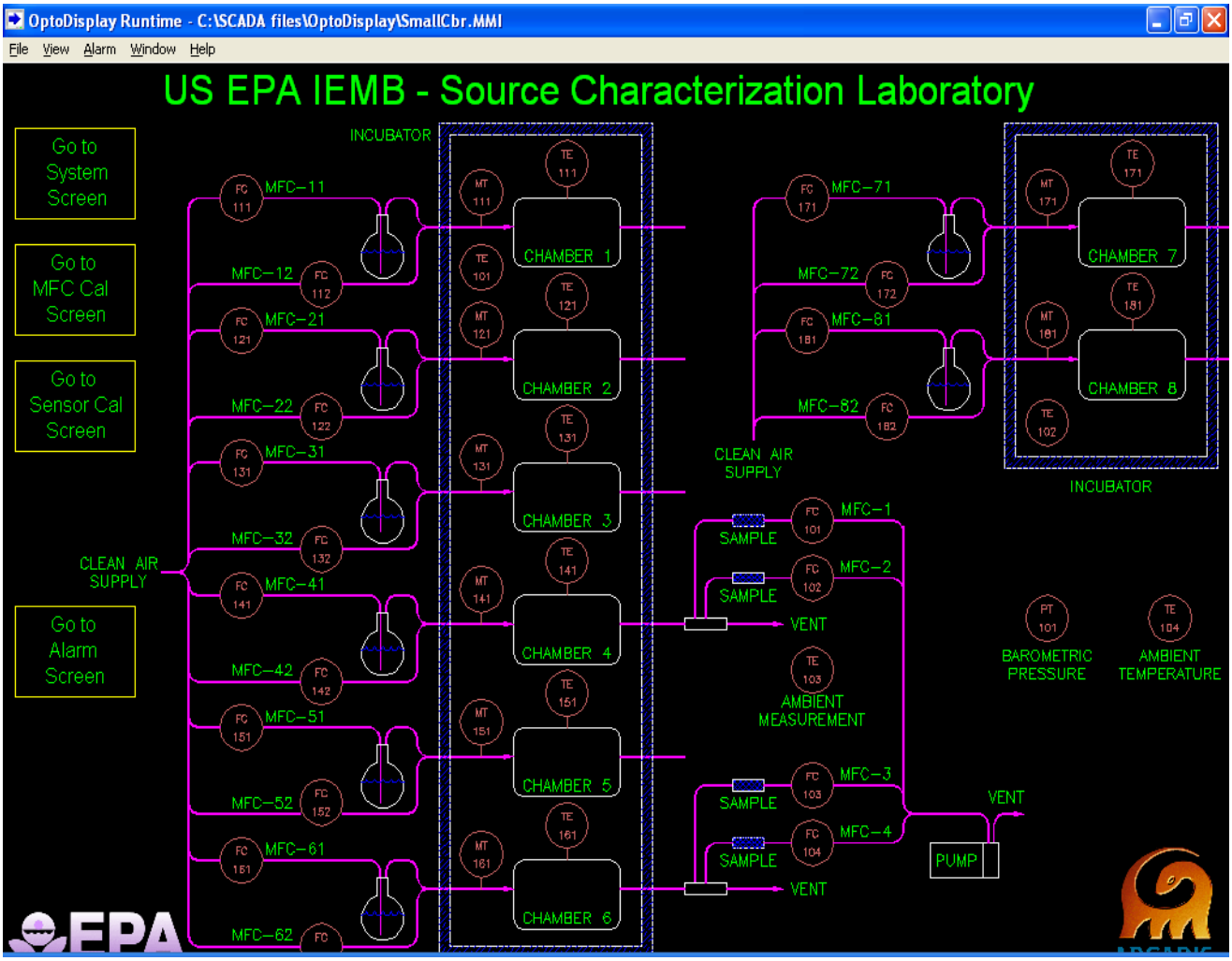
OptoDisplay Operation in Automatic Mode

The OPTO system can be operated automatically (as well as manually, see OptoDisplay Operation in Manual Mode section for details) to allow the user to set the desired environmental parameters for the small chamber system and allow the OPTO system to make incremental adjustments to maintain those parameters. Once system parameters (ACH and %RH) are set, the system will automatically maintain those parameters by adjusting the proportional airflows

through the 'Wet' and 'Dry' mass flow controllers for each chamber. Prior to setting a chamber's parameters for automatic mode operation, the user must know the required chamber temperature in degrees Celsius (°C), ACH, and RH. Follow the steps below to adjust OPTO system environmental parameters automatically.

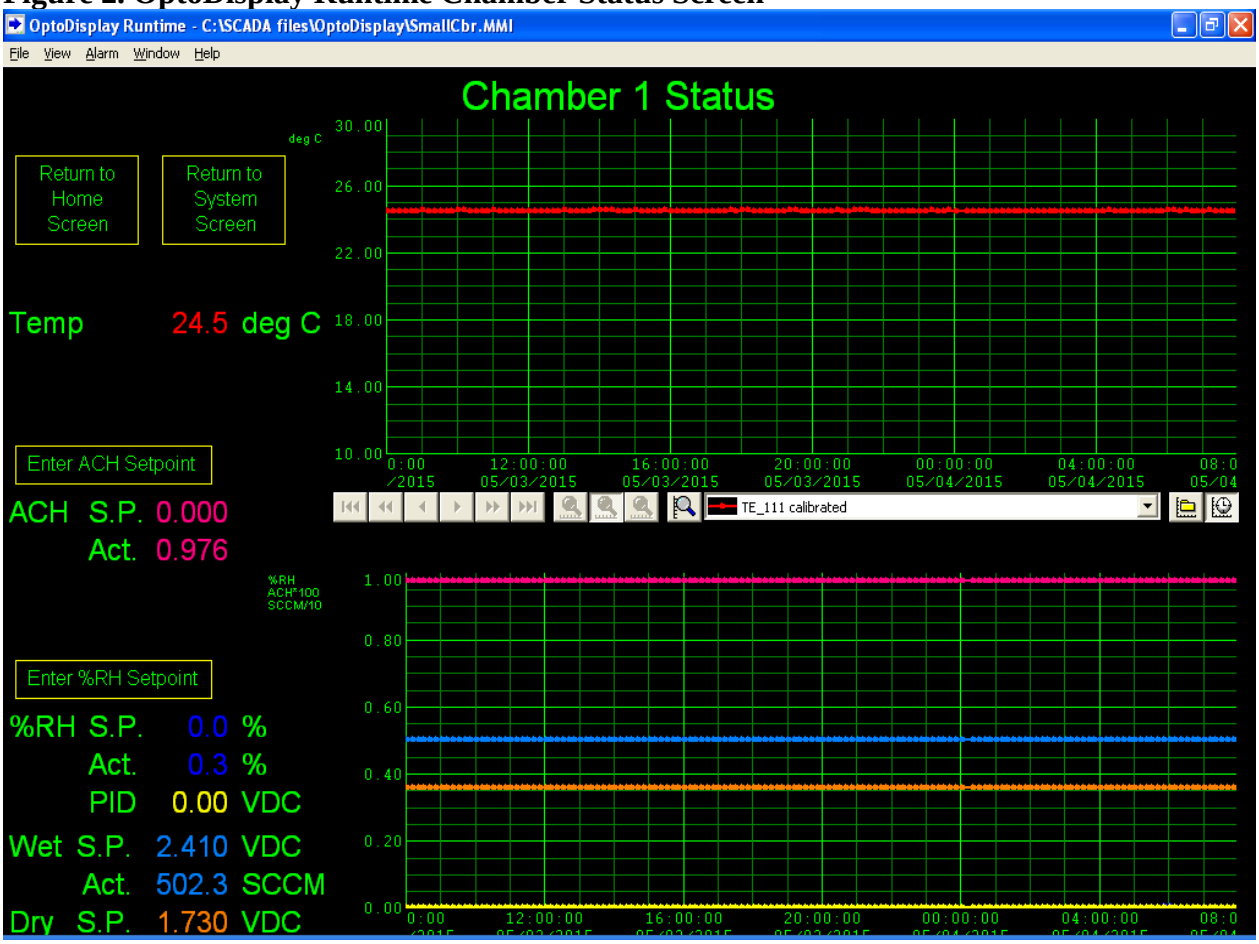
1. Turn on the DAS controlling computer.
2. At the prompt, press the "control, alt, and delete" keys simultaneously to log on to Windows XP. Enter the network password "Welcome 1," and then press ENTER on the keyboard to continue.
3. Select the OptoDisplayRuntime icon from the desktop, and the Opto Display DAS will then load.
4. An Event Log Viewer Screen will then appear. This screen shows where logged data will be stored.
5. Select "Close" and the Home Screen (Figure 1) will be displayed. This is a detailed diagram of the clean air flow system to the chambers.

Figure 1. OptoDisplay Runtime Home Screen



6. To enter the system display and control page for each individual chamber's status from this screen, point the cursor to the desired chamber diagram on the Home Screen and click once or press the "Page Up" key on the keyboard.
7. The Chamber # Status (Figure 2) page is now displayed. To scroll through each chamber page, continue to press the "Page Up/Page Down" keys until you have reached the desired chamber page.

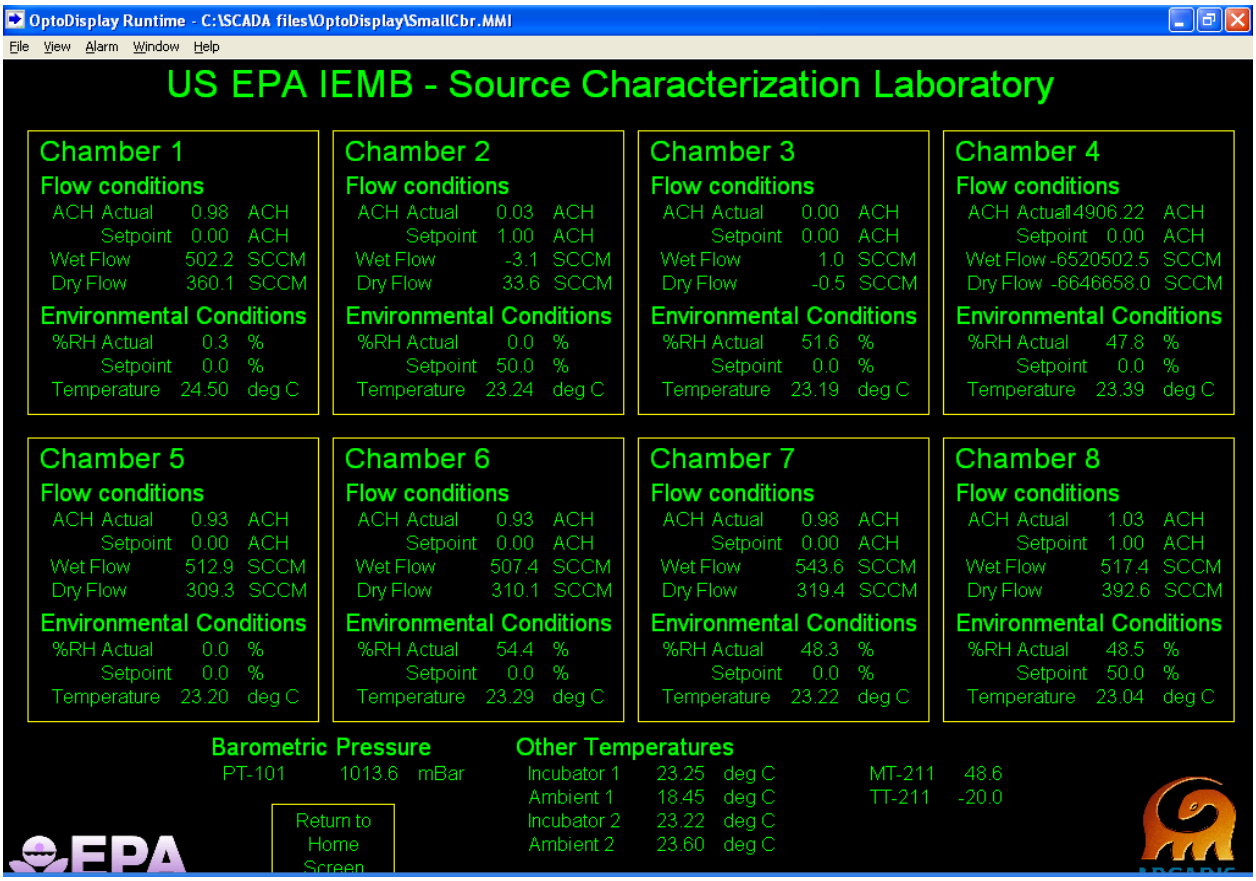
Figure 2. OptoDisplay Runtime Chamber Status Screen



The Chamber # Status window graphically displays the temperature, relative humidity, and air exchange rate for each individual chamber. From this screen, the chamber's air exchanges per hour (ACH) can be set by pointing the cursor in the "Enter ACH Setpoint" box. Once selected, a gray data entry box appears. Enter an ACH set point between 0.1 and 3 air changes per hour. Set the chamber's RH by pointing the cursor to the "Enter %RH Setpoint" box and entering in the desired %RH. The OPTO system will adjust both parameters so that criteria are met. It is best to set the chamber ACH and %RH at least 12 hours before the start of a test to allow the system's RH and temperature to change and stabilize within the accepted limits specified in the test plan. These parameters can be monitored visually on the OptoDisplay's Runtime Chamber Status Screen (see Figure 2).

The last page is the System Screen (Figure 3). Access the System Screen by pressing the "Go to System Screen" button on the Home Screen. This page displays the operating parameters of all eight chambers, barometric pressure, and laboratory and incubator temperatures. This is a display page only; no set points can be changed on this screen.

Figure 3. OptoDisplay Runtime System Screen



OptoDisplay Operation in Manual Mode

The OPTO system can be operated manually (as well as automatically, see OptoDisplay Operation in Automatic Mode section for details) to allow the user to manually set the environmental parameters for the small chamber system and lock said parameters once set. Once system parameters are set, they will not change unless the user changes them or the system is switched back into automatic mode. Prior to setting a chamber's parameters manually, the user must know the required chamber temperature in degrees Celsius (°C), ACH, and RH. Follow the steps below to adjust OPTO system environmental parameters manually.

Set up the small chamber to be used for testing in the incubator according to MOP-802. Set the incubator temperature to the test plan's prescribed chamber temperature. Allow the incubator temperature at least three hours to stabilize before continuing.

Follow steps 1-5 from the previous section OptoDisplay Operation in Automatic Mode.

1. Once in the Home Screen, click on the Go to System Screen button in the upper left to open the System Screen (Figure 3).
2. On the System Screen, identify the chamber to be set up. Note the chamber's RH in the laboratory notebook for later use when setting the chamber RH. Measure and record that chamber's inlet wet and dry flows using the Gilibrator at the chamber's inlet manifold.
3. Calculate the chamber's current ACH using the combined wet and dry flows previously measured by the Gilibrator as the inlet flow in the following equation:

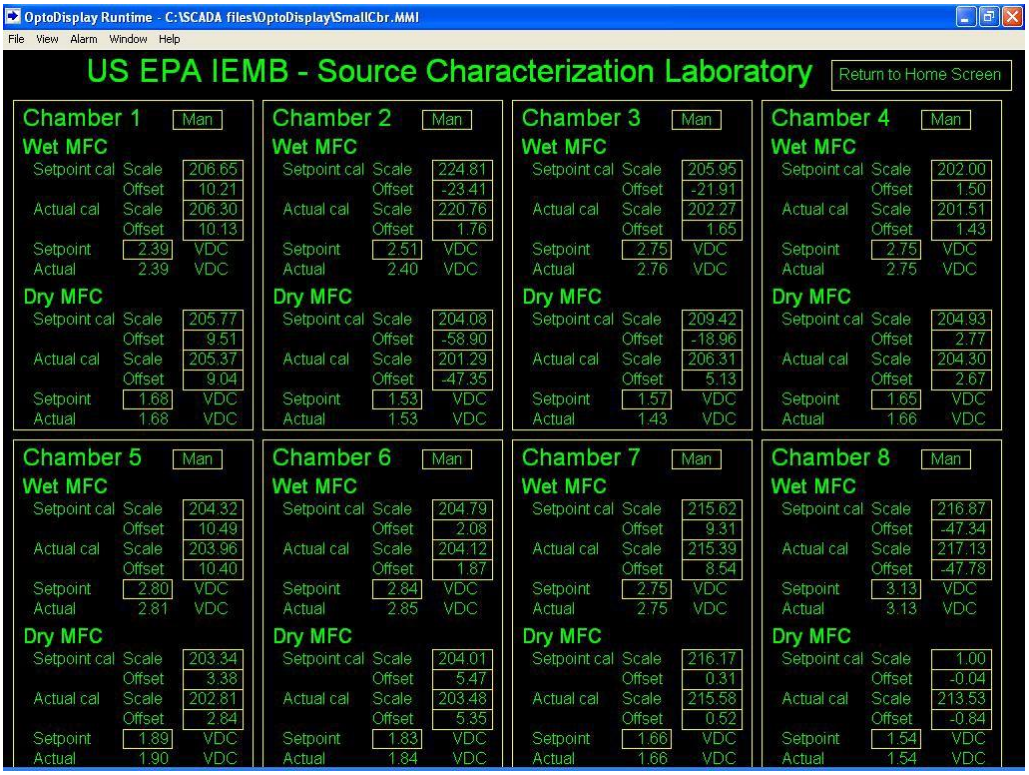
$$\underline{FF \times 60}$$

$$AAAAAA = \underline{1000}$$
$$VV$$

Where: F is the chamber's inlet air flow in milliliters per minute (mL/min)
V is the chamber volume in cubic meters (m3).

4. Compare the calculated ACH to the test plan's specified ACH to determine whether flow rates need to be increased, decreased, or not adjusted at all.
5. Click the Return to Home Screen button in the lower left. In the Home Screen, click the Go to MFC Cal Screen button on the left. A text box requesting a password will open. Type "tufts" and click OK.
6. In the MFC Cal Screen (Figure 4) locate the box containing information on the chamber being set up. At the top of the chamber information box, click the Auto box. The box should now say Man. The OPTO system's control for that chamber has been switched into manual mode.

Figure 4. OptoDisplay Runtime MFC Cal Screen



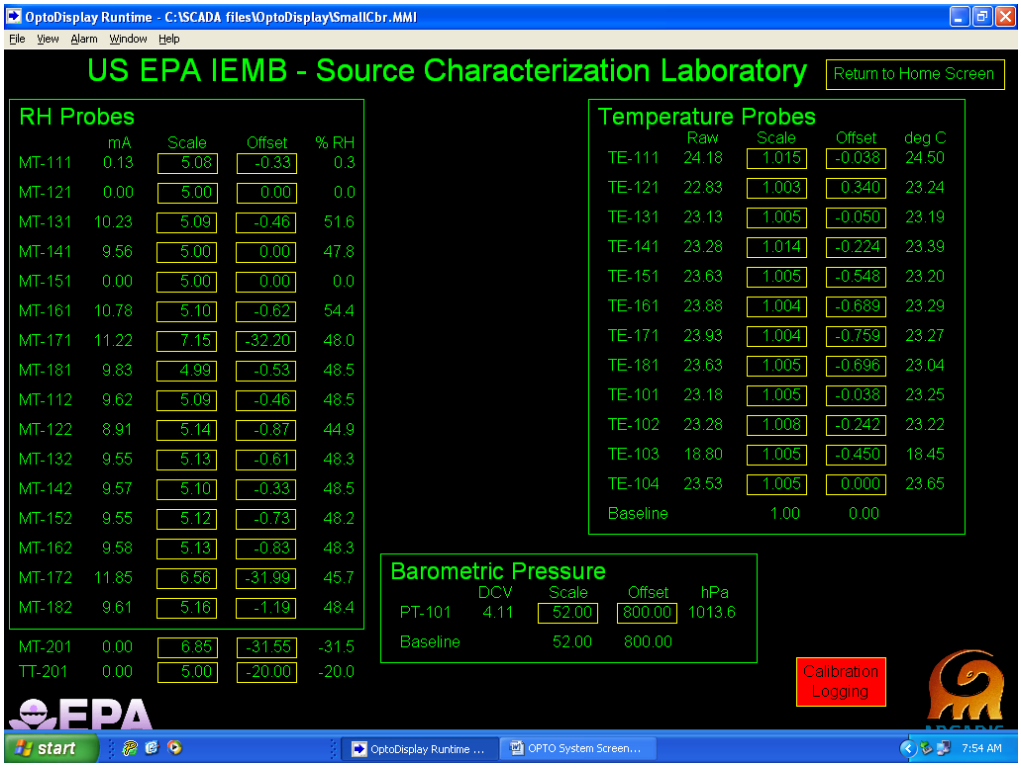
To increase or decrease the chamber ACH in manual mode:

7. Click the Setpoint box in the Wet MFC section. Using the previous set point as a guide, increase/decrease the wet flow controller's voltage setting. Click OK. The new set point should now register in the Setpoint box of the Wet MFC section.
8. Repeat the previous step for the Setpoint box in the Dry MFC section. Make sure the new set points are displayed in the Setpoint box. Allow the system's MFCs approximately five minutes to adjust to the new system flows before continuing.
9. Disconnect the wet and dry inlet lines from the chamber and measure the chamber's inlet flows again using the Gilibrator. The flows should have increased or decreased depending on how the MFC set points were adjusted.
10. Recalculate the chamber's ACH with the new flows and determine whether or not to adjust flows further.
11. If flows do need to be adjusted, repeat Steps 9 through 11 until the desired ACH has been achieved. When the ACH is set, reconnect the chamber's inlet air supply to the inlet manifold so the chamber can begin to stabilize to the test plan's specified environmental parameters. Allow this air to purge through the chamber overnight before continuing.
12. After the ACH is set and the chamber has stabilized, the chamber RH parameters must be set by changing the proportional wet and dry flows entering the chamber while keeping the total ACH constant.
13. From OPTO's Home Screen, click the Go to System Screen button in the upper left. Locate the information box for the chamber of interest. In the Environmental Conditions section, the %RH Actual reading will be displayed. Record the chamber's %RH reading

and then click Return to Home Screen.

14. In the Home Screen, click the Go to MFC Cal Screen button. Enter the password “tufts” and click OK.
15. To increase or decrease the chamber RH:
16. To increase chamber RH, increase the wet flow voltage and decrease the dry flow voltage by equal increments. To decrease the chamber RH, decrease the wet flow voltage and increase the dry flow voltage by equal increments.
17. Click the Setpoint box in the Wet MFC section. Using the previous set point as a guide, increase/decrease the wet flow controller’s voltage setting. Click OK. The new set point should now register in the Setpoint box of the Wet MFC section.
18. Repeat the previous step for the Setpoint box in the Dry MFC section. If wet flows were decreased, then increase the dry flows; if wet flows were increased, then decrease the dry flows. Make sure the new set points are displayed in the Set point box. Allow the system several minutes to stabilize before continuing.
19. Measure the chamber’s inlet flows using the Gilibrator. The total inlet flow should be equal to previous measurements recorded prior to adjusting the flows for RH. If the total inlet flow is not equal to the previous measurement, adjust wet or dry voltages so the RH remains at the target set point, but air flows are either increased or decreased to the desired ACH.
20. Recalculate the chamber’s ACH with the new flows from the Gilibrator (measured in Step 17) using the ACH equation, above. If the newly calculated ACH was altered by more than 5% in adjusting for RH, re-adjust the wet and dry MFC voltage set points to re-establish the ACH specified in the test plan. Once MFC voltage set points have been adjusted for RH and the chamber ACH is at the desired flow, allow the chamber at least eight hours to stabilize and then recheck the chamber RH on OPTO’s System Screen. If the RH is within test plan QC limits, it does not need to be adjusted. Allow the system to purge overnight before continuing with test preparations and then recheck the environmental parameters to ensure they are remaining stable. If the RH does need to be adjusted further, repeat Steps 16-18 until the RH has stabilized within test plan QC limits.
21. If multiple chambers are being set up using the OPTO, the Sensor Cal Screen (Figure 5) can be used to monitor multiple temperature and RH probes simultaneously. To access the Sensor Cal Screen, go to the Home Screen (Figure 1) and then click Go to Sensor Cal Screen. Make sure to verify the identification of the probes to be monitored. Each probe is tagged with an identification label for this purpose.

Figure 5. OPTO Display Runtime Sensor Cal Screen



Downloading OPTO Data

Each day beginning at midnight, the DAS automatically creates new files and begins logging data to them. Data are saved in the following files (where YY is the last two digits of the current year, MM is the current month and DD is the current day. For example, May 4, 2015 would be 150504):

RdYYMMDD.h00: This file contains all of the temperature, relative humidity and air exchange data for all eight of the chambers and current barometric pressure data. Table 2 outlines the format of the data stored in this text file.

RdYYMMDD.h03: This file contains the temperature data for the incubator and ambient thermocouples. Table 3 outlines the format of the data stored in this text file.

Table 2. Format of File RdYYMMDD.h00

A	B	C	D	E	F to Z	AA
Date YYMMDD	Time HH:MM:SS	Chamber 1 Temp. (°C)	Chamber 1 RH (%)	Chamber 1 ACH	Chambers 2 to 8	Barometric Pressure (mBar)

Table 3. Format of File RdYYMMDD.h03

A	B	C	D	E	F
Date YYMMDD	Time HH:MM:SS	TE-101 Temp. Incubator 1 (°C)	TE-102 Temp. Ambient 1 (°C)	TE-103 Temp. Incubator 2 (°C)	TE-104 Temp. Ambient 2 (°C)

Logged environmental data files are stored in the OPTO PC's hard drive at C:\DATA\. Files are then periodically backed up on an external hard drive; however, the data will also permanently remain on the OPTO PC's hard drive for availability. Files are in text format and can be imported into MS Excel for data reduction. An Excel file titled "Small Cbr Lab 2.xlsm" contains a macro located on OPTO PC desktop that will automatically download all of the vital data into an Excel notebook for each test.

Double-click on the Small Cbr Lab 2.xlsm file icon. When the file opens, select the Developer tab at the top of the screen. Click on the Macro button in the upper left. A text box will open with a selection named "Enviro_Data". Highlight this selection and click Run. A text box will open. Enter the Chamber #, start date, start time, end date, and end time. Press the Enter key. The macro will download environmental data from the OPTO system and provide an Excel spreadsheet and chart for the specified chamber and time interval.

Operation of the Clean Air System for the Small Chamber Laboratory (MOP 806) – June 2016

U.S. EPA Office of Research and Development
National Risk Management Research Laboratory
Air Pollution Prevention and Control Division

Miscellaneous Operating Procedure 806
Operation of the Clean Air System for the Small Chamber Laboratory

Authored by
Matt Allen, Jacobs Technology 6/22/16
Date

Approved by

EPA Team Leader
Xiaoyu Liu Date

APPCD QA Manager
Bob Wright Date

Jacobs Project Manager
Matthew Allen 6/22/16 Date

Jacobs QA Officer
Zora Drake-Richman 6/22/16 Date

Keywords: Small chamber, clean air system

MISCELLANEOUS OPERATING PROCEDURE (806) FOR OPERATION OF THE CLEAN AIR SYSTEM FOR THE SMALL CHAMBER LABORATORY – June 20, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Bob Wright
Jacobs	Matt Allen
	Gary Folk
	Zora Drake-Richman
Laboratory Copy	Maintained by Jacobs
Electronic Filing	To be determined

Revision Record

Revision	Date	Responsible Person	Description of Change
0	Unknown	Unknown	Initial document
1	Aug 2003	Unknown	Updated after move to new facility
2	June 2016	Matt Allen	Updated with new equipment, new system schematic, and inserted title page, approvals page, and distribution list

MISCELLANEOUS OPERATING PROCEDURE NO. 806

TITLE

OPERATION OF THE CLEAN AIR SYSTEM FOR THE SMALL CHAMBER LABORATORY.

SCOPE

Procedures to produce and supply a source of clean volatile organic compound (VOC) free air to the small chambers.

PURPOSE

This procedure provides a reproducible method for producing VOC free air.

ACRONYMS AND ABBREVIATIONS

DAS	Data Acquisition System
°C	Degrees Celsius
FEP	Fluorinated ethylene propylene
FV	Flow Valve
in.	inch(es)
MFC	Mass Flow Controller
mL	milliliter(s)
µm	micron
MOP	Miscellaneous Operating Procedure
PI	Pressure Indicator
psig	pounds per square inch gage
PTFE	polytetrafluoroethylene
SCCAS	Small Chamber Clean Air Supply
SLPM	Standard Liters per Minute
VOC(s)	Volatile Organic Compound(s)

PROCEDURE

Introduction
The clean air system is a critical part of the small chamber laboratory, because the research performed requires an endless supply of clean conditioned air to the chambers. This supply of air must be free of most volatile organic compounds (VOC). Table 1 lists the components of the small chamber clean air supply.

Table 1. Components of the Small Chamber Clean Air Supply

Component	Manufacturer	Model/Serial #
Filter/Regulator Assembly	Wilkerson	Model – l B18-03-FK00
Particulate Filter (15 µm)	Swagelok®	Model – B-6F-15
Compressor	Gardner Denver Thomas	Air tank: Manchester, TX Size: 10 gallons Model – TA-6102 Motor: Thomas Dry Running Articulated Piston Compressor Model – 8-164551-03; SN: BU1-065
Refrigerated Air Dryer	Hankinson	Model – 8010—SS SN: 0302A-15-9010-49N
Timed Electric Auto Drain Valve	Speedaire	Model – 6Z948 1-60-minute adjustable drain cycle time.
Compressed Air Dryer	Hankison	Model – SSRD10-300
Toggle Shut-off Flow Valve	Swagelok®	Model – SS-1GS4
Carbon Trap	Supelco	Model – 24565
Moisture Trap	Supelco	Model – 23992
Molecular Sieve 5A refill for Supelco moisture trap. Mesh size is 8-12 mesh.	Supelco	Model – 20298
Coalescing Hydrocarbon Filter	Speedaire	Model – 6ZC24
Pure Air Generator	AADCO	Model – 737-11A
Coalescing Filter/Regulator	Norgren	Model – B736-2AK-API-RMG
Pressure Gauge	U.S. Gauge	60 psi (or equivalent)
Mass Flow Controllers (16)	Tylan (1)	Model – FC-260; 1 SLPM

Component	Manufacturer	Model/Serial #
	Teledyne Hastings (15)	Model – HFC-E-202: 1 SLPM
Data Acquisition System	OPTO 22 Software	
Water Bath Tub (no temperature controls on tub)	Blue M	Model – MR-324C1; SN: M2-340
Constant Temperature Bath	Fisher Thermo Scientific	Model – Neslab RTE 7
1000 mL Round Bottom Flask	Prism Glass	Model – PRG-5795-03
Midget Impinger Bubbler	Prism Glass	Model – PRG-5030-23
¼-in OD PTFE of FEP tubing	Cole Parmer	Model – WU-95231-02
¼-in OD Stainless Steel tubing	Restek	Model - 29035

Air Supply

Figure 1 shows a diagram of the complete Small Chamber Clean Air System (SCCAS). The air supply to the system must meet several critical criteria, one of which is that the supply air must come from an oil-free source. Air is supplied to the SCCAS from the laboratory house air system. House air inlet pressure is approximately 90 psig. Another criterion is that the air supply to the pure air generator must be supplied at a pressure of at least 70 psig.

From the house air supply, 3/8" copper tubing extends from the system outlet to a filter/regulator assembly with a moisture drain and a pressure indicator (PI) (Wilkerson Model - B18-03-FK00). From the filter/regulator assembly, air continues into a 15 µm particulate filter (Swagelok® Model B-6F-15), where particulates larger than a 15 µm diameter are filtered from the air supply. From the particulate filter, supply air proceeds through 3/8" copper tubing to a refrigerated air dryer (Hankinson Model 8010—SS). The Hankinson is also equipped with a timed electric auto drain valve (Speedaire Model 6Z948) that is set to drain for 5 seconds every hour. A Swagelok® union tee is attached to the outlet of the Hankinson. One arm of the union tee continues with the clean air system and the other arm is equipped with a shut-off toggle valve to provide the lab with a source of high-pressure unconditioned air. Currently, the lab uses an oil- free Thomas dry running articulated piston compressor attached to a 10-gallon air tank as a backup air supply. The compressor air tank is maintained at a pressure of approximately 90 psig when in use.

A carbon trap (Supelco Model 24565), a moisture trap (Supelco Model 23992), and a hydrocarbon coalescing filter (Speedaire Model 6ZC24) further purify the air prior to its introduction into the pure air generator (Aadco Model 737-11A). The carbon and moisture traps should be replaced or recharged on an annual basis. Purified air exits the pure air generator via ¼" stainless steel tubing at approximately 35 psig.

A ¼" Swagelok® union tee is attached to the clean air system downstream of the pure air generator which routes the air into two streams, one for the small chambers 1-6 air supply and a second for small chambers 7, 8, the air supply to the two micro-chamber units, and the water bath circulation pump. Each airline from this point has a toggle shut-off flow valve (FV). The line to

chambers 1-6 passes through a second coalescing filter/regulator (Norgen Model B736-2AK-API-RMG) and a shut-off valve for each chamber's airflow before entering the mass flow controllers. The chamber 7/8/micro-chamber line passes through a 60-psi pressure gauge (U.S. Gauge) and a toggle shut-off flow valve to each chamber's airflow before entering the chamber 7 and 8 mass flow controllers and the micro-chamber units.

Conditioning of Chamber Airflow

From the pure air generator, the VOC free air continues to the wet and dry mass flow controllers that are controlled by the OPTO 22 software data acquisition system (DAS) (See MOP 803). The air coming from the mass flow controller (MFC) designated as "WET" continues through a fritted glass bubbling impinger into a 1-liter round bottom flask filled with deionized water. The flask is submerged into a water bath maintained at a temperature of 18.5°C. All Swagelok® fittings attached to the glass impingers and bulbs are polytetrafluoroethylene (PTFE) and utilize PTFE ferrules. The humidified air continues from the flask to a PTFE Swagelok® union tee where the air from the MFC designated as "DRY" mixes with the humidified air. PTFE or fluorinated ethylene propylene (FEP) tubing is used for routing conditioned air from the MFCs to the chambers. The humidified air next enters the incubator system and travels through a coil of ¼" stainless steel tubing to equilibrate to the temperature of the incubator before passing through a Swagelok® union tee containing the relative humidity probe for the chamber's inlet air supply. The humidity, airflow and air exchange rates are all controlled to this point. Figure 2 shows the clean air system through the chambers. MOP 803 details how to set the air exchange rate and the humidity for each of the eight chambers.

Figure 1. Clean Air System for Small Chamber Laboratory

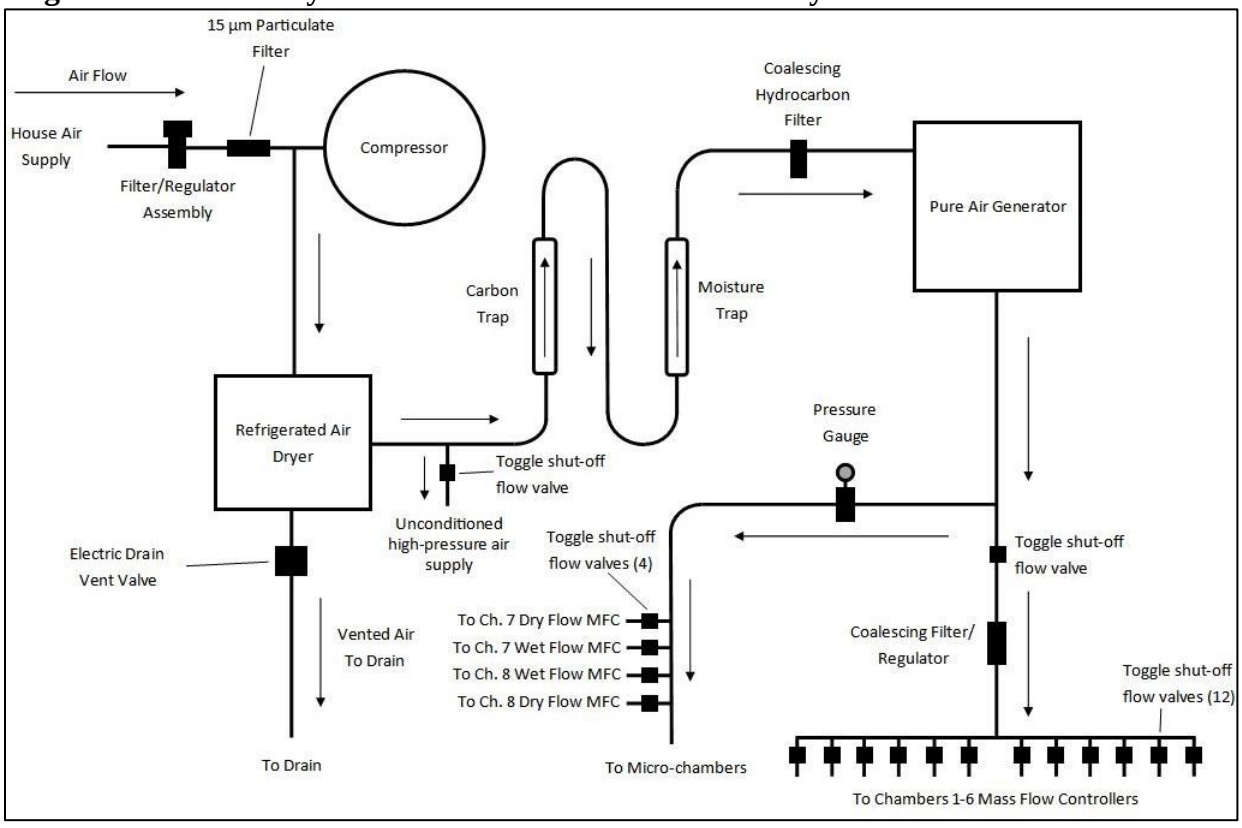
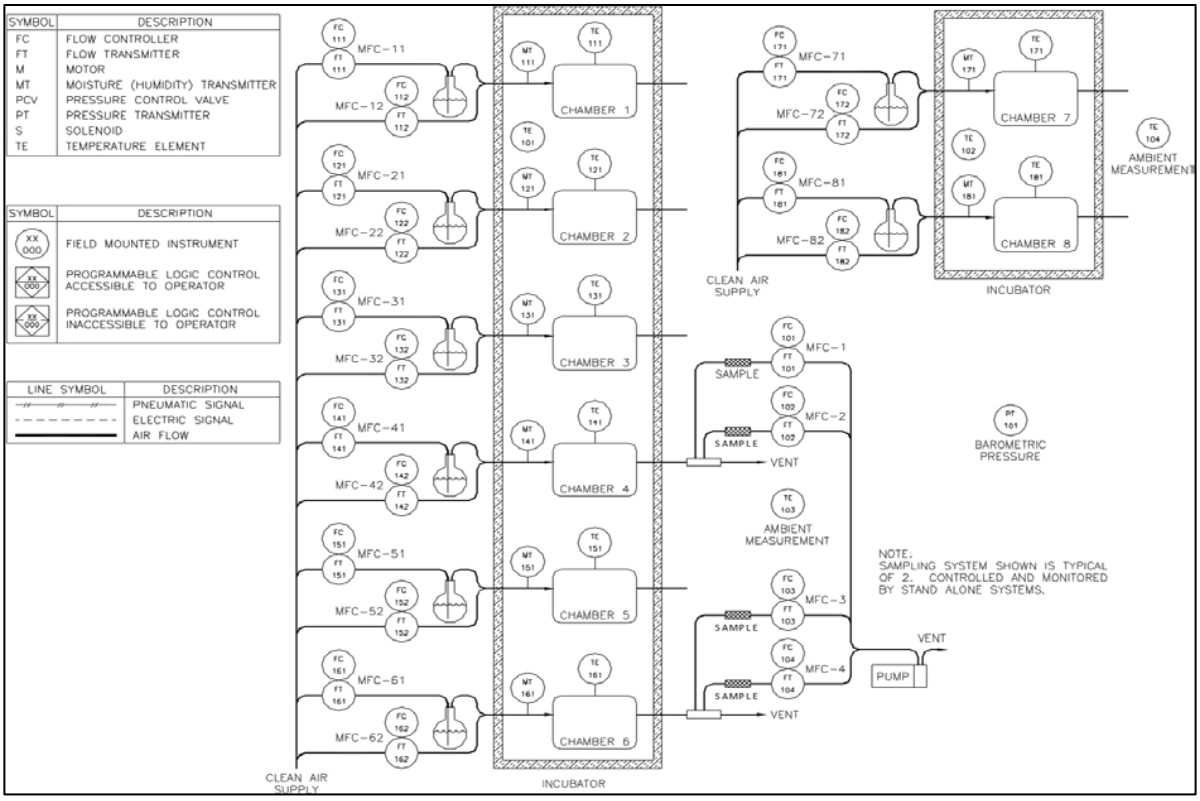


Figure 2. Clean Air System Continued through the Chambers



Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes (MOP 812) – September 2016

U.S. EPA Office of Research and Development
National Risk Management Research Laboratory
Air Pollution Prevention and Control Division

Miscellaneous Operating Procedure 812
Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes

Authored by Matt Allen, Jacobs (Work Assignment Leader) 09/16/2016 Date

Approved by

EPA Team Leader

Xiaoyu Liu Date: 2016.09.16 12:59:16 -04'00' Date

APPCD QA Manager Libby Nessley Date

Jacobs QA Officer

Zora Drake-Richman 09/16/2016 Date

Keywords: DNPH, Aldehydes, Formaldehyde, silica gel cartridge

MISCELLANEOUS OPERATING PROCEDURE 812
SAMPLING AND EXTRACTION PROCEDURES FOR DNPH-COATED SILICA GEL CARTRIDGES USED TO DETERMINE AIR CONCENTRATIONS OF FORMALDEHYDE AND OTHER ALDEHYDES – September 15, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Bob Wright
Jacobs	Matt Allen
	Zora Drake-Richman
Laboratory Copy	Maintained by X. Liu
Electronic Filing	Scientific Data Share L:\LAB\NRML_Public\Tire Crumb Rubber\QA Documents\MOPs\Approved MOP-SOPs\MOP 812

Revision Record

Revision	Date	Responsible Person	Description of Change
0	Unknown	Unknown	Initial release
1	February 2003	Unknown	
2	2012	Robert Pope	Changed Title from "Collecting and Desorbing Air Samples on DNPH-Silica Gel Cartridges" to "Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes" and Updated Sampling/Extraction Procedure
3	Feb 2014	Matt Allen	Updated MOP from 2012 revision
4	June 2016	Matt Allen	Updated definitions and section on air sampling
5	August 2016	Matt Allen	Revisions made base on EPA QA review comments. QLOG No. G-APPCD- 0016211, QTRAK No. 11037_15, QA Category B.
6	September 2016	Xiaoyu Liu	Revisions made in Section C based on NRMRL TSA QA audit report (QA Track: 16607/A30052)

TITLE

Sampling and Extraction Procedures for DNPH-Coated Silica Gel Cartridges Used to Determine Air Concentrations of Formaldehyde and Other Aldehydes

PURPOSE

This MOP describes the sampling and extraction procedures for determining air concentrations of formaldehyde and other aldehydes from environmental small chamber effluent and ambient samples using 2, 4-dinitrophenylhydrazine (DNPH)-silica gel cartridges.

SUMMARY OF METHOD

A stream of air sample exiting an environmental small chamber or at an ambient location is directed through a DNPH-silica gel cartridge using a vacuum pump. Aldehydes in the air are absorbed and derivatized by DNPH. The DNPH cartridge is then extracted with acetonitrile and the aldehyde derivative is analyzed via high performance liquid chromatography (HPLC) to determine the concentration of the aldehyde present in the air sample.

DEFINITIONS

Ambient location	any open space
°C	degree(s) Celsius
Chamber effluent	the air exiting the source chamber through a glass 3-port sampling manifold
DAS	data acquisition system
°	degree(s)
DNPH Cartridge	silica gel cartridge coated with 2,4-dinitrophenylhydrazine
FEP	Fluorinated ethylene propylene
HPLC	High performance liquid chromatography
ID	Inner Diameter
L	liter(s)
MFC	mass flow controller
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
µg	microgram(s)
µm	micrometer(s)
MOP	Miscellaneous Operating Procedure
OD	outer diameter
Other Aldehydes	Other aldehydes would include but are not limited to the following compounds: Acetaldehyde, Acrolein, Acetone, Propionaldehyde, Crotonaldehyde, *n*-Butyraldehyde, Benzaldehyde, Isovaleraldehyde, Valeraldehyde, *o*-Tolualdehyde, *m*- & *p*-Tolualdehyde, Hexaldehyde, and 2, 5-Dimethylbenzaldehyde.

%	Percent
PTFE	Polytetrafluoroethylene
QAPP	Quality Assurance Project Plan
Sampling Pump System	Consists of a vacuum pump connected to a stainless-steel manifold with four ports. Each port contains a toggle valve and mass flow controller (MFC) connected to a ¼" OD sampling line with a 2" section of silicon tube on the end not connected to the MFC. The flow rate of each mass flow controller is adjusted by a mass airflow controller box. Each of the four sampling lines is given a unique designation, i.e., sample pump line 1is labeled (P1), etc.
SOP	Standard Operating Procedure

RELATED DOCUMENTS

- ASTM D5197-09 Standard Test Method for Determination of Formaldehyde and Other Carbonyl Compounds in Air (Active Sampler Methodology). ASTM International. 2010.
- MOP 801: *Preparation of small (53-L) chambers for testing.*
- MOP 802: *Operation of small emissions chambers during testing.*
- MOP 803: *Operation of the Opto Display Software Data Acquisition System (DAS) in the small chamber laboratory.*
- MOP 806: *Operation of the clean air system for the small chamber laboratory.*
- MOP 826: *Operation of the Agilent 1200 HPLC for analysis of DNPH-Carbonyls.*
- MOP 827: *High-performance Liquid Chromatography (HPLC) calibration standard preparation procedure.*
- SOP 6404: *Collecting air samples from the small environmental chambers using Carbopack™ X Sorbent.*
- SOP 6405: *Glassware and chamber cleaning procedure.*
- Waters Sep-Pak DNPH-Silica Cartridge Care and Use Manual. Waters Corporation. 2009.

EQUIPMENT

Note: All equipment, unless otherwise noted, is located in lab E-378A.

Table 1 lists sampling system components and Table 2 lists the supplies necessary for the extraction of the DNPH cartridges.

Table 1. Sampling System Components

Component	Manufacturer	Model #	Manufacturer's Location
Vacuum Pump	Welch	2565B-50	Skokie, Il
Mass Flow Controllers (4)	Coastal Instruments	FC-260	Burgaw, NC
Mass Flow Control Box[a]	Porter Instrument Co.	CM4	Hatfield, PA
Gilibrator Primary Flow Calibrator	Sensidyne	800286	Clearwater, FL
Flexible FEP (fluorinated ethylene propylene) or PTFE (polytetrafluoroethylene) Tubing	Fisher Scientific	----	Hampton, NH
Flexible Silicone Tubing	Fisher Scientific	----	Hampton, NH
3-Port Glass Sampling Manifold	Prism Research Glass	----	Raleigh, NC
P-touch Labeler	Brother	PT-2100	Bridgewater, NJ

[a] or equivalent four-channel mass flow control box

Table 2. Extraction Supplies

Item	Manufacturer	Part #	Supplier	Manufacturer's Location
DNPH silica gel cartridge – Short Body, 55-105µm	Waters	WAT037500	Waters	Milford, MA
Acetonitrile, HPLC Grade (99.9% purity)	Fisher Scientific	A998-4	Fisher Scientific	Hampton, NH
5¾" Glass Pasteur Pipettes	Fisher Scientific	13-678-20B	Fisher Scientific	Hampton, NH
5 mL Glass Syringe	Popper & Sons, Inc	5019	Popper & Sons, Inc	Staunton, VA
5 mL Class A Volumetric Flasks	Corning, Inc	5630-5	VWR	Corning, NY

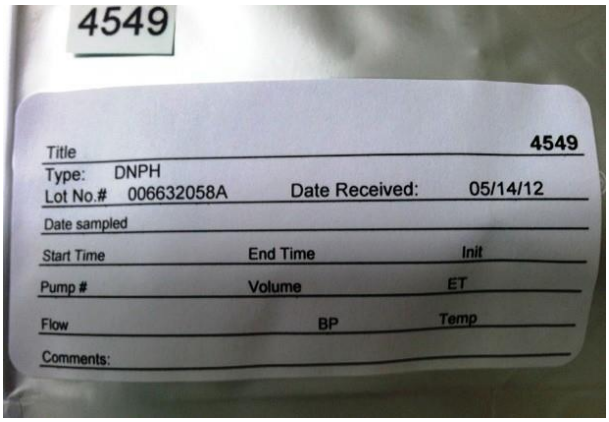
PROCEDURE

A. *DNPH Cartridge Storage and Handling*

DNPH cartridges (Figure 1) must be stored at temperatures below 4 ºC, and unopened cartridges can be used for up to six months from the date of receipt provided they are stored at or below the 4°C temperature (http://www.waters.com/webassets/cms/support/docs/wat037506.pdf, last accessed August 2, 2016). The boxes are dated with the date received and placed in the freezer located in E378-A. Labels are generated with a unique number for each cartridge and affixed to the individually pre-packaged sealed aluminum pouch. Using the P-touch labeler, a separate individual label with a number that matches the package label is generated and attached to the pouch (Figure 2). This label will be used to identify the extraction solution vial. After sampling, the DNPH cartridge will be placed back in the original pouch and re-sealed with the pouch zip-lock closure. The sample will be stored in the freezer until it is extracted as detailed below. Two Frigidaire freezer/refrigerators are used to store DNPH cartridges in laboratories E378-A (model number FFU11FK0DW2) and E375-A (model number FFU21M7HWL). Temperature dial settings on these two units are: 5 for FFU11FK0DW2 and 3.5 for FFU21M7HWL to achieve an approximate -15°C temperature.

Figure 1. DNPH cartridge

Figure 2. DNPH cartridge label



B. *Air Sampling Process*

1. Turn on the sampling pump system and flip the toggle switches to open airflow to the mass flow controllers. Allow 30 minutes for the pump system to stabilize before use.
2. Remove a DNPH cartridge from the freezer and allow 10 minutes for it to reach room temperature before opening.
3. Calculate the air sample volume to be collected on the DNPH cartridge. Maximum sampling volume can be calculated by dividing the expected effluent chamber air formaldehyde concentration by the DNPH cartridge's formaldehyde capacity (75 µg). When the expected formaldehyde air concentration is not known, a sample volume of at least 10 L should be collected.

$$V = (_{MM}{}^{CC})$$

Where: V = Air Volume Sampled through DNPH Cartridge (L) C = Expected Analyte Air Concentration (µg/L) M = Target Analyte Mass on the Cartridge (µg)

Note:
After sampling and extraction, formaldehyde concentrations will be determined by HPLC analysis according to MOP 826 and MOP 827.

4. Connect the fluorinated ethylene propylene (FEP) or polytetrafluoroethylene (PTFE) tubing from the sampling pump system to a Gilibrator using a 2" section of silicone tubing. Place a 'dummy' DNPH cartridge on the end of the pump line before the Gilibrator to simulate test conditions. Verify that vacuum pump flows match those specified in the project test plan or QAPP. Record three measurements of the sampling pump air flow taken with the Gilibrator on each pump line to be used for sampling. Gilibrator and mass flow controller calibration reports are stored in laboratory E378-A, and in the project L: drive folder. The sampling system setup is shown in Figure 3.

Note: *The total sampling vacuum flow (including duplicate DNPH cartridge sampling as well as any other samplers being used at the same time) should not exceed 70 % of the total air flow exiting the chamber system. The chamber system's typical flow range is 900-1000 mL/min (1.0 ACH through a 53-L chamber volume), which allows for an approximate 600-700 mL/min maximum sampling flow through the DNPH cartridge. However, flow rates of other samplers being used simultaneously must be accounted for and those flow rates subtracted from the DNPH sampling flow to determine the maximum sampling flows for the DNPH. A sample volume of at least 10 L should be collected. The total concentration of all aldehydes collected on the cartridge should not exceed that of the DNPH concentration (1 mg/cartridge); each cartridge has a formaldehyde sampling capacity of 75µg (Waters). If aldehyde concentrations*

exceed this level, re-sampling should occur at lower flow rates and/or for a shorter sampling period to bring sample concentrations into the HPLC calibration range.

Figure 3. DNPH sampling cart setup

5. Remove the DNPH cartridge from its pouch. Remove both end caps. Save the pouch and caps.
6. Connect the small tapered end of the DNPH cartridge to the 1st port of the glass sampling manifold using flexible PFA tubing and a 2" segment of silicone tubing as a connector. Connect the larger end of the DNPH cartridge to the sampling pump line with FEP or PTFE tubing and a 2" silicone connector. Record the time. Record the atmospheric pressure and ambient temperature from the Opto environmental data collection "System Screen"

(see MOP 803).

7. Record the sampling information on the sample label and in the Excel notebook on the small chamber test computer.
8. When the sampling period is complete, remove the pump tubing from the DNPH cartridge first and then remove the cartridge from the sampling exhaust manifold. Replace the end caps and reseal the DNPH cartridge back in the pouch. Record the date, time, atmospheric pressure, and ambient temperature as stated in Step 7. Store exposed DNPH cartridges in the freezer until time of extraction. Extraction of the cartridge must occur within two weeks of sample collection.

C. Extraction of DNPH Cartridge

1. Remove any DNPH cartridges to be extracted from the refrigerator and allow at least 10 minutes to equilibrate to room temperature.
2. Clean all glassware by rinsing with acetonitrile or isopropyl alcohol and drying in the oven (also see SOP 6405).
3. Set out a clean 5 mL volumetric flask, 5-¾" Pasteur pipette and 5 mL glass syringe. The clean pipette and syringe can each be placed in an open 15 mL polypropylene tube for easy accessibility.
4. Remove a DNPH cartridge from pouch. Remove end caps and dispose.
5. Place the small end of the DNPH cartridge down into the 5 mL volumetric flask.
6. Remove the plunger from the 5 mL glass syringe and place the syringe down into the large end of the DNPH cartridge that is facing upward.
7. While holding the plunger in hand to avoid contamination, use the pipette to transfer acetonitrile into the syringe barrel up to the 4 mL mark. Place the pipette back in its tube and replace the lid of the acetonitrile immediately to prevent contamination.
8. Use the plunger to push the acetonitrile through the DNPH cartridge at a rate not to exceed 3 mL/min (gradual drops, no stream). Upon completion, remove the syringe from the top of the DNPH cartridge and place back in its tube.
9. Use the pipette to bring the flask up to volume with acetonitrile. Discard any excess acetonitrile remaining into the pipette into the waste container.
10. Use the pipette to mix the contents of the volumetric flask by aspirating the eluate 20 times.
11. Transfer 1.5 mL of the extract solution with the pipette into a labeled 2 mL amber HPLC injection vial.
12. An extraction blank without DNPH and with DNPH cartridges are recommended for each batch of DNPH cartridges extraction.
13. Refrigerate until time of analysis.
14. Analyze the samples within two weeks of extraction by HPLC (MOP 826).

Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls (MOP 826) – September 2016

Miscellaneous Operating Procedure 826
Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls

Authored by

09/16/2016

Matt Allen, Jacobs Technology Date

Approved by

EPA Team Leader

Date: 2016.09.16 13:01:52 -04'00'

Xiaoyu Liu Date

EPA QA Manager

Date: 2016.09.16 13:06:22 -04'00'

Libby Nessley Date

Jacobs QA Officer 09/16/2016

Zora Drake-Richman Date

Keywords: HPLC, DNPH, Aldehydes, Calibration

MISCELLANEOUS OPERATING PROCEDURE (826) FOR OPERATION OF THE AGILENT 1200 HPLC FOR ANALYSIS OF DNPH-CARBONYLS – September 15, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Ken Krebs
Jacobs	Matt Allen
Laboratory Copy	Maintained by Xiaoyu Liu
Electronic Filing	APPCD SharePoint

Revision Record

Revision	Date	Responsible Person	Description of Change
2	June 2011	Xiaoyu Liu	Update of MOP 826 from August 2003
3	Feb 2014	Matt Allen	Update of MOP 826 from June 2011
3.1	3/31/2014	Matt Allen	Revisions in response to EPA QA review comments
4	8/8/2016	Matt Allen	Updated document header date, approvals page, distribution list, removed “a”, “b” and “c” from referenced MOP numbers to reflect the most current MOP version, and updated HPLC instrument method parameters
5	8/9/2016	Matt Allen	Updated HPLC instrument injection volume, definitions, HPLC PC software requirements document, revised DCC recovery range from 85-115% to 75-125%, and made minor grammatical changes.
6	8/11/2016	Matt Allen	EPA QA re-reviewed V5 Added minor clarifications to the Sequence Setup section for the Sequence Parameters screen.
7	8/15/2016	Matt Allen	Revisions in response to EPA QA review comments
8	9/15/2016	Xiaoyu Liu	Revisions made in Section D based on NRMRL TSA QA audit report (QA Track: 16607/A30052)

MISCELLANEOUS OPERATING PROCEDURE NO. 826

TITLE

Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls

PURPOSE

This MOP will provide a procedure outlining the proper use and operation of the Agilent 1200 High-Performance Liquid Chromatography (HPLC) for the analysis of DNPH-carbonyl samples.

SUMMARY OF METHOD

This MOP provides instruction to the laboratory analyst in powering on the HPLC system, preparing the HPLC for analysis, creating analytical run sequence tables, creating and/or modifying an analytical method (if necessary), running an analytical sequence, and analyzing data acquired from the HPLC.

DEFINITIONS

- Air Pollution Prevention and Control Division (APPCD)
- High-Performance Liquid Chromatography (HPLC)
- Daily Calibration Check (DCC)
- Diode-Array Detector (DAD)
- DNPH: 2,4-Dinitophenylhydrazine
- Milliliter (mL)
- Miscellaneous Operating Procedure (MOP)

RELATED DOCUMENTS

- MOP 827: *High-performance Liquid Chromatography (HPLC) standard preparation procedure*
- MOP 812: *Sampling and extraction procedures for DNPH-coated silica gel cartridges used to determine air concentrations of formaldehyde and other aldehydes*

EQUIPMENT AND SUPPLIES

Table 1: Equipment, Reagents, and Other Supplies to Operate the Agilent 1200 HPLC

Component	Manufacturer	Model #	Location
1200 Series ALS Auto-Sampling Unit	Agilent Technologies, Inc.	G1329A	Waldbronn, Germany
Thermostat for 1200 ALS /Fraction Collector	Agilent Technologies, Inc.	G1330B	Waldbronn, Germany
1200 Thermostated Column Compartment	Agilent Technologies, Inc.	G1316A	Waldbronn, Germany
1200 Series Diode Array Detector	Agilent Technologies, Inc.	G1315B	Waldbronn, Germany
1200 Series Micro Vacuum Degasser	Agilent Technologies, Inc.	G1379A	Osaka, Japan
1200 Binary Pump	Agilent Technologies, Inc.	G1312A	Waldbronn, Germany
PC, Windows 2000, or XP capable ‡	Dell	Optiplex GX270 (or equivalent)	Round Rock, TX
HP Laser Jet Printer	Hewlett Packard	P1505n	Boise, ID
2 wide mouth amber glass bottles, 1000 mL with PTFE cap and tubing port	VWR	89199-074 (or equivalent)	Suwanee, GA
Aldehyde/Ketone-DNPH Stock Standard-15, 15 µg/mL each analyte in acetonitrile (diluted to 6.0 µg/mL per target analyte to prepare Daily Calibration Check standard)	Cerilliant	ERA-020	Round Rock, TX
Acetonitrile – HPLC/ACS grade, 0.2 µm filtered	Fisher	998-4	Raleigh, NC
Water – HPLC grade, submicron filtered	Fisher	W5SK-4	Raleigh, NC

‡ PC hardware requirements for operation of ChemStation HPLC software can be found online at: http://www.chem.agilent.com/Library/Support/Documents/F01010.pdf (last accessed August 9, 2016)

PROCEDURAL STEPS

A. *Preparation of the HPLC System*

1. Typically, the HPLC system is not powered down. If the system has been turned off, restore the power to the main components: the degasser, the binary pump, the auto-sampler, the temperature-controlled column compartment, and the diode-array detector (DAD). The power buttons are located on the lower left corner of each module.
2. Fill the solvent bottles located on top of the degasser module. Fill bottle A1 with HPLC grade water and bottle B1 with reagent grade acetonitrile, both to the 1000 mL mark on each container.
3. Open “Instrument 1 Online” from the HPLC computer’s desktop.
4. Once the software has loaded and is ready, left click on the solvent bottle icon

located on the primary ChemStation Instrument Control screen Figure 1 (Appendix). Click “Solvent Bottles Filling”. The window in Figure 2 (Appendix) should appear.

5. Set the volume of bottles A1 and B1 to 1.00 liter. Make sure the two check boxes at the bottom of the solvent bottles filling window are checked.
6. Click “OK.”
7. From the “Method” drop-down menu (Figure 1) select “Load Method”. Choose the designated method for DNPH analysis, e.g. DNPH-ALL.M and click “OK”. For detailed information and method parameters, see section B.
8. Before starting the system, visually inspect the reagent lines to ensure there are no air bubbles in the reagent lines. If air bubbles are present, purge the lines of the bubbles before proceeding by disconnecting the reagent line at the degasser inlet and using a 50 mL syringe with a threaded tip to draw the air out of the tubing. Open and close the binary pump’s main valve located on the front of the pump module to help purge air bubbles downstream from the degasser.
9. To start operation of the system, go to the “Instrument” drop-down menu at the top of the screen (Figure 1).
10. Select “System On.”
11. Wait for the DAD signal to stabilize before running any samples. The instrument’s signal plot line located in the Online Plot window of the instrument control screen should be plotting parallel to the horizontal axis of the chromatogram (Figure 1). System stabilization may take up to 30 minutes. Verify pump pressure is between 90-100 bar on the instrument’s control screen.

B. *Method Setup*

Table 2 lists an example of proper method parameters for analyzing DNPH-derivatized carbonyls. This method is already saved in ChemStation. If the method is not available, a new method must be created. If so, click “Method” and then “New Method”. Follow the on-screen instructions and fill in the appropriate method parameters according to the table. When an existing method’s parameters are modified or a new method is created, the instrument must be calibrated using the new method parameters. The instrument must be calibrated before analysis of samples can occur. Refer to “MOP 827 - High Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure” for details on these procedures.

Table 2: DNPH Analysis Method Parameters (DNPH-ALL.M)

Parameter	Setting		
Column	Eclipse XDB-C18 4.6 x 150 mm, 5 µm		
Column Temperature	40°C		
Mobile Phase Flow Rate	1.0 mL/min		
Solvent A/B	Water/Acetonitrile		
Solvent Gradient Program	**Start Time (min)**	**% Water (A)**	**% Acetonitrile (B)**
	0	40	60
	7	40	60
	12	25	75
	15	10	90
	16	10	90
	16.5	40	60
Total Run Time	20.5 min (18-minute run time; 2.5 minutes post run time)		
Injection Volume	10 µL		
ALS (Injector) Temperature	5°C		
Detector Wavelength	UV at 360 nm absorbance		

C. *New Run Sequence*

1. From the "Sequence" drop-down menu (Figure 1) select "New Sequence" to reset all sequence parameters and the sequence table.
2. Select Solvent Bottle Filling Option (Figure 2) to determine the need to refill solvents before starting the sequence.
3. From the "Sequence" drop-down menu (Figure 1) select "Sequence Parameters".
4. Once the window in Figure 3 appears, fill in the following information:
    a. Operator name or initials,
    b. Folder name for the data in the subdirectory box in the form of the analytical run date (e.g. YYYYMMDD),
    c. Select the "Auto" setting,
    d. Run the method "According to Runtime Checklist",
    e. Check the "Post-Sequence Command Macro" box,
    f. From the dropdown box under the shutdown section, select "STANDBY", and,
    g. Click "OK"
5. From the "Sequence" drop-down menu (Figure 1) select "Sequence Table". The window seen in Figure 4 will come up on the screen.
6. Fill in the required portions of the sequence table:
    a. Vial tray location number,
    b. Sample name from the sample's ID label,
    c. Method name (e.g. DNPH-ALL.M),
    d. Injections/vial,
    e. Sample type (This field should automatically fill in the term "Sample"),

f. Data file name, and,
g. Injection amount.

7. For every sequence, a solvent blank and Daily Calibration Check standard, (the mid-level calibration standard [see MOP 827]), must be analyzed. As an example, the solvent blank can be named YYYYMMDD-SLVTBLK and the DCC can be named YYYYMMDD-DCC where YYYY is the four-digit year, MM is the two-digit month, and DD is the two-digit day. The solvent blank and DCC need to be re-analyzed approximately every 15 samples when running a large sequence.
8. Once all of the samples for the run have been added to the sequence table, click "OK".
9. Save the sequence by choosing "Sequence" (Figure 1) then "Save Sequence". The sequence can be, but does not have to be, named in the following format: YYYYMMDD.s, where YYYY is the four-digit year, MM is the two-digit month, and DD is the two-digit day.
10. Begin the run by pushing the green "Start" button located above the vial tray illustration on the main ChemStation screen (Figure 1).

D. *Data Analysis*

1. Open the "Instrument 1 Offline" program using the icon on the desktop of the HPLC computer. Be sure that the "Data Analysis" module is selected on the lower left side of the program window, as shown in Figure 5.
2. Using the data analysis window on the left side of the screen, locate the directory for the data. All data are initially stored on C:/Chem32/1/Data under the desired data file path and subdirectory indicated in the sequence parameters mentioned in Section C (see Figure 3). A sequence table should appear on the screen with the sample (Figure 5).
3. Double click on the sample to be analyzed. The sample's analysis chromatogram will be displayed on the screen.
4. Target analyte peaks should be integrated automatically based on their characteristic retention times. Visually verify that ChemStation has correctly integrated each peak. If peaks are not integrated correctly such as integration at incorrect retention times, integration of co-eluted peaks as well as the target analyte peak, integration of incorrect peaks, integrations not following the baseline response, or peaks exhibiting unusual characteristics such as significant shoulders or co-eluted peaks use the "Auto-Integrate" function. In this situation, click the "Auto-Integrate" button (Figure 5). The command line on the bottom of the program indicates when integration is complete, typically a few seconds. If Auto-Integration does not correct the integration, it will be necessary to manually zoom in on each peak and perform a manual integration. Manual integrations should follow the response baseline across the peak being integrated, remain within the retention window for the method, and not include co-eluted peaks.
5. To export integration results, right click on the sample name of the desired sample (Figure 5), arrow down to "Export" then click "to CSV File."
6. Select "Integration Results" and check the box for "write to clipboard" and click

"OK."

7. In the new window, check the boxes for "RetTime" and "Area." Click "OK." The integration results have now been placed on the computer's clipboard.
8. Paste the integration results into a blank MS Excel worksheet.
9. Copy the target peaks and areas from the list and paste the selected data into the appropriate analysis workbook in MS Excel.

E. *Quality Assurance/Quality Control*

- A solvent blank must be run at the beginning every analytical sequence to ensure there is no systemic contamination affecting sample results.
- No compounds should be detected in the solvent blank. If any of the target analytes can be quantified in the solvent blank, the source of contamination must be determined and corrective action taken. These corrective actions include: reanalyzing the solvent blank, preparation of a new solvent blank followed by its analysis, preparation of new HPLC grade water and acetonitrile reagents, or troubleshooting the instrument.
- DCC standards must be run at the beginning of every analytical sequence and then after every 10-15 samples thereafter in the sequence.
- DCC recovery must be between 75-125%. If the DCC for the run is not within range, corrective actions need to be taken. These corrective actions include: reanalyzing the DCC and subsequent samples, recalibrating the HPLC, or troubleshooting the instrument.

Figure 1. Main Screen of ChemStation

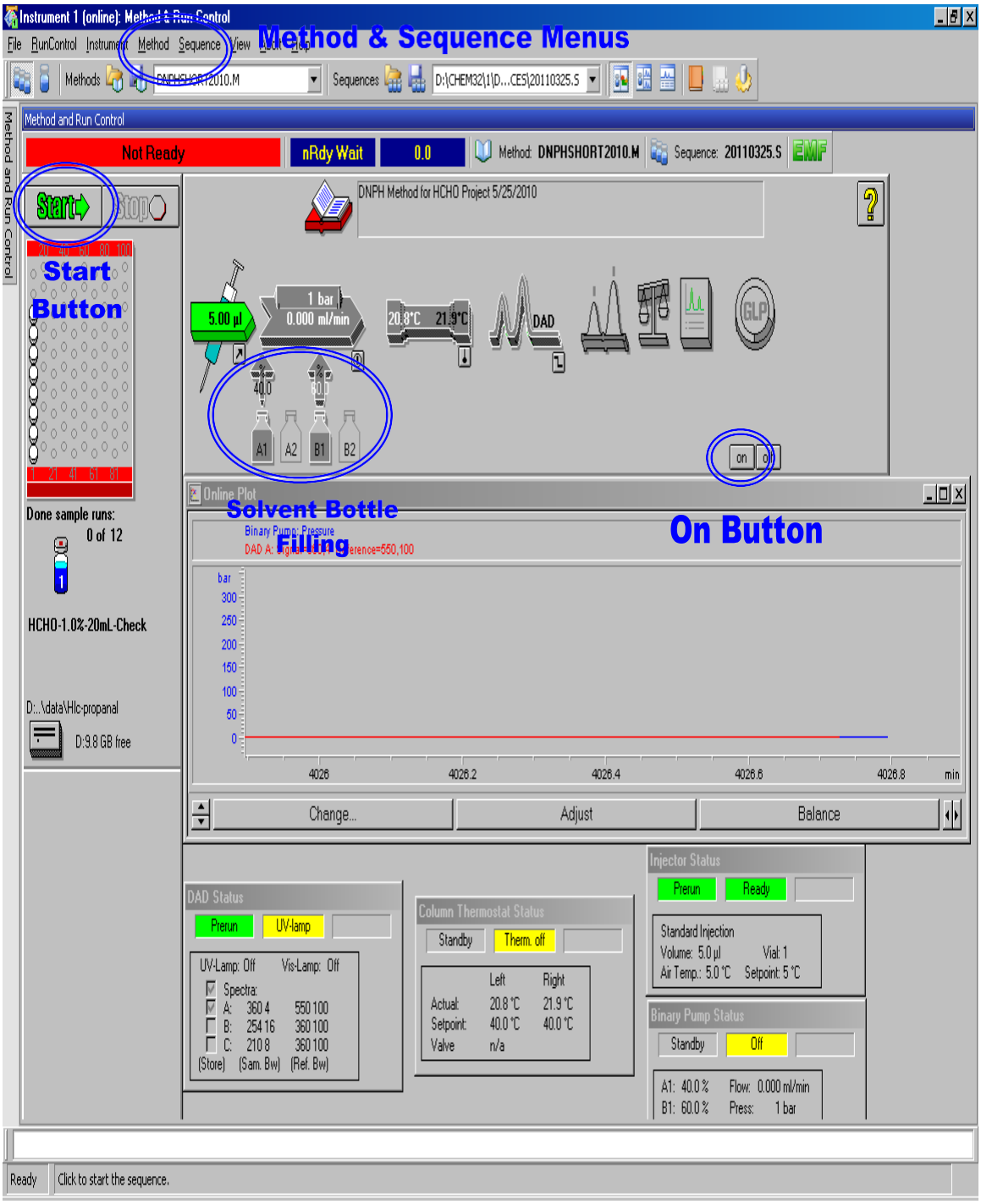


Figure 2. Solvent Bottle Filling Setup

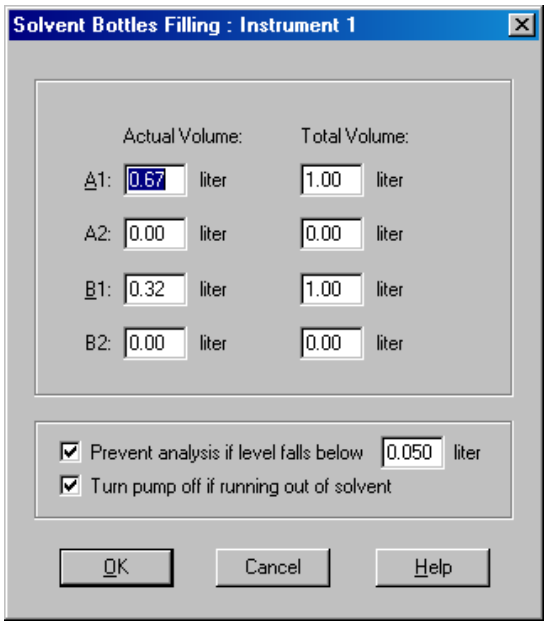


Figure 3. Sequence Parameters Setup

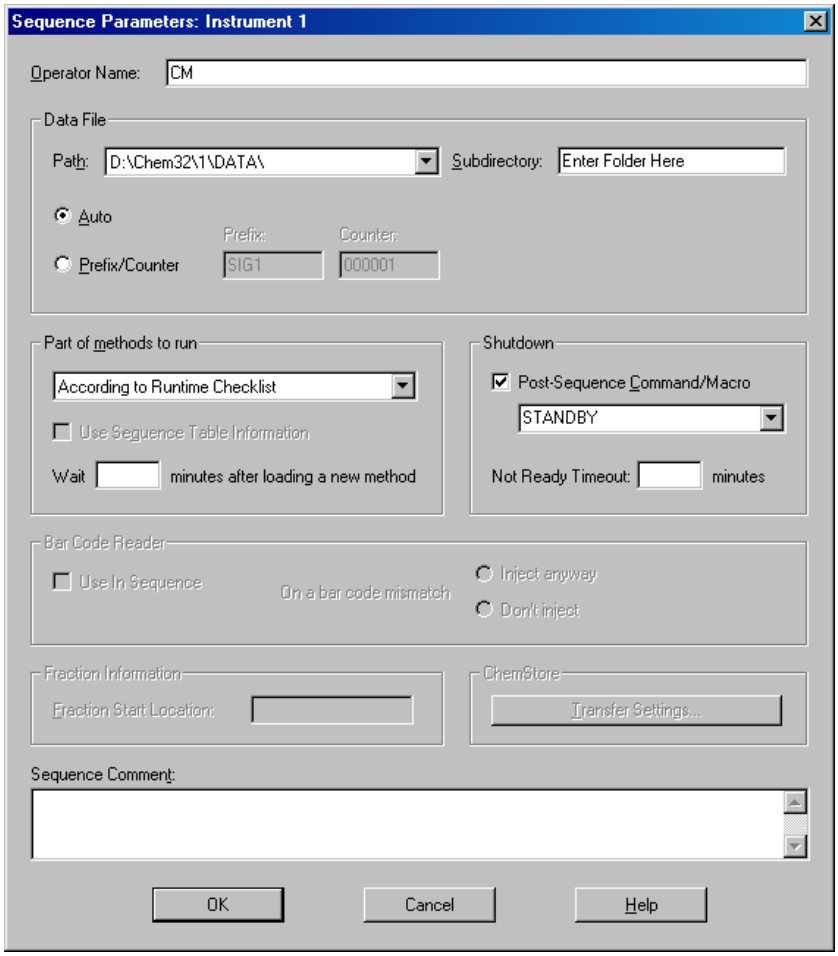


Figure 4. Example Sequence Table

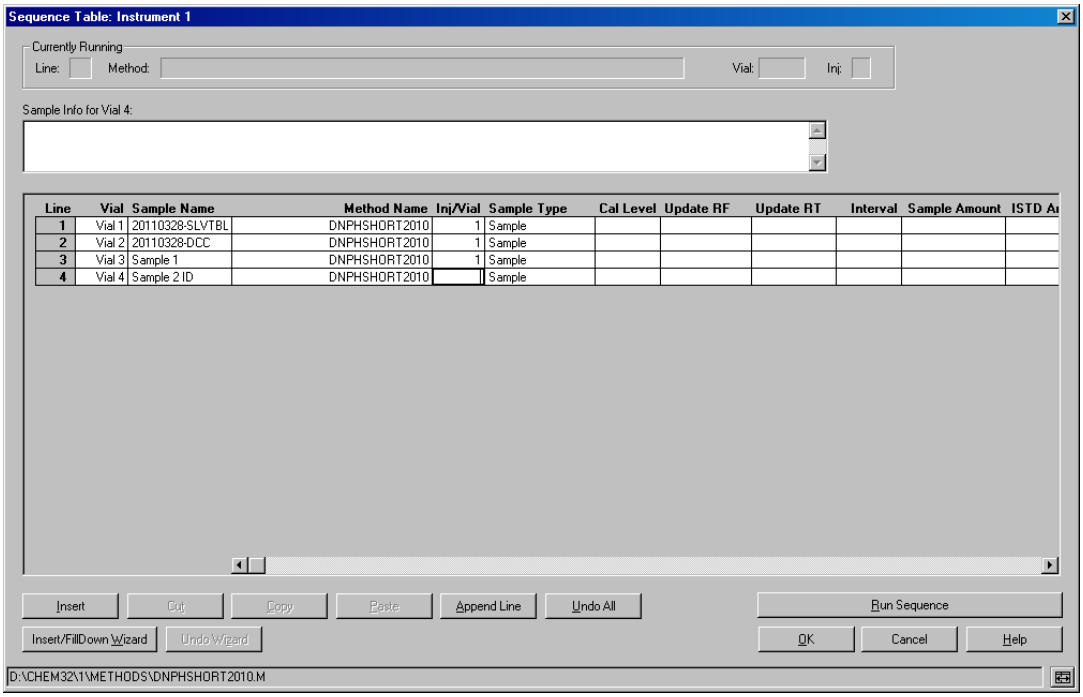
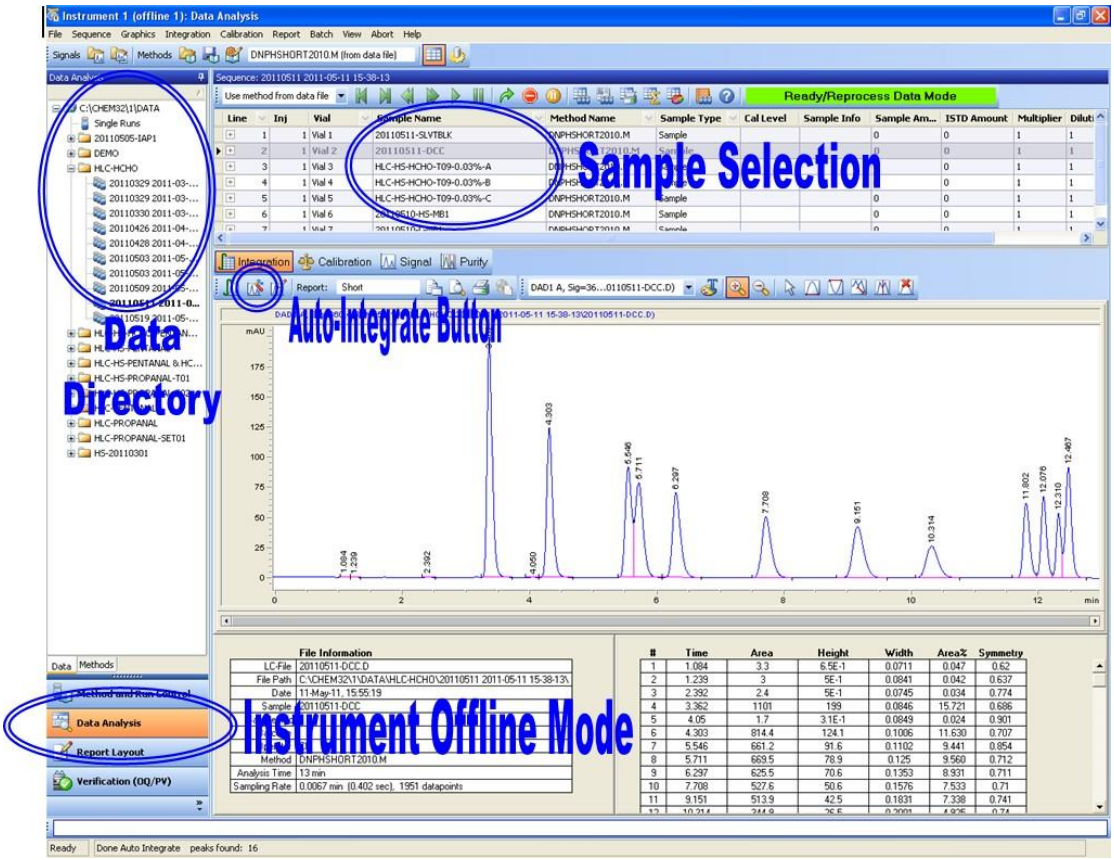


Figure 5. Offline Data Analysis Screen



High Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure (MOP 827) – August 2016

Miscellaneous Operating Procedure 827
High Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure

Authored by

08/09/2016

Matt Allen, Jacobs Technology Date

Approved by

EPA Team Leader Xiaoyu Liu
Date: 2016.08.17 11:14:37 -04'00'

EPA QA Manager Laura Nessley
Date: 2016.08.17 12:19:29 -04'00'

Keywords: HPLC, Calibration, DNPH

MISCELLANEOUS OPERATING PROCEDURE (827) FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) CALIBRATION STANDARD PREPARATION PROCEDURE – August 17, 2016

TABLE OF CONTENTS

Distribution List	
APPCD	Xiaoyu Liu
	Ken Krebs
Jacobs	Matt Allen
	Zora Drake
Laboratory Copy	Maintained by Xiaoyu Liu
Electronic Filing	APPCD SharePoint

Revision Record

Revision	Date	Responsible Person	Description of Change
2.0	April 2011	X. Liu	Update of MOP 827 from August 2003
2.1	June 1, 2011	X. Liu	Revised based on QA comments
3.0	Feb 2014	M. Allen	Update of MOP 827 from June 2011
3.1	3/31/2014	M. Allen	Revisions in response to EPA QA review comments
3.1	8/8/2016	M. Allen	Updated document header date, approvals page, and distribution list
4	8/9/2016	M. Allen	Submitted for EPA QA re-review of V3.1 Updated document revision number, date, calibration stock standards, MOP references, added definitions, and revised QA/QC recoveries from 85-115% to 75-125% and %RSD limit to 25%.
5	8/16/2016	M. Allen	Revised based on QA comments

MISCELLANEOUS OPERATING PROCEDURE No. 827

TITLE

High-Performance Liquid Chromatography (HPLC) Calibration Standard Preparation Procedure

PURPOSE

The purpose of this MOP is to explain the procedure for the preparation of carbonyl-DNPH standards for calibrating the Agilent 1200 HPLC. This MOP also describes the procedure for preparing the internal audit program (IAP) standard.

SUMMARY OF METHOD

This MOP provides a procedure for preparing calibration and IAP standards for the analysis of carbonyl- DNPH. The two stock standards used are Cerilliant ERA-020 and Supelco CRM4M7285. Both are multi-component aldehyde/ketone-DNPH stock standards. One will be used to prepare the calibration standards, and the other will be used to prepare the IAP standards. The concentration of each analyte in the solution is 15 µg/mL. After standard preparation and analysis, the calibration of the instrument will range from 0.03 to 15.00 µg/mL.

The IAP standard verifies the concentration of calibration standards by comparing the instrument's calibration standard response to the instrument's IAP standard response. IAP standards must be made from a source other than the manufacturer of the calibration standard.

DEFINITIONS

- American Chemical Society (ACS)
- DNPH: 2,4-Dinitrophenylhyrazine
- High Performance Liquid Chromatography (HPLC)
- Internal Audit Program (IAP)
- Microliter (µL)
- Milliliter (mL)
- Miscellaneous Operating Procedure (MOP)
- Polytetrafluoroethylene (PTFE)

RELATED DOCUMENTS

MOP 826: *Operation of the Agilent 1200 HPLC for analysis of DNPH-Carbonyls*

EQUIPMENT

- TO11/1P-6A aldehyde/ketone-DNPH Mix Stock Standard (CRM4M7285), 15 µg/mL (Supelco)
- ERA-020 Aldehyde/Ketone-DNPH Stock Standard, 15 µg/mL (Cerilliant Corporation)
- Gas Tight Syringes (SGE or Hamilton; 10 µL, 50 µL, 250 µL, 500 µL, and 2.5 mL)

- 2 mL amber screw top vials with PTFE/silicone septa (Supelco)
- 4 mL amber screw top vials with PTFE/silicone septa (Supelco)
- Vortex Mixer (Fisher Scientific)
- Acetonitrile – HPLC/ACS grade, 0.2 μm filtered (Fisher # 998-4 or equivalent)

PROCEDURAL STEPS

A. *Preparation of Calibration Standards*

The following Table 1 gives an example of calibration standards to be prepared for calibration using Supelco CRM4M7285.

Table 1. Preparation of Calibration Standards

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Volume of CRM4M7285 (μL)	5	10	40	160	260	400
Volume of Acetonitrile (μL)	2495	360	360	240	140	0
Total Volume (μL)	2500	370	400	400	400	400
Final Concentration (μg/mL)	0.03	0.405	1.50	6.00	9.75	15.0

- ***Note:*** *Each syringe used should be rinsed a minimum of five times with HPLC grade acetonitrile before use. The syringe should also be flushed five times with air to ensure that the stock standard is not diluted inside the syringe by residual acetonitrile in the Luer-Lok fitting.*
- ***Note:*** *Use appropriate size gas tight syringes to prepare each concentration of the standards. The calibration standards will be more accurate if the measured volumes are not in the lower part of the syringes' ranges.*

1. Label six new 2 mL amber vials with PTFE-silicone septa caps with the naming scheme detailed in Section C. An additional 4 mL vial will be needed for Standard 1.
2. Open a new ampoule of TO11/1P-6A aldehyde/ketone-DNPH Mix stock standard (CRM4M7285) and transfer the solution to a 2 mL vial.
3. Following the measurements in Table 1, add the listed volume of acetonitrile to each level of concentration standard's vial using a gas tight syringe.
    a. Since the final volume of Standard 1 is approximately 3 mL, prepare Standard 1 in a 4 mL vial then transfer an aliquot into the 2 mL vial for analysis
4. Following the Table, add the listed volume of TO11/1P-6A aldehyde/ketone-DNPH Mix stock standard (CRM4M7285) to a 2 mL vial using a gas tight syringe. The calibration standards will be more accurate if the measured volumes are not in the lower parts of the syringes' ranges
5. Cap the vial and mix thoroughly using a vortex mixer.
6. Label the standard according to Section C.
7. Repeat the procedure for all five standards.
8. Store all solutions in the freezer when not in use.

B. *Preparation of Internal Audit Program (IAP) Sample*

The IAP must be prepared by a person other than the person who created the calibration standards. Since stock standard CRM4M7285 is used as the calibration standard as shown in Table 1, ERA-020 Aldehyde/Ketone-DNPH Stock Standard will be used for IAP stock standard. The initial concentration is 15 µg/mL formaldehyde-DNPH. The desired concentration for analysis should be between 0.03 µg/mL and 15 µg/mL.

1. Add 1.0 mL of acetonitrile to a 2 mL vial using a gas tight syringe.
2. Add between 4 µL and 2000 µL of the ERA-020 Aldehyde/Ketone-DNPH Stock Standard, 15
3. µg/mL formaldehyde-2, 4-DNPH standard.
4. Do not inform the primary analyst of the concentration until they have verified the results.
5. Label the solution according to Section C.
6. Store the solution in the freezer when not in use.

C. *Naming of Standards*

As an example, calibration standards can be named “YYYYMMDD-STD-X”, where YYYY is the four-digit year, MM is the two-digit month, and DD is the two-digit day. “STD” indicates that the sample is a calibration standard. “X” is the number of the standard. IAP standards can be named “YYYYMMDD-IAP-X”, where YYYY is the four-digit year, MM is the two-digit month, and DD is the two-digit day. “IAP” indicates that the solution is the internal audit program sample. “X” is the number of the IAP.

D. *Analyze Samples*

- Analyze the calibration standards and IAP according to MOP 826 - Operation of the Agilent 1200 HPLC for Analysis of DNPH-Carbonyls.
- Perform triplicate injections of each sample at each calibration concentration level (1-6).
- Perform triplicate injections of the IAP standard after all the calibration injections are complete.
- Analytes are calibrated by quantifying peak areas against their characteristic retention times at each calibration level, as shown in Table 2 as an example. Note: Retention times listed could vary from one calibration to the next. The peak order could vary from one method to another method.

Target Analyte	Peak #	Retention Time (min)
Formaldehyde-DNPH	1	3.33
Acetaldehyde-DNPH	2	4.25
Acrolein-DNPH	3	5.47
Acetone-DNPH	4	5.63
Propionaldehyde-DNPH	5	6.21
Crotonaldehyde-DNPH	6	7.60
n-Butyraldehyde-DNPH	7	8.99
Benzaldehyde-DNPH	8	10.09
Isovaleraldehyde-DNPH	9	11.76
Valeraldehyde-DNPH	10	12.21
o-Tolualdehyde-DNPH	11	12.60
m- & p-Tolualdehyde-DNPH	12	12.89
Hexaldehyde-DNPH	13	14.61
2,5-Dimethylbenzaldehyde-DNPH	14	14.76

E. *Data Reduction*

- Calculate the response factor (RF) of each analyte for every injection as follows:

$$RF = \frac{A_C}{A_R}$$

Where A_C is the concentration of the analyte in the sample and A_R is the integrated peak area of the analyte of interest.

- Calculate the average response factor (RF) for each analyte as follows:

$$\overline{RF} = \frac{\sum RF}{n}$$

- Calculate the percent relative standard deviation (% RSD) of the RF of the triplicate injections and the % RSD of the overall average RF value as follows:

$$\% RSD = \frac{SD}{\overline{RF}} \times 100$$

$$\sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})}{}}$$

F. *Quality Assurance/Quality Control*

- The % RSD of the triplicate RF values of each standard for a single analyte must be less than 25 %.
- The % RSD of the average RF value of a single analyte must be less than 25 %.
- If the % RSD of either parameter is above the limit, corrective actions must be taken. These corrective actions may include reanalyzing the standard, preparing the standard again, and troubleshooting the instrument.
- The IAP must be between 75-125 % of the actual value. If the concentration of the analyte in the IAP is outside this value, corrective action must be taken. These corrective actions may include reanalyzing the IAP, preparing the IAP again, re-analysis of the calibration data and calibration curve, recalibration of the instrument, preparing a new set of calibration standards, and troubleshooting the instrument

Setup and Operation of the Markes Micro-Chamber Thermal Extractor and Humidifier Accessory (SOP 6401) – May 2016

United States Environmental Protection Agency

INTERNAL NRMRL PROCEDURE

Setup and Operation of the Markes Micro-Chamber Thermal Extractor and Humidifier Accessory (May 2, 2016)

NRMRL
Standard Operating Procedure (SOP)

Title:	**Setup and Operation of the Markes Micro-Chamber Thermal Extractor and Humidifier Accessory**
Division/Staff:	**NRMRL/AEMD/DSBB**
SOP Number:	**NRMRL-6401**
Former SOP Number: (if applicable)	NA
Effective Date:	5/02/2016

SOP Author(s)	
Name: Eric Morris*	Name: Matt Allen
Name: Xiaoyu Liu (PI)	Name:
Name:	Name:

QA Approval	
Name: Libby Nessley	Signature/Date:

SHEM Approval	
Name:	Signature/Date:

Management Approval	
Name:	Signature/Date:
Name: NRMRL Director (optional)	Signature/Date:

* Primary Author

STANDARD OPERATING PROCEDURE (6401) FOR SETUP AND OPERATION OF THE MARKES MICRO-CHAMBER THERMAL EXTRACTOR AND HUMIDIFIER ACCESSORY – May 2016

TABLE OF CONTENTS

1.0 INTRODUCTION

This SOP provides a written, repeatable procedure for operation and use of the Markes 120 and 250 series Micro-Chamber/Thermal Extractor and Humidity Accessory.

2.0 SCOPE

This SOP is for the use of National Risk Management Research Laboratory (NRMRL) laboratory personnel for the proper setup, operation and maintenance of the Markes Micro- Chamber/Thermal Extractor and Humidity Accessory.

3.0 DEFINITIONS

Term	Definition
°C	Degrees Celsius
EPA	U.S. Environmental Protection Agency
LED	Light-emitting diode
µ-CTE	Micro-Chamber Thermal Extractor™
min	minute
mL	milliliter
NRMRL	National Risk Management Research Laboratory
psi	Pounds per square inch
PUF	Polyurethane foam
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
T0	Time zero, test start time
v	volt
VOC	Volatile organic compound
SVOC	Semi-volatile organic compound

4.0 ROLES AND RESPONSIBILITIES

Name	Title	Description of Work
Matt Allen	Work Assignment Leader/Senior Scientist	Oversees micro-chamber testing operations. Reviews and validates micro-chamber test data. Prepares data reports to EPA for micro-chamber data. Serves as technical lead for micro-chamber work on the project.
Eric Morris	Chemist	Perform micro--chamber testing duties including: chamber and sampling systems set up, collection of environmental data, test air sampling, data entry, provide input for micro-chamber data reports to EPA, prepare chain-of custody documents for sample transfer to analytical laboratories, and adhere to all project safety and Quality Assurance (QA) procedures.

5.0 PROCEDURES

5.1 Method Summary

This procedure outlines the proper setup, operation, sampling, and cleanup of the Markes Micro- chamber/Thermal Extractor™ with Humidifier Accessory.

5.2 Sample Preservation, Containers, Handling, and Storage

Samples should be handled and stored as outlined in the project Quality Assurance Project Plan (QAPP).

5.3 Health and Safety Precautions

The Markes micro-chamber/thermal extractor™ (µ-CTE) testing should be done in a fume hood to reduce exposure to any (S)VOCs emissions. Metal chamber surfaces of the µ-CTE could be hot depending on the test conditions. The µ-CTE should be cooled to room temperature before touching, cleaning, or servicing.

5.4 Interferences

The µ-CTE is a powerful concentrator of VOCs and SVOCs the µ-CTE should be used and stored in a clean laboratory environment with minimal atmospheric concentration of organic vapors. Ensure the µ-CTE is clean before testing using the procedure outlined in SOP 6405.

5.5 Reagents and Supplies

- Deionized Water
- ORBO™ 1000 Precleaned Small PUF Cartridge (Sigma Aldrich # 20557)

- ORBO™ 1000 Precleaned Small PUF Plug (Sigma Aldrich # 20600-U)
- Aluminum weigh boats
- Pure air

5.6 Equipment/Apparatus

- Markes four chamber (µ-CTE) or six chamber (M-CTE250) micro-chamber/thermal extractor™
- Markes Humidifier Accessory, this includes a regulator/gauge, tubing, check valve, bottle humidifier, rotameter, and hygrometer (Markes # M-HUMID-MCTE)
- Sartorius Extend GK 1203 balance, or equivalent Polypropylene Caps
- Gilibrator Primary Flow Calibrator (Sensidyne # 850109-1) Digital thermometer (OMEGA # HH-25KC)
- Aadco 737 series Pure Air Generator (Aadco model # 737-11)

5.7 Procedure

5.7.1. The µ-CTE and the Humidifier Accessory Setup

1. Refer to figure 5-1 below for setup schematic and figure 5-2 for the air and electrical connections to the µ-CTE.
2. Connect the airline from the pure air generator to the step-down regulator inlet.
3. Fill Bottle humidifier with 350-400 mL of high-purity water and replace cap.
4. Connect the Bottle humidifier to the step-down regulator. The outlet of the step- down regulator is connected to the inlet of the bottle humidifier with a line that contains a check valve.
5. Connect the outlet of the bottle humidifier to the inlet of the rotameter.
6. Connect the outlet of the rotameter to the high-flow gas inlet port on the back of the µ-CTE.
7. Verify the µ-CTE power switch is in the off position. The switch is in the back of the unit.
8. Connect electrical cord to the connector on the back of the µ-CTE and plug into 115-volt (v) power supply.

Figure 5-1. Schematic of Micro-Chamber/thermal Extractor with Humidifier Accessory.

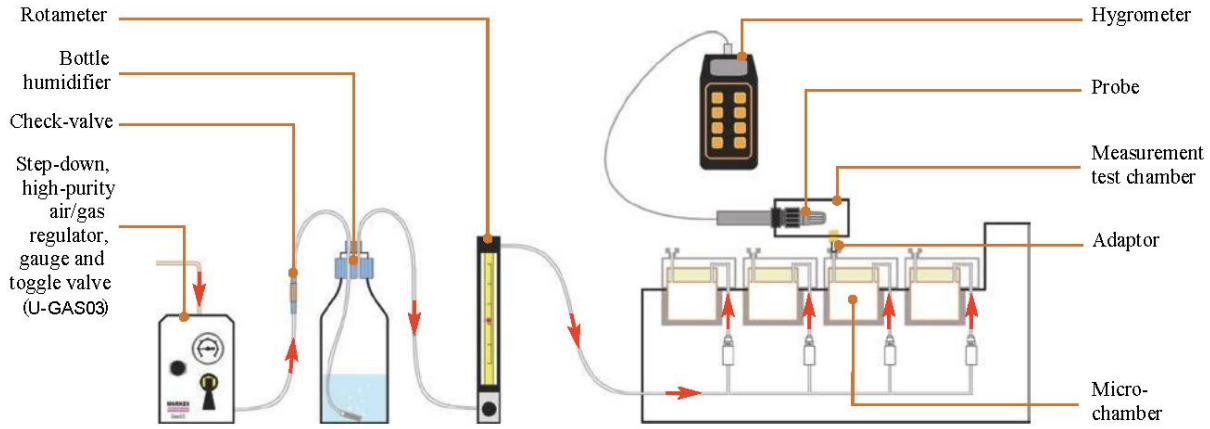


Figure 5-2 µ-CTE gas inlets – Left low-flow and Right high-flow and power connection and switch

5.7.2 Operation of µ-CTE

1. Switch power to on position. Power switch is on the back of the unit.
2. Turn on the system gas with the toggle valve on the step-down regulator. See figure 5-1
3. Set gas regulator to the desired pressure. Pressure should not exceed 20 pounds per square inch (psi) with the Humidifier Accessory attached. A pressure of 14 psi gives an approximate relative humidity of ~50% at 23oC and a pressure of 20 psi gives an approximate relative humidity of ~42% at 23oC.

4. Adjust the Rotameter to the specified test flow rate. A flow rate between 10-90 mL/min is possible with the Humidifier Accessory attached. The flow rate is checked at the chamber tube connector outlet with a Gilibrator primary flow calibrator (Sensidyne, 16333 Bay Vista Drive Clearwater, Florida).
5. Set the micro-chamber/thermal extractor™ temperature to the desired test temperature. The temperature is changed with the following procedure. Press the “P” button once, see figure 5-3. The display will flash “SP1” on the red light- emitting diode (LED) readout. Use the up and down arrow buttons to change the set point to the desired temperature on the green LED readout. Press the “P” button once to save the set point. Allow at least 15 minutes for the unit to equilibrate to the new temperature set point.
6. After the temperature reaches equilibrium, the pan temperatures are verified by measuring each chamber with a digital thermometer. Record pan temperature data in the laboratory notebook. If the pan temperature is out of the specified test value the set point will be adjusted until it is in specification.
7. The flow rates are verified by measuring each chamber outlet with the Gilibrator. Record the pan flow rate data in the laboratory notebook. The relative humidity percentage is verified by measuring each chamber outlet with a hygrometer. Record the pan relative humidity data in the laboratory notebook. If out of test specifications adjust until back into specification.
8. Check the water level in the bottle humidifier. Add deionized water if needed.
9. When ready to start test load test material. See section 5.7.3

Figure 5-3 Temperature controller interface

5.7.3 Sample loading

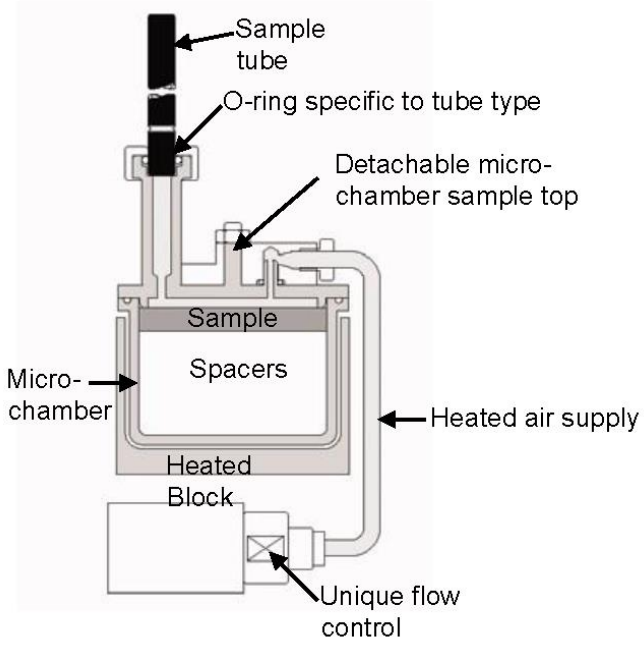
1. Don gloves
2. Check and verify calibration and operation of balance.
3. Weigh aluminum weigh boat and record this weight in the project laboratory notebook.
4. Add the test sample material to the aluminum weigh boat. Record mass of the sample and the weigh boat in the laboratory notebook.
5. Flip the micro-chamber cover’s locking latch to the unlocked position and open the chamber cover.

6. Pour the sample from the weigh boat into the micro-chamber pan. Make sure the material is spread evenly across the base of the micro-chamber pan. Use a clean stainless-steel spatula to spread material if it is necessary.
7. Close and latch chamber lid.
8. Reweigh the weight boat and record this weight in the project laboratory notebook.
9. Repeat steps 3 through 8 until all the micro-chambers are filled with test sample material.
10. Record the time when all the chamber lids are closed in the test laboratory notebook. This is the test start time, T0.

5.7.4 Chamber Cleaning

1. Cool down the µ-CTE to room temperature if needed.
2. Open each micro-chamber cover.
3. Remove the micro-chamber pan by lifting out of the chamber.
4. Remove the micro-chamber pan O-ring.
5. Properly dispose of the contents of the micro-chamber according to approved safety procedures.
6. Remove the micro-chamber lid. Unscrew the cap from the tube connector and remove the O-ring. Remove the nut and washer from the pan lid. Gently pull the pan lid away from the chamber cover. Remove the O-ring from gas inlet on the pan lid.
7. Repeat steps 2 thru 5 until all micro-chambers and lids are removed.
8. Wash the micro-chamber, lid and O-rings with soap and water as detailed in SOP- 6405 “GLASSWARE AND CHAMBER CLEANING PROCEDURE”.

Figure 5-4. Chamber diagram



5.7.5 Chamber Assembly

1. Verify micro-chamber and lid pieces were cleaned.
2. Don gloves
3. Place the micro-chamber pan into the µ-CTE unit.
4. Replace the micro-chamber pan O-ring seal into the groove on the top lip of each micro-chamber pan.
5. Place the gas inlet O-ring on the lid.
6. Align the tube connector, securing stud and gas inlet with their corresponding orifices.
7. Gently push the lid into the chamber cover until it can go no further.
8. Start nut on securing stud. Do not tighten nut. Take care not to cross threat the nut to prevent damage to the securing stud.
9. Close chamber cover and lock.
10. Finish tightening the nut on the securing stud.
11. Place O-ring into tube connector and attach tube connector cap.
12. Repeat steps 3 thru 11 until all chambers are finished.

6.0 CALCULATIONS

Not applicable

7.0 QUALITY ASSURANCE/QUALITY CONTROL

Record all sample and reagent test information in the test laboratory note book. Test information includes pretest and posttest micro-chamber flows and temperature. The barometric pressure and laboratory temperature are also record for the pretest and posttest checks. Ensure all reagents are not expired and save any Certificates of Analysis that are sent with any reagents. Check the calibration dates on any equipment used and ensure it is up to date.

8.0 REFERENCES

- SOP 6405: *Glassware and Chamber Cleaning Procedure*

9.0 REVISION HISTORY

Revision #	Description	Effective Date
0	Initial release	4/25/2016
1	Minor revision based on EPA QA review comments, QLOG No. G-APPCD-0030052, QTRAK No. 16007_04, QA Category B.	5/2/2016

Chain of Custody Procedures for the Receipt and Transfer of Samples (SOP 6402) – May 2016

United States Environmental Protection Agency

INTERNAL NRMRL PROCEDURE

Chain of Custody Procedures for the Receipt and Transfer of Samples (May 2, 2016)

NRMRL
Standard Operating Procedure (SOP)

Title:	**Chain of Custody Procedures for the Receipt and Transfer of Samples**
Division/Staff:	**NRMRL/AEMD/DSBB**
SOP Number:	**NRMRL-6402**
Former SOP Number: (if applicable)	NA
Effective Date:	5/02/2016

SOP Author(s)	
Name: Eric Morris*	Name: Matt Allen
Name: Xiaoyu Liu (PI)	Name:
Name:	Name:
QA Approval	
Name: Libby Nessley	Signature/Date:
SHEM Approval	
Name:	Signature/Date
Management Approval	
Name:	Signature/Date:
Name: NRMRL Director (optional)	Signature/Date:

* Primary Author

STANDARD OPERATING PROCEDURE (6402) FOR CHAIN OF CUSTODY PROCEDURES FOR THE RECEIPT AND TRANSFER OF SAMPLES – May 2016

TABLE OF CONTENTS

1.0 INTRODUCTION

This SOP provides a written procedure for the receipt or transfer of samples using a chain of custody (COC) form.

2.0 SCOPE

This SOP is for the use of National Risk Management Research Laboratory (NRMRL) laboratory personnel for the proper receipt and transfer of sample materials in the Indoor Air Quality Laboratory.

3.0 DEFINITIONS

Term	Definition
COC	Chain of Custody
EPA	U.S. Environmental Protection Agency
ID	Identification
NRMRL	National Risk Management Research Laboratory
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
WACOR	Work Assignment Contraction Officer Representative
SOP	Standard Operating Procedure

4.0 ROLES AND RESPONSIBILITIES

Name	Title	Description of Work
Matt Allen	Work Assignment Leader / Senior Scientist	Oversees chamber testing operations. Reviews and validates chamber test data. Prepares data reports to EPA for test data. Serves as technical lead for all chamber work on the project. Receives sample test material from EPA WACOR. Completes COC forms for receipt of test materials and sampling tubes and submission of sampling tubes to analytical laboratories.
Eric Morris	Chemist	Perform chamber testing duties including: chamber and sampling systems set up, test air sampling, receive test sample materials from EPA WACOR, and adhere to all project safety and Quality Assurance (QA) procedures. Completes COC forms for receipt of test materials and sampling tubes and submission of sampling tubes to analytical laboratories.

Jacobs Laboratory Personnel	Technician	Perform chamber testing duties including: chamber and sampling systems set up, test air sampling, prepare chain- of custody documents for sample transfer to analytical laboratories, and adhere to all project safety and QA procedures.

5.0 PROCEDURES

5.1 Method Summary

This method outlines a procedure for properly transferring or receiving sample or test materials using a COC and the proper method to generate, complete, and archive the COC.

5.2 Sample Preservation, Containers, Handling, and Storage

Samples or materials should be handled and stored as outlined in the Quality Assurance Project Plan (QAPP).

5.3 Health and Safety Precautions

Wear gloves, safety glasses, and lab coat when handling samples and materials.

5.4 Interferences

Not applicable

5.5 Reagents and Supplies

- Chain of Custody QAPP
- Project folder
- Samples or materials being transferred

5.6 Equipment/Apparatus

Computer with network access Printer

5.7 Procedure

5.7.1 Receipt of Samples or Test Materials

1. Compare the sample or material IDs on COC to the materials delivered. Verify the material delivered matches the items list on the COC.
2. After the items are verified, have the person delivering the items sign the "Relinquished" section of the COC along with the time and date when the items were relinquished.
3. As the receiver of the items, sign the COC in the "received by" section of the COC along with the time and date of when the items were transferred.
4. Store samples or test material according to procedures outlined in the QAPP.
5. Make an electronic copy of the COC and store in the project folder on the EPA network.

6. Place the hard copy of the COC in the project folder in the laboratory.

5.7.2 Transfer of Samples

1. Collect samples together. Package samples according to parameters outlined in the QAPP.
2. Fill out the Chain of Custody form. An example is shown in figure 5.2-1. Items on COC include the sample IDs, project number and name, the laboratory analyzing the sample, when the samples were collected, and any other information pertinent to the analysis of the samples.
3. Verify the information of the COC form matches the sample information.
4. Sign the "collected by" section of the COC.
5. Transfer the samples using the procedure outlined in section 5.7.1

Figure 5.2-1 Sample Chain of Custody

JACOBS

109 T.W Alexander Drive
RTP, NC 27709

Report to: | Bill to:

Chain of Custody Record

PROJECT NUMBER	P0#			No. of Containers	Analysis Required		LABORATORY:		
PROJECT NAME							LAB ADDRESS		
COLLECTED BY (SIGNATURE)							REPORT FORMAT (CIRCLE ALL REQUIRED) ELECTRONIC/VERBAL/FAX/HARDCOPY		
SAMPLE ID	RUSH FACTOR	SAMPLE MATRIX	DATE/TIME				REMARKS	LAB ID NO. (for lab use only)	
REMARKS							RELINQUISHED BY:	DATE	TIME
RECEIVED BY:	DATE	TIME	RELINQUISHED BY:	DATE	TIME		RECEIVED BY:	DATE	TIME

5.8 Calculations

Not applicable

5.9 Quality Assurance/Quality Control

Verify samples or materials being transferred match the items list on the COC. Verify COC is signed and dated. Store samples or materials according to the QAPP.

6.0 REFERENCES

Not applicable

7.0 REVISION HISTORY

Revision #	Description	Effective Date
0	Initial Version	4/25/2016
1	Minor revision based on EPA QA review comments, QLOG No. G-APPCD-0030052, QTRAK No. 16007_05, QA Category B.	5/2/2016

Collecting Air Samples from the Markes Micro-Chambers Using PUF Plugs (SOP 6403) – May 2016

United States Environmental Protection Agency

INTERNAL NRMRL PROCEDURE

Collecting Air Samples from the Markes Micro-Chambers Using PUF Plugs (May 2, 2016)

NRMRL
Standard Operating Procedure (SOP)

Title:	**Collecting Air Samples from the Markes Micro-chambers Using PUF Plugs**
Division/Staff:	**NRMRL/AEMD/DSBB**
SOP Number:	**NRMRL-6403**
Former SOP Number: (if applicable)	NA
Effective Date:	5/02/2016

SOP Author(s)	
Name: Eric Morris*	Name: Matt Allen
Name: Xiaoyu Liu (PI)	Name:
Name:	Name:
QA Approval	
Name: Libby Nessley	Signature/Date
SHEM Approval	
Name:	Signature/Date:
Management Approval	
Name:	Signature/Date:
Name: NRMRL Director (optional)	Signature/Date:

* Primary Author

STANDARD OPERATING PROCEDURE (6403) FOR COLLECTING AIR SAMPLES FROM THE MARKES MICROCHAMBERS USING PUF PLUGS – May 2016

TABLE OF CONTENTS

1. INTRODUCTION

This SOP provides a written, repeatable procedure for the collection of air samples on polyurethane foam (PUF) plug samplers from the Markes micro-chamber/thermal extractor system.

2. SCOPE

Collection of air samples on PUF plugs during tests with Markes micro-chamber/thermal extractor emissions chambers and for general indoor and outdoor ambient air sampling.

3. DEFINITIONS

Term	Definition
DAS	Data Acquisition System
°C	Degrees Celsius
EPA	U.S. Environmental Protection Agency
ID	identification
LDPE	Low-density polyethylene
min	minute
mL	milliliter
NRMRL	National Risk Management Research Laboratory
PUF	Polyurethane foam
QA	Quality Assurance
SOP	Standard Operating Procedure

4. ROLES AND RESPONSIBILITIES

Name	Title	Description of Work
Matt Allen	Work Assignment Leader / Senior Scientist	Oversees microchamber testing operations. Reviews and validates microchamber test data. Prepares data reports to EPA for microchamber data. Serves as technical lead for microchamber work on the project.
Eric Morris	Chemist	Perform microchamber testing duties including: chamber and sampling systems set up, collection of environmental data, test air sampling, data entry, provide input for microchamber data reports to EPA, prepare chain-of custody documents for sample transfer to analytical laboratories, and adhere to all project safety and QA procedures.

5. PROCEDURES

5.1 Method Summary

This method is for the collect of VOC and SVOC samples using small PUF cartridges with the Marks micro-chamber/thermal extractor unit with the Marks Humidification System. This method gives guidance for the setup of sampling and recovery of the PUF cartridges.

5.2 Sample Preservation, containers, Handling, and Storage

Samples should be handled and stored as outlined in the Quality Assurance Project Plan.

5.3 Health and Safety Precautions

The Marks micro-chamber/thermal extractor (µ-CTE) testing should be done in a fume hood to reduce exposure to any (S)VOCs emissions. Metal chamber surfaces of the µ-CTE could be hot depending on the test conditions. The µ-CTE should be cooled to room temperature before touching, cleaning, or servicing.

5.4 Interferences

The µ-CTE is a powerful concentrator of VOCs and SVOCs the µ-CTE should be used and stored in a clean laboratory environment with minimal atmospheric concentration of organic vapors. Ensure the µ-CTE is clean before testing using the procedure outlined in SOP-6405.

5.5 Reagents and Supplies

Component	Manufacturer	Model #	Location	Description
ORBO™ 1000 Precleaned Small PUF Cartridge	Sigma Aldrich	20557	St. Louis, MO	Collect micro-chamber air emissions
Aluminum Foil	Fisher Brand	01-213-101	Waltham, MA	Aluminum Foil for covering PUF Cartridge
Ziploc™ Sandwich bags	SC Johnson	876057	Racine, WI	Plastic bags for sample storage

5.6 Equipment/Apparatus

Component	Manufacturer	Model #	Location	Description
Four chamber Micro-chamber/Thermal extractor unit	Markes	µ-CTE™	Cincinnati, OH	Emissions chamber generation system
Six chamber Micro-chamber/Thermal extractor unit	Markes	M-CTE250™	Cincinnati, OH	Emissions chamber generation system
Humidification System	Markes	M-HUMID-MCTE	Cincinnati, OH	Generate moisture for RH control

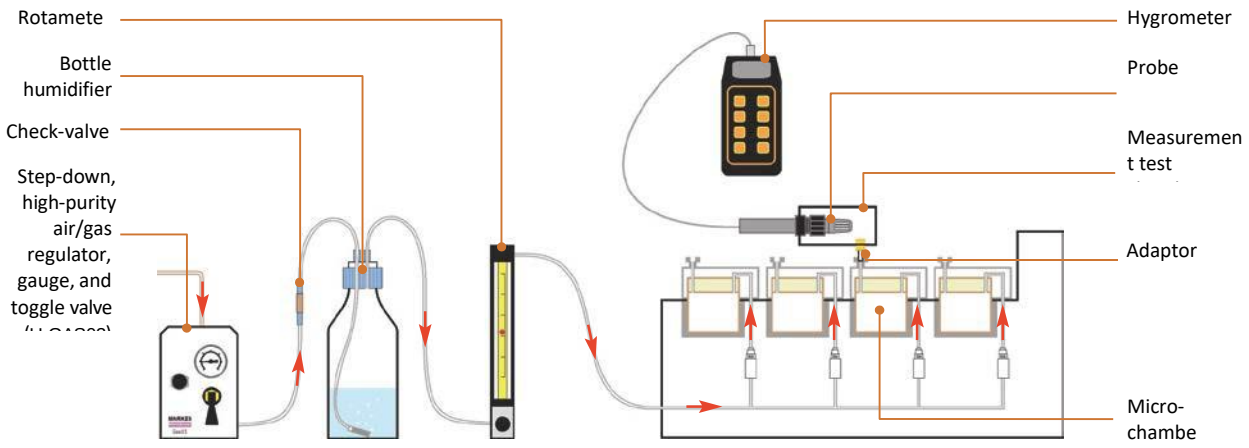
Opto Control System	Opto 22	B3000	Temecula, CA	Communication hub for MFCs, RH probes, Thermocouples
Gilibrator	Sensidyne	800286	Clearwater, FL	Used to determine air rate of sampling pump lines
Pure Air Generator	Aadco	737	Cleves, OH	Generate pure air for testing

5.7 Procedure

5.7.1 Pretest Setup

1. Before each chamber test, an Excel notebook is created on the chamber test computer. All chamber setup and operational parameters as well as the sampling information are recorded in the notebook. The pertinent parameters include the subject of the test, test date, test start time, test end time, test duration, air exchange rate, relative humidity of the chamber, and chamber air flow rate. The sampling information includes sample identification (ID), sampling data, sampling start time, sampling end time, sampling time, elapsed sampling time, sample volume, sampling flow rate (each measurement and average of triplicate measurements), sampling duration, sampling flow correct factor, barometric pressure, and temperature while sampling. All sampling information should also be written on the sampling label. The detailed sorbent sample label will be discussed in the project QAPP.
2. The sampling system consists of the clean air supply system, Humidifier Accessory (regulator gauge, humidifier bottle, rotameter, and tubing), and Markes micro- chamber/thermal extractor unit (see Figure 5-1).

Figure 5-1 Schematic of Micro-Chamber/thermal Extractor with Humidifier Accessory.



5.7.2 Set and Check Sampling Air Flows

1. Using the Gilibrator primary flow calibrator (Sensidyne # 850109-1) measure the micro- chambers' air flows at the exhaust port of each micro-chamber. Adjust the air flow to the desired rate for the micro-chambers using the rotameter.
2. Once the flows are set, attach a flow check PUF sampling tube to the Gilibrator and recheck the flow at each micro-chamber's exhaust port.
3. Collect 3 air flow measurements, in milliliters per minute (mL/min) and record on the sample label and in the electronic test notebook including the ambient temperature and barometric pressure reading from the OPTO Display Data Acquisition System (DAS) screen (see MOP 803 for details on operation of the OPTO system). Repeat this process for all micro-chambers to be used for sampling.

5.7.3 Sample Collection

1. Don nitrile gloves.
2. Remove the ORBO™ 1000 Precleaned Small PUF Cartridge (Sigma Aldrich # 20557) from the manufacturer's packaging. Remove the red low-density polyethylene (LDPE) cartridge end caps and place caps aside for use later.
3. Place the ¼-inch tapered end of the glass sampling cartridge into the micro-chamber exhaust/sampling port. Verify the exhaust port O-ring has remained properly seated in the sampling port with the introduction of the glass cartridge. Tighten the exhaust/sampling port collar down to finger tight. Do not over tighten the collar. Over tightening could result in a broken glass sampling cartridge.
4. Record the time the PUF sampler was placed in the exhaust/sampling port in the laboratory notebook.
5. Collect micro-chamber emissions on the PUF sampler for the predetermined length of time according to the test plan.
6. After sampling period is complete, remove the PUF from the exhaust/sampling port. Record the time the sampler was removed in the laboratory notebook. Wrap the glass sampling cartridge in clean aluminum foil and seal the ends of the cartridge with the red LDPE caps and place the sample into a plastic Ziploc™ bag for storage. The sample may be stored for 7 days in the refrigerator at ≤ 4°C before analysis.

5.8 Calculations

Not Applicable

5.9 Quality Assurance/Quality Control

Record all sample and reagent test information in the test laboratory note book. Test information includes pretest and posttest micro-chamber flows and temperature. The barometric pressure and laboratory temperature are also record for the pretest and posttest checks. Ensure all reagents are not expired and save any Certificates of

Analysis that are sent with any reagents. Check the calibration dates on any equipment used and ensure it is up to date.

6. REFERENCES

- MOP 803: *Operation of the OPTO Display Software Data Acquisition System (DAS) in the small chamber laboratory*
- SOP 6405: *Glassware and chamber cleaning procedure*

7. REVISION HISTORY

Revision #	Description	Effective Date
0	Initial Release	4/25/2016
1	Minor revision based on EPA QA review comments, QLOG No. G-APPCD-0030052, QTRAK No. 16007_06, QA Category B.	5/2/2016

Collecting Air Samples from the Small Environmental Testing Chambers Using Carbopack™ X Sorbent Tubes (SOP 6404) – September 2016

United States Environmental Protection Agency

INTERNAL NRMRL PROCEDURE

Collecting Air Samples from the Small Environmental Testing Chambers Using Carbopack™ X Sorbent Tubes (September 15, 2016)

NRMRL
Standard Operating Procedure (SOP)

Title:	Collecting Air Samples from the Small Environmental Testing Chambers Using Carbopack^{TM} X Sorbent Tubes
Division/Staff:	**AEMD/DSBB**
SOP Number:	**NRMRL-SOP-6404**
Former SOP Number: (if applicable)	N/A
Effective Date:	09/15/2016

SOP Author(s)	
Name: Eric Morris *	Name: Matt Allen
Name: Xiaoyu Liu (PI)	Name:
Name:	Name:

QA Approval	
Name: Libby Nessley	Signature/Date:

SHEM Approval	
Name:	Signature/Date:

Management Approval	
Name:	Signature/Date:
Name: NRMRL Director (optional)	Signature/Date:

* Primary Author

STANDARD OPERATING PROCEDURE (6403) FOR COLLECTING COLLECTING AIR SAMPLES FROM THE SMALL ENVIRONMENTAL TESTING CHAMBERS USING CARBOPACK™ X SORBENT TUBES - SEPTEMBER 2016

TABLE OF CONTENTS

1. INTRODUCTION

This SOP provides a written, repeatable procedure for the collection of volatile organic compound (VOC) air samples using Carbopack™ X sorbent tubes with small emission chambers.

2. SCOPE

This SOP is for the use of NRMRL laboratory personnel for a reproducible method for collecting VOCs from air samples with volumes greater than 50 mL on Carbopack™ X sorbent tubes. Common Carbopack™ X VOC target analytes, their boiling points, and their breakthrough volumes are shown. This list is not all inclusive, rather illustrates the types of compounds which can be collected on the Carbopack™ X sample tubes.

Compound Name	CAS #	Boiling Point (°C)	Breakthrough Volume (L)
1,2-Dichloro-1,1,2,2-tetrafluoroethane (CFC 14)	76-14-2	3.8	NL
1,3-Butadiene	106-99-0	-4.5	20
Trichlorofluoromethane (CFC 11; Freon-11; R-11)	75-69-4	23.7	NL
1,1-Dichloroethene (1,1-DCE)	75-35-4	32	20
3-Chloropropene	107-05-1	44.5	20
1,1,2-Trichloro-1,2,2-trifluoroethane (CFC 113; Freon-113)	76-13-1	48	NL
1,1-Dichloroethane	75-34-3	57.0	100
cis-1,2-Dichloroethene (1,2-DCE)	156-59-2	60.2	100
1,2-Dichloroethane (DCE)	107-06-2	83.5	100
1,1,1-Trichloroethane	71-55-6	74.1	100
Benzene	71-43-2	80.1	100
Carbon Tetrachloride (CCl4)	56-23-5	76.7	100
1,2-Dichloropropane	78-87-5	97.0	100
Trichloroethene (TCE)	79-01-6	87.0	100
1,1,2-Trichloroethane (1,1,2-TCA)	79-00-5	114	100
Toluene	108-88-3	111	100
Tetrachloroethene (PERC)	127-18-7	121	100
Chlorobenzene (MCB)	108-90-7	132	100
Ethylbenzene	100-41-4	136	100

Compound Name	CAS #	Boiling Point (°C)	Breakthrough Volume (L)
m,p-Xylene	m-108-38-3; p-106-42-3	138	100
Styrene	100-42-5	145	100
o-Xylene	95-47-6	144	100
4-Ethyltoluene	622-96-8	162	100
1,3,5-Trimethylbenzene	108-67-8	165	100
m-Dichlorobenzene (mDCB)	541-73-1	173	NL
p-Dichlorobenzene (pDCB)	106-46-7	173	NL
o-Dichlorobenzene (oDCB)	95-50-1	180	NL

NL – Not listed by Supelco

3. DEFINITIONS

Term	Definition
CAS	Chemical Abstracts Service
C	Celsius
CB	Control box
DAS	Data acquisition system
°	Degree(s)
EPA	United States Environmental Protection Agency
FEP	Fluorinated ethylene propylene
ID	Identification or Inner Diameter
MFC	Mass flow controller
mL	milliliter(s)
MOP	Miscellaneous Operating Procedure
NRMRL	National Risk Management Research Laboratory
#	Number
OD	Outer Diameter
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
SOP	Standard Operating Procedure
VOC	Volatile Organic Compounds

4. ROLES AND RESPONSIBILITIES

Name	Title	Description of Work
Matt Allen	Work Assignment Leader/Senior Scientist	Oversees chamber testing operations. Reviews and validates chamber test data. Prepares data reports to EPA for chamber data. Serves as technical lead for chamber work on the project.
Eric Morris	Chemist	Performs chamber testing duties including: chamber and sampling systems setup, collection of environmental data, test air sampling, data entry, provide input for chamber data reports to EPA, prepare chain of custody documents for sample transfer to analytical laboratories, and adhere to all project safety and Quality Assurance (QA) procedures.

5. PROCEDURES

5.1 *Method Summary*

This method outlines the setup, sampling method and parameters, and handling of Carbopack™ X sorbent tubes used to sample VOCs and SVOCs from small environmental chambers.

5.2 *Sample Preservation, Containers, Handling, and Storage*

Clean Carbopack™ X tubes are supplied to the emission chamber testing laboratory by the analytical laboratory. They are capped with brass Swagelok® caps and stored in screw cap sealed glass culture tubes. All glass tubes are put into paint cans and stored in freezers in E378-A or E375. The cleanliness of the Carbopack™ X tubes should be checked prior to delivery to the emission chamber testing laboratory by analytical laboratory personnel. Upon receipt by the emission chamber testing laboratory, the clean tubes should be ready for use. Completed and signed chain of custody forms should come with clean Carbopack™ X tubes. Tubes should be stored in freezer with temperature < 4°C if not to be used within a day. On second and subsequent uses, the tubes will generally not require further conditioning as above. However, tubes with an immediate prior use indicating high levels of pollutant trace gases should be reconditioned prior to continued usage.[1]

Chain of custody forms should be completed after sample collection and prior to submittal of the Carbopack™ X tubes to the analytical laboratory. Tubes should be stored in freezer at <4°C prior to submittal to the analytical laboratory.

5.3 *Health and Safety Precautions*

Wear nitrile gloves, safety glasses, and laboratory coat when performing tasks outlined in this SOP.

5.4 *Interferences*

Multiple interferences can contribute to inaccurate target analyte adsorption onto the Carbopack™ X tube sorbent bed. Factors of concern in the Indoor Air Quality (IAQ) laboratory are listed below.

5.4.1 Artifact Interference

Artifact interference occurs when residual target analyte(s) are not thoroughly desorbed from the sampling tube sorbent material. Tubes must be properly conditioned, capped, and stored to reduce the occurrences of artifact interference. Blank tubes should be capped with ungreased, Swagelok®-type, metal screw-caps and combined PTFE ferrules. The screw caps should be tightened by hand and then an extra 1/4 turn with a wrench. Batches of blank, sealed tubes should be stored and transported inside a suitable multi-tube container [1].

Analytical laboratory personnel will be responsible for the conditioning and Quality Control (QC) verification that tubes are in fact clean prior to supplying the IAQ laboratory with tubes.

Long-term storage of Carbopack™ X tubes can result in low levels of artifact interference. Quantifiable VOC concentrations can be detected after as little as 1-2 months of storage with artifact concentrations increasing over time [1]. Carbopack™ X tubes supplied to the IAQ laboratory for emission chamber test sampling should be used within 30 days of receipt in the laboratory.

Certain types of thermal desorption sorbent tubes can exhibit benzaldehyde, phenol, and acetophenone artifacts when used for sampling in 100-500 ppb ozone atmospheres. However, Carbopack™ X samplers have not been reported to exhibit this artifact formation, and have even shown acceptable levels of chemically stable VOC target analyte recoveries after storage for a year or more, including under high ozone concentrations [1].

5.4.2 Water and Humidity Interference

Supelco product literature states Carbopack™ X sorbent material is hydrophobic, and thus considered suitable for use in environments with high levels of humidity [4]. However, the tubes should be allowed to equilibrate to room temperature prior to sampling. If the sorbent material is at a lower than ambient temperature during sampling, moisture can be retained due to condensation.

5.4.3 Temperature Interference

The generally accepted sampling temperature range for most carbonaceous sorbent materials is 0-40°C [1]. This should not pose any problems to sampling from the 53-L small emissions chambers as the air flow temperatures at the chambers' exhaust sampling manifolds have been observed to be lower than 40°C, even when chamber temperatures are at 60°C.

5.5 *Reagents and Supplies*

Item	Manufacturer	Model #	Location	Description
Carbopack™ X thermal desorption samplers	Supelco	28686-U	St. Louis, MO	Sample tube containing sorbent
Silicon tubing ¼ in ID	Masterflex	96410-17	Vernon Hills, IL	Connecter tubing
1/4 –in OD PTFE or FEP tubing	Cole Parmer	WU-95231-02	Vernon Hills, IL	tubing

5.6 *Equipment/Apparatus*

Component	Manufacturer	Model #	Location	Description
Gilibrator Primary Flow Calibrator	Sensidyne	800286	Clearwater, FL	Used to determine air flow rate in sampling pump lines
Mass Flow Controller	Tylan General	FC-260	San Diego, CA	Control sampling flow. Range 0-1000 mL/min.
Mass Flow Control box	Tylan General	RO-28	San Diego, CA	Control sampling rate of mass flow controller
Standard Duty Vacuum Pump with vacuum gauge	Welch	2567B-50	Niles, IL	Vacuum for sampling. Gauge reads vacuum pressure up to 30 psi.
Opto Control System	Opto 22	B3000	Temecula, CA	Communication hub for MFCs, RH probes, thermocouples
Glass sampling manifold	Prism Glass	EPA-MFD-5-port	Raleigh, NC	5-port glass sampling port used to introduce sampling media to the chamber's effluent airstream
Sampling system valves (MFC and vacuum pump)	Swagelok®	SS-1GS4	Wake Forest, NC	Toggle valve that allows for vacuum flow from the pump to be turned on/off at each individual MFC or to all MFCs

All sampling system components such as MFCs, temperature sensors, barometer, and flow meters (Gilibrator) must have a current calibration to collect data. Small chamber system, and sampling system, components including temperature, relative humidity and mass flow controllers are calibrated once a year by the EPA Metrology Laboratory. Current calibration reports are stored in laboratory E378-A, and electronically in the project L: drive folder. Their procedures are documented in:

- FV-0201.1 GENERAL-Molbloc.doc
- TH-0301.0 GENERAL-Hart Dry Well.doc
- TH-0300.0 GENERAL-RH.doc
- PR-0400.0 GENERAL-Mensor.doc

These metrology laboratory files are located on the NRMRL intranet at: L:\Lab\NRML_Metlab\METLAB MOPs – ACTIVE.

Current calibration reports will be kept on file on the NRMRL intranet in the project folder at: L:\Lab\NRML_Public\Tire Crumb Rubber\QA Documents\Calibration Reports and Certificates.

5.7 *Procedure*

5.7.1 Preliminary Test Set-up

1. All SOPs and MOPs referenced in this document are found in an SOP folder kept in the laboratory. Electronic copies can be found on the network in the NRMRL intranet share drive at L:\Lab\NRML_Public\Tire Crumb Rubber\QA Documents\MOPs.
2. Before each chamber test, an electronic notebook is created in Excel on the small chamber laboratory computer. This electronic notebook will contain data and information regarding test parameters and provide a summary of the activities of each test. Each test notebook will contain the following tabs: Summary, Narrative, and SampleTimeVol (Sample Time and Volume). Pertinent test parameters and information include the subject of the test, test name, test date, test start time, test end time, test duration, air velocity, chamber air exchange rate and relative humidity, chamber air flow rate. Sampling information includes sorbent type, sorbent tube ID, sample ID, sampling data, sampling start time, sampling end time, sampling time, elapsed sampling time, sample volume, sampling flow rate (each measurement and average of triplicate measurements), sampling duration, sampling flow correction factor, ID of the mass flow controller (MFC), barometric pressure and temperature while sampling. All sampling information should also be written in the laboratory notebook and on the sample label. See Figure 1 for an example of a sorbent sample label.

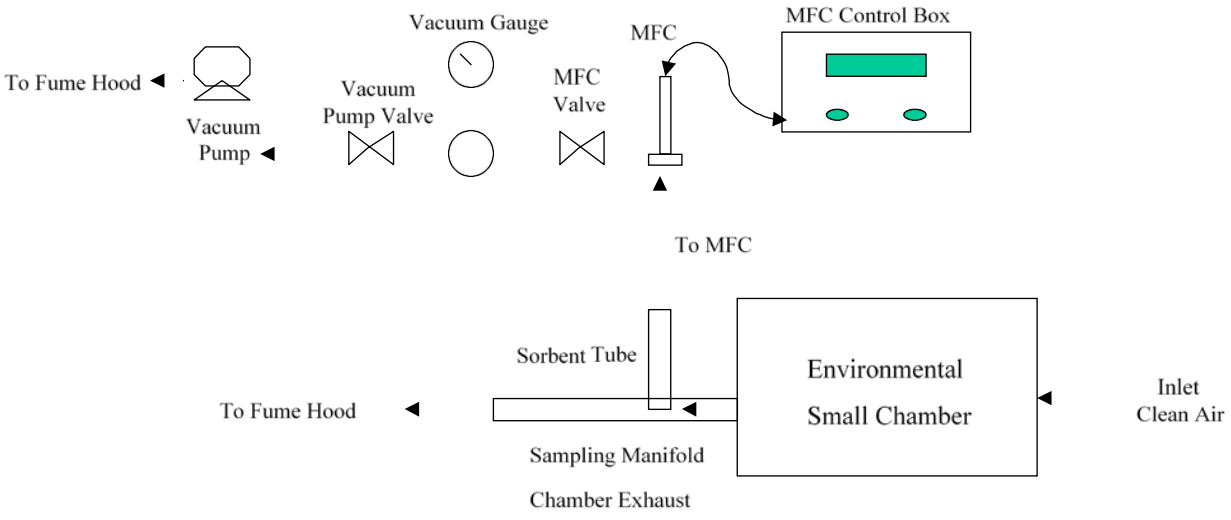
Figure 1. Example Carbopack™ X Sample Label

Title: TCRS-VOC-SCh-SE-T1-CX-C# CX-		CX-001
Type: Carbopack X Tube #	Chamber Temp: ° C Exp Date:	
Date sampled	Tire Material ID:	
Start Time	End Time	Init
Pump #	Volume	SD
Start Flow:	BP	Temp
End Flow:	BP	Temp

Data will be entered into this notebook manually by the laboratory analyst completing the applicable work. Electronic notebooks will be quality control reviewed by the laboratory technical lead. Upon completion of the test, each electronic notebook will be quality control reviewed by the laboratory technical lead and archived on the NRMRL intranet share drive at L:\Lab\NRML_Public\Tire Crumb Rubber\Data\Completed Test Notebooks.

3. Set up the sampling system as outlined in MOP-802. The sampling system consists of a pump, MFC, mass flow controller control box (CB), and a valving system (see Figure 2).

Figure 2. Schematic of Sampling System



1. Prior to sampling, consult the project QAPP or test plan for details on sampling flow rates. Typical Carbopack™ X tube sampling flow rates are 50-100 mL/min for 5-120 minutes, resulting in a 0.25-12 L sample volume. Using a Gilibrator primary flow calibrator flow-measuring device, set the mass flow controller to the

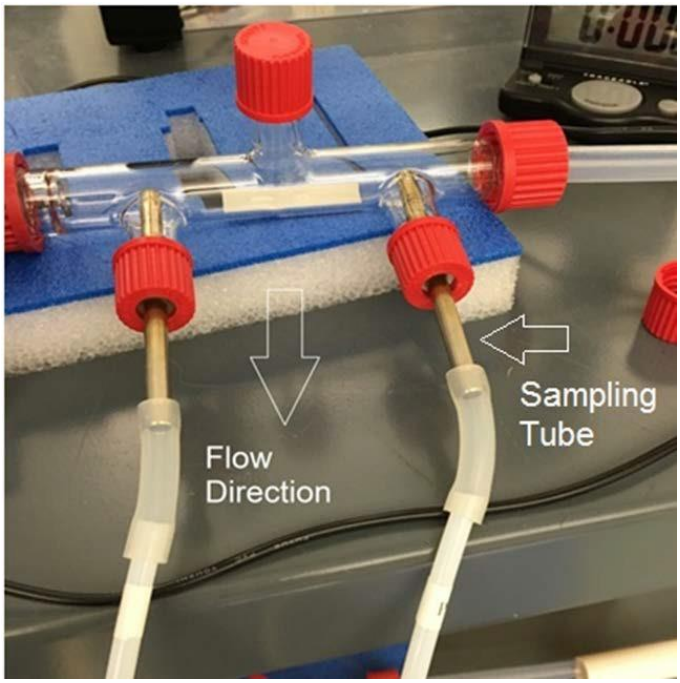
desired flow rate.

2. Once the flow is set, attach a Carbopack™ X flow check tube to the Gilibrator and recheck the flow. The check tube is attached to the vacuum pump line with a ¼-inch ID Masterflex tubing on the side without the two grooves. The tube is connected to the Gilibrator with a short piece of a ¼-inch ID Masterflex tubing. For tubes manufactured by Supelco, place the Supelco symbol end with the two grooves facing towards the manifold.
3. Collect and manually record triplicate flow measurements on the sample label, the laboratory notebook, and in the electronic test notebook, including the ambient temperature and barometric pressure reading from the OPTO data acquisition system (DAS) display screen. Repeat this process for all MFCs that will be used for sampling.

5.7.2 Sample Collection

1. Carbopack™ X tubes are received in the laboratory preconditioned and ready to use. No additional tube preparation is necessary. Remove the preconditioned tubes from the freezer and allow them to equilibrate to ambient temperature before any sampling is started. Prior to sampling, consult the QAPP or test plan's sampling schedule for details on sampling time, sampling duration, sampling flow rates, and sampling volumes. This will provide the correct number of Carbopack™ X tubes which will need to be taken from the freezer. One Field Blank should accompany at least every 10 test samples collected. Field Blanks are tubes which have been uncapped, exposed to laboratory air, and recapped. They will be processed through the same procedures as test samples prior to submittal to the analytical laboratory.
2. To collect a sample: remove the brass compression fittings from each end, slide the Carbopack™ X tube through the red sampling cap and gasket, screw the tube and red cap onto the sample manifold until snug, with the tube end located in the middle of the manifold tube, attach the ¼-in polytetrafluoroethylene (PTFE) or fluorinated ethylene propylene (FEP) vacuum line with a 1 ½ inch long ¼ inch ID Masterflex line as the connecter to the other end of the sorbent tube. For tubes manufactured by Supelco, place the Supelco symbol end with the two grooves facing towards the manifold. See Figure 3 for details on the Carbopack™ X sampling setup. Record the sampling information from section 5.7.1 on the label and in the electronic notebook.

Figure 3. Carbopack™ X Sampling Setup with Sampling Pump Tubing



3. After sampling period is complete, remove the vacuum line, then remove the sample and seal the ends of the Carbopack™ X with the brass compression fitting end caps and return the sample to the glass storage tube. The sample may be stored for up to 14 days at <4 °C if analysis will not occur immediately following sampling. See Figure 4 for an example of a Carbopack™ X sampling tube inside the capped glass culture tube.

Figure 4. Carbopack™ X Sampling Tube in Capped Glass Culture Tube

4. Collect and record three sampling line flow measurements on the sample label and in the electronic test notebook, including the ambient temperature and barometric pressure reading from the OPTO data acquisition system (DAS) Display screen. Repeat this process for all MFCs that were used for sampling.

5.8 *Calculations*
Not applicable

5.9 *Quality Assurance/Quality Control*
Record all sample and test information in the test laboratory notebooks. Test information includes pretest and posttest chamber flows, temperature, barometric pressure, laboratory temperature, and Carbopack™ X ID information. Refer to the calibration report notebook for instrument calibration date(s). Test data will be reviewed by emission chamber laboratory technical personnel to ensure data quality and accuracy in the electronic notebooks.

6. REFERENCES

- Compendium Method TO-17: *Determination of volatile organic compounds in ambient air using active sampling onto sorbent tubes*, Office of Research and Development
- U.S. Environmental Protection Agency, Cincinnati, OH, 1999.
- MOP 802: *Setup and operation of small environmental emissions chambers during testing.*

- Supelco Technical Report: A Tool for Selecting an Adsorbent for Thermal Desorption Applications. Sigma Aldrich. 2001. https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Supelco/General_Information/t402025.pdf
- Carbopack™ X TD Tube for Fenceline Monitoring. Sigma Aldrich. 2016. http://www.sigmaaldrich.com/analytical-chromatography/air-monitoring/thermal-desorption/fence-line-monitoring.html

7. REVISION HISTORY

Revision #	Description	Effective Date
0	Initial release	
1	Made minor grammatical changes to procedural steps to clarify processes. Inserted details to better describe the preliminary setup, flow checks, sampling, and QA/QC. Inserted Figure 2 detailing the sampling setup.	
2	Made corrections based on EPA QA review comments, QLOG No. G-APPCD-0030052, QTRAK No. 16007_11, QA Category B.	8/8/2016
3.	Revisions made in Section 5.7.3 based on NRMRL TSA QA audit report (QA Track: 16607/A30052)	9/15/2016

Glassware and Chamber Cleaning Procedure (SOP 6405) – May 2016

United States Environmental Protection Agency

INTERNAL NRMRL PROCEDURE

Glassware and Chamber Cleaning Procedure (May 11, 2016)

NRMRL
Standard Operating Procedure (SOP)

Title:	**Glassware and Chamber Cleaning Procedure**
Division/Staff:	**NRMRL/AEMD/DSBB**
SOP Number:	**NRMRL-6405**
Former SOP Number: (if applicable)	NA
Effective Date:	05/11/2016.

SOP Author(s)	
Name: Eric Morris*	Name: Matt Allen
Name: Xiaoyu Liu (PI)	Name:
Name:	Name:

QA Approval	
Name: Libby Nessley	Signature/Date:

SHEM Approval	
Name:	Signature/Date:

Management Approval	
Name:	Signature/Date:
Name: NRMRL Director (optional)	Signature/Date:

* Primary Author

STANDARD OPERATING PROCEDURE (6405) FOR GLASSWARE AND CHAMBER CLEANING PROCEDURE (MAY 2016)

TABLE OF CONTENTS

1. INTRODUCTION

This SOP provides a written, repeatable procedure cleaning of glassware and testing chambers in the Indoor Air Quality Small Chamber Laboratory.

2. SCOPE

This procedure is the small and micro-chamber cleaning procedure in the Indoor Air Quality Small Chamber Laboratory.

3. DEFINITIONS

Term	Definition
°C	Degrees Celsius
EPA	U.S. Environmental Protection Agency
PPE	Personnel Protective Equipment
HASAP	Hazard Assessment Safety Action Plan
NRMRL	National Risk Management Research Laboratory
QA	Quality Assurance
SOP	Standard Operating Procedure

4. ROLES AND RESPONSIBILITIES

Name	Title	Description of Work
Laboratory Personnel	Chemist/Technician	Wash glassware, environmental emissions testing chambers, and chamber parts according to SOP 6405

5. PROCEDURES

5.1. *Method Summary*

This method is for give guidance for the proper cleaning of chambers and glassware associated with chamber testing.

5.2. *Sample Preservation, containers, Handling, and Storage*

Not Applicable

5.3. *Health and Safety Precautions*

Wear gloves, safety glasses and lab coat when preforming tasks outlined in this SOP. Refer to the project Hazard Assessment Safety Action Plan (HASAP) for details on proper PPE to be worn during chamber cleaning procedures. A hardcopy of the project

HASAP is kept in laboratory E378-A; an electronic copy will be kept on the NRMRL share drive at: L:\Lab\NRML_Public\Tire Crumb Rubber\Safety\HASAP.

5.4. *Interferences*

Not Applicable

5.5. *Reagents and Supplies*

Component	Manufacturer	Model #	Location	Description
Sparkleen™ 1Detergent	Fisherbrand™	S701101	Waltham, MA	Detergent for washing glassware, chamber, and chamber parts
Aluminum Foil	Fisherbrand™	01-213-101	Waltham, MA	Aluminum foil for covering cleaned items
Organic Solvent	Various	Various	Various	Solvent for rinsing chamber and chamber parts if needed. Solvent type varies depending on test method.

5.6. *Equipment/Apparatus*

Component	Manufacturer	Model #	Location	Description
Bottle brushes	Various	Various	Various	Brushes to clean various small items
Dry Oven	Cole-Parmer Lab Companion	OF-22	Vernon Hills, IL	Oven to dry wet items
Sonicator	Branson	2510	Danbury, CT	Sonicate parts

5.7. *Procedure*

1. Don appropriate personal protective equipment (PPE) including nitrile gloves and lab coat. Goggles or a face splashguard may be necessary while working with the 53-L chambers.
2. Fill a wash tub in the sink with hot soapy water using Fisherbrand™ Sparkleen™ (or equivalent) detergent and soak the small or micro-chamber parts and chamber components.
3. Since the 53-Lsmall chambers and their lids are too large to soak in the sink, wash the chambers and lids with hot soapy water using Techlite tissue wipes or equivalent next to the sink. Use bottle brushes to clean the Swagelok openings on the chamber lid. Proceed to Step 5.
4. Wash all small or micro-chamber parts with hot soapy water using Techlite tissue

wipe or bottle brushes depending on the item being cleaned. Proceed to Step 5.

5. Rinse all items three times with hot tap water using the hose attached to the faucet. To rinse the 53-L chambers, tip them upright onto their side with the chamber lip extending over the edge of the sink basin, so all rinse water flows into the sink basin.
6. Complete this step in a fume hood. If chamber and/or glassware is to be used in sample collection associated with a specific analytical method, place the items in a clean stainless-steel beaker filled with the solvent used for extraction or preparation of samples in that analytical method. Sonicate the items for ten minutes. Remove items from the solvent and place on aluminum foil. Allow the solvent to evaporate completely before continuing cleaning procedure. Since the 53-L small chambers are too large to place in the Sonicator the chambers and lids will be wiped three times with the cleaning/extraction solvent and a Techlite tissue wipe. Allow the solvent to evaporate completely before continuing cleaning procedure.
7. Rinse all items three times with deionized water using the hose attached to the deionized water faucet.
8. Dry chamber parts in a convection oven at ≥105°C for at least 2 hours. O-rings, small chambers and lids, and any temperature sensitive items are wiped with Techlite tissue wipes and allowed to air dry on clean aluminum foil.
9. Cover cleaned items with aluminum foil until use.

5.8. *Calculations*
Not Applicable

5.9. *Quality Assurance/Quality Control*
Not Applicable

6. REFERENCES

Not Applicable

7. REVISION HISTORY

Revision #	Description	Effective Date
0	Initial Release	5/4/2016
1	Minor revision based on EPA QA review comments, QLOG No. G-APPCD-0030052, QTRAK No. 16007_08, QA Category B.	5/11/2016