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United States Environmental Protection Agency

ATSDR AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

CDC CENTERS FOR DISEASE CONTROL AND PREVENTION

Synthetic Turf Field Recycled Tire Crumb Rubber Research Under the Federal Research Action Plan

FINAL REPORT PART 2– EXPOSURE CHARACTERIZATION APPENDICES VOLUME 2

Centers for Computational Toxicology and Exposure, Environmental Measurement and Modeling, Environmental Solutions and Emergency Response, and Public Health and Environmental Assessment, Office of Research and Development

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EPA/600/R-24/020.2
April 2024

Synthetic Turf Field Tire Crumb Rubber Research Under the Federal Research Action Plan

Final Report Part 2 – Exposure Characterization Appendices

Volume 2

April 16, 2024

By

U.S. Environmental Protection Agency / Office of Research and Development (EPA/ORD)

Centers for Disease Control and Prevention / Agency for Toxic Substances and Disease Registry (CDC/ATSDR)

Disclaimer

This document has been reviewed by the U.S. Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry and approved for release. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Preferred citation: U.S. EPA & CDC/ATSDR. (2024). *Synthetic Turf Field Recycled Tire Crumb Rubber Research Under the Federal Research Action Plan Final Report: Part 2 - Exposure Characterization (Volumes 1 and 2)*. (EPA/600/R-24/020). U.S. Environmental Protection Agency, Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry.

Foreword

The U.S. Environmental Protection Agency (EPA) Office of Research and Development (ORD) and the Centers for Disease Control and Prevention (CDC) Agency for Toxic Substances and Disease Registry (ATSDR) have worked collaboratively to complete the research activities on synthetic turf playing fields under the "Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds" (FRAP). The Agencies have released the research activities' results in two parts. The Part 1 Report (U.S. EPA & CDC/ATSDR, 2019) summarizes the research effort to characterize tire crumb rubber, which includes characterizing the components of, and emissions from, recycled tire crumb rubber. The exposure characterization report (Part 2 – this report) summarizes the potential exposures that may be experienced by users of synthetic turf playing fields with recycled tire crumb rubber infill, such as how people come in contact with the materials, how often and for how long. It includes the results from a supplemental biomonitoring study conducted by CDC/ATSDR. This Part 2 exposure characterization report completes FRAP efforts with respect to playing fields.

The study is not a risk assessment; however, the results of the research described in the FRAP reports will advance our understanding of exposure to inform the risk assessment process. We anticipate that the results from this multi-agency research effort will be useful to the public and interested stakeholders to understand the potential for human exposure to chemicals found in recycled tire crumb rubber used on synthetic turf fields.

This report has been prepared to communicate to the public the research objectives, methods, results and findings for the exposure characterization research conducted as part of the Federal Research Action Plan. The report has undergone independent, external peer review in accordance with EPA and CDC policies. A response-to-peer review comments document accompanies the release of the Part 2 report.

The mission of the EPA is to protect human health and the environment so that future generations inherit a cleaner, healthier environment that supports a thriving economy. Science at EPA provides the foundation for credible decision-making to safeguard human health and ecosystems from environmental pollutants. ORD is the scientific research arm of EPA, whose leading-edge research helps provide the solid underpinning of science and technology for the Agency. ORD supports six research programs that identify the most pressing environmental health research needs with input from EPA offices, partners and stakeholders.

CDC works 24/7 to protect America from health, safety and security threats, both foreign and in the United States. ATSDR is a non-regulatory, environmental public health agency that was established by Congress under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980. ATSDR protects communities from harmful health effects related to exposure to natural and man-made hazardous substances by responding to environmental health emergencies; investigating emerging environmental health threats; conducting research on the health impacts of hazardous waste sites; and building capabilities of and providing actionable guidance to state and local health partners.

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Acknowledgments

Contract support to the EPA was provided by Jacobs Technology, Inc under Contract EP-C-15-008, the Eastern Research Group, Inc. under Contract EP-C-12-029, Oregon State University under Purchase Order EP-18-Z-000023, and Pegasus Technical Services under Contract EP-C-15-010. Special acknowledgements are given to Justicia Rhodus of Pegasus Technical Services for technical editing, and to Attainx, contractor to the EPA for graphics and media support under Contract 68HERH23D0009_G&M. Authors and contributors included student service contractors to EPA Larissa Hassinger under Contract EP-D-15-003, and Oak Ridge Institute for Science and Education (ORISE) participants Gregory Grissom, James McCord, and Sandra Utile-Okechukwu under an interagency agreement with the Department of Energy. Larry McMillan, Lillian Alston and Richard Walker were supported under the Senior Environmental Employment Program.

Special acknowledgements are given to the external peer reviewers who reviewed the draft report under contract EP-C-17-017 with the Eastern Research Group, Inc.

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Acronyms and Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ACH	Air change per hour
ADI	Acceptable daily intake
ADPA	Acetone-diphenylamine condensation product
AEMD	Air and Energy Management Division
ANOVA	Analysis of variance
ANSI	American National Standards Institute
APHC	U.S. Army Public Health Center
ASTM	American Society for Testing and Materials
ASTSWMO	Association of State and Territorial Solid Waste Management Officials
ATSDR	Agency for Toxic Substances and Disease Registry
BHA	Butylated hydroxyanisole
BTEX	Benzene, toluene, ethylbenzene, xylenes
°C	Degrees Celsius
CAES	Connecticut Agricultural Experiment Station
Cal-OEHHA	California Office of Environmental Health Hazard Assessment
CalEPA	California Environmental Protection Agency
CalOSHA	California Division of Occupational Safety and Health
CAS	Chemical Abstracts Service
CASE	Connecticut Academy of Science and Engineering
CDC	Centers for Disease Control and Prevention
CDEP	Connecticut Department of Environmental Protection
CDPH	Connecticut Department of Public Health
CFU	Colony forming units
CICAD	Concise International Chemical Assessment Documents
cm	Centimeter
COC	Chemicals of concern
COPC	Chemicals of potential concern
CPSC	Consumer Product Safety Commission
CSF	Cancer slope factor
CV	Coefficient of variance
d	day
DAD	Diode array detector
DAS	Data acquisition system
DBA + ICDP	Sum of Dibenz[a,h]anthracene and Indeno(1,2,3-cd)pyrene
DCC	Daily calibration checks
DDC	Direct dermal contact
ddPCR	Droplet digital polymerase chain reaction
DGI	Dust and gas inhalation
DNA	Deoxyribonucleic acid
DQI	Data quality indicators
ECHA	European Chemicals Agency
ECR	Excess cancer risk

EHHI	Environment and Human Health, Inc.
EOHSI	Environmental and Occupational Health Sciences Institute
EPA	U.S. Environmental Protection Agency
EU	European Union
FDEP	Florida Department of Environmental Protection
FLM	Fence line monitor
FR	Federal Register
FRAP	Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds
g	Gram
GC/MS	Gas chromatography/mass spectrometry
GC/TOFMS	Gas chromatography/time-of-flight mass spectrometry
GS/MS/MS	Gas chromatography/tandem mass spectrometry
h	Hour
h^{-1}	Per hour
HEAST	Health Effects Assessment Summary Table
HHRA	Human health risk assessment
HI	Hazard index
HPLC	High performance liquid chromatography
HR-ICPMS	High resolution magnetic sector inductively coupled plasma mass spectrometer
IAP	Internal audit program
IARC	International Agency for Research on Cancer
ICP/AES	Inductively coupled plasma-atomic emission spectrometry
ICP/MS	Inductively coupled plasma/mass spectrometry
ICP-OES	Inductively coupled plasma – optical emission spectrometry
IDL	Instrument detection limit
IOAA	Immediate Office of the Assistant Administrator
IPCS	WHO International Programme on Chemical Safety
IRIS	U.S. EPA Integrated Risk Information System
ISRI	Institute of Scrap Recycling Industries, Inc.
JTI	Jacobs Technology, Inc.
KEMI	Swedish Chemicals Inspectorate
kg	Kilogram
L	Liter
LC/MS	Liquid chromatography/mass spectrometry
LIMS	Laboratory Information Management System
LOD	Limit of detection
LOQ	Limit of quantitation
LRGA	Literature Review and Data Gaps Analysis
m	Meter
mg	Milligram
MADL	Maximum allowable dose levels
Max	Maximum
MCL	Maximum contaminant limit
MDL	Method detection limit

mecA	Gene for methicillin resistance
mg	Milligram
min	Minute
mL	Milliliter
MOS	Margin of safety
MQL	Method quantifiable limit
MQL	Minimum quantitation level
MRL	Minimum reportable limit
MRL	Minimum risk level
MRM	Multiple reaction monitoring
MRSA	Methicillin-resistant *Staphylococcus aureus*
N/A	Not applicable/Not available
NAAQS	National Ambient Air Quality Standards
NCCT	U.S. EPA National Center for Computational Toxicology
NCEA	U.S. EPA National Center for Environmental Assessment
NCEH	CDC National Center for Environmental Health
ND	Nondetect
NERL	U.S. EPA National Exposure Research Laboratory
NFL	National Football League
ng	Nanogram
NHEERL	U.S. EPA National Health and Environmental Effects Research Laboratory
NHTSA	National Highway Traffic Safety Administration
NIOSH	National Institute for Occupational Safety and Health
NIPH	Norwegian Institute of Public Health
NIST	National Institute of Standards and Technology
NOAEL	No observed adverse effect level
NOEC	No observable effects concentration
NOEL	No observable effects limit
NR	Not reported
NRMRL	U.S. EPA National Risk Management Research Laboratory
NSRL	No significant risk level
NTP	U.S. National Toxicology Program
NYDEC	New York Department of Environmental Conservation
NYDOH	New York Department of Health
OCHP	U.S. EPA Office of Children's Health Protection
OEHHA	Office of Environmental Health Hazard Assessment
OLEM	U.S. EPA Office of Land and Emergency Management
OMB	U.S. Office of Management and Budget
ORAU	Oak Ridge Associated Universities
ORCR	U.S. EPA Office of Resource Conservation and Recovery
ORD	U.S. EPA Office of Research and Development
ORISE	Oak Ridge Institute for Science and Education
OSHA	Occupational Safety and Health Administration
OSP	U.S. EPA Office of Science Policy
OTOS	N-Oxydiethylenedithiocarbamyl-N`-oxydiethylenesulfenamide

OTR	Off-the-road
PAH	Polyaromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PQL	Practical quantification limit
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	Permissible exposure limit
PM	Particulate matter
PNEC	Predicted no effect concentration
POP	Priority organic pollutants
ppbv	Parts per billion by volume
ppm	Parts per million
PQAM	Program Quality Assurance Manager
PPRTV	Provisional peer-reviewed toxicity value
PRA	Paperwork Reduction Act
PSA	Particle size analysis
PUF	Polyurethane foam
QA	Quality assurance
QAM	Quality assurance manager
QAPP	Quality assurance project plan
QMP	Quality management plan
QC	Quality control
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
REL	Recommended exposure limit/Reference exposure levels
RfC	Reference concentration
RfD	Reference dose
RH	Relative humidity
RIVM	Netherlands National Institute for Public Health and the Environment
RM	Rubber mulch
RMA	Rubber Manufacturers Association
RNA	Ribonucleic acid
RPD	Relative percent difference
rRNA	Ribosomal ribonucleic acid
%RSD	Percent relative standard deviation
RTP	Research Triangle Park (North Carolina)
RWC	Rain water contact
SA/BW	Surface area to body weight ratio
SBR	Styrene-butadiene rubber
SEE	Senior Environmental Employee
SEM	Scanning electron microscopy
SF	Slope factor
SOP	Standard operating procedure
SPME	Solid-phase microextraction
SSC	Student Services Contractor
STC	Synthetic Turf Council

STEL	Short term exposure limit
Sum15PAH	Sum of 15 of the 16 EPA ‘priority’ PAHs
SumBTEX	Sum of benzene, toluene, ethylbenzene, m/p-xylene, and o-xylene
SVOC	Semivolatile organic compound
TCC	Tire Crumb Characterization
TCLP	Toxicity characteristic leaching procedure
TCR	Tire crumb rubber
TCRS	Tire Crumb Research Study
TLV	Threshold limit value
TOF	Time of flight
TOFMS	Time-of-flight mass spectrometry
TPE	Thermoplastic elastomers
TSA	Technical systems audit
TSP	Total suspended solids
TWA	Time weighted average
TWP	Tire wear particles
UCHC	University of Connecticut Health Center
µg	Microgram
µm	Micrometer
µL	Microliter
UR	Unit risk
URL	Uniform resource locator
U.S.	United States of America
U.S. EPA	United States Environmental Protection Agency
UV	Ultraviolet spectrometry
VID	Video identification number
VOC	Volatile organic compound
WDOH	Washington State Department of Health
WHO	World Health Organization
WM	Wood mulch
XRF	X-ray fluorescence spectrometry
yr	Year

Appendix A
Biomonitoring Study

Supplemental Biomonitoring Study of Exposure During Activities Conducted on Synthetic Turf Fields with Tire Crumb Rubber Infill

Summary Report

April 16, 2024

Prepared by

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Acronyms and Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CPSC	Consumer Product Safety Commission
CRE	Creatinine
DLS	Division of Laboratory Sciences
EPA	U.S. Environmental Protection Agency
FRAP	Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds
GLM	Generalized Linear Model
Ln	Natural log
LOD	Limit of detection
NCEH	National Center for Environmental Health
NHANES	National Health and Nutrition Examination Survey
No.	Number
OMB	U.S. Office of Management and Budget
PAH	Polycyclic Aromatic Hydrocarbon
SG	Specific Gravity
STC	Synthetic Turf Council
U.S.	United States of America
U.S. EPA	United States Environmental Protection Agency
U.S. HHS	United States Department of Health and Human Services
1-NAP	1-Hydroxynaphthalene
1-PHE	1-Hydroxyphenanthrene
1-PYR	1-Hydroxypyrene
2-FLU	2-Hydroxyfluorene
2-NAP	2-Hydroxynaphthalene

2 & 3-PHE	Sum of 2-Hydroxyphenanthrene and 3-Hydroxyphenanthrene
3-FLU	3-Hydroxyfluorene

1. Executive Summary

In 2016, the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (CDC/ATSDR) and the U.S. Environmental Protection Agency (EPA), in collaboration with the Consumer Product Safety Commission (CPSC), launched a multi-agency research effort known as the Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds (FRAP). To address the public's concerns about the use of recycled tire crumb rubber on synthetic turf fields, the FRAP focused on assessing potential human exposure, which includes conducting research activities to characterize the chemicals associated with recycled tire crumb rubber and to identify the ways in which people may be exposed to those chemicals based on their activities on synthetic turf fields [EPA and CDC/ATSDR 2019].

In 2022, CDC/ATSDR conducted a biomonitoring study to supplement the FRAP's pilot-scale human exposure study conducted in 2017 [EPA and CDC/ATSDR 2024]. This report summarizes key findings as they relate to the following objectives:

- **Objective 1**: Expand upon the FRAP's pilot-scale study by including a larger sample size of synthetic turf with recycled tire crumb rubber infill users and a comparison group of natural grass field users.
- **Objective 2:** Examine potential associations in pre- and post-activity urinary polycyclic aromatic hydrocarbon (PAH) metabolite concentrations with field type (i.e., synthetic turf fields with recycled tire crumb rubber infill and natural grass fields).
- **Objective 3**: Compare study participants' urinary concentrations to those found in the noninstitutionalized general U.S. population using National Health and Nutrition Examination Survey (NHANES) data.

CDC/ATSDR's biomonitoring study included questionnaire administration to participants and the collection of pre- and post-activity urine sampling. Results compiled from a total of 161 study participants aged between 7–77 years are included in this report. The 7 urinary PAH metabolites assessed at the CDC National Center for Environmental Health (NCEH) Division of Laboratory Sciences (DLS) included: 1-hydroxynaphthalene (1-NAP), 2-hydroxynaphthalene (2-NAP), 2-hydroxyfluorene (2-FLU), 3-hydroxyfluorene (3-FLU), 1-hydroxyphenanthrene (1-PHE), 1-hydroxypyrene (1-PYR), and the sum of 2-hydroxyphenanthrene and 3-hydroxyphenanthrene (2 & 3-PHE).

Notably:

- Pre- and post-activity differences in urinary PAH concentrations were not associated with field type (i.e., synthetic turf with recycled tire crumb rubber infill and natural grass fields).
- Pre- and post-activity differences in urinary PAH concentrations varied by statistical method and urinary dilution adjustment (i.e., specific gravity, creatinine) method.

- The best predictor of post-activity urinary PAH concentration was pre-activity concentration.
- Except for 2-NAP, pre-activity PAH concentrations were lower than those in the general U.S. population (NHANES 2015-2016).

These results indicate recycled tire crumb rubber infill users and natural grass field users experienced similar differences in pre- and post-activity PAH concentrations. Importantly, CDC/ATSDR's biomonitoring study as detailed in this report is not a risk assessment. However, combined with the initiatives of the FRAP, this study's findings contribute to the extensive research portfolio regarding the use of tire crumb rubber infill in playing fields.

2. Introduction

There are more than 12,000 synthetic turf fields installed in the United States [STC et al. 2016]. Millions of people use and/or work at these synthetic turf fields across a range of settings, including municipal and county parks; schools, colleges, and universities; professional sports stadiums and practice fields; and military installations. Approximately 95% of synthetic turf fields utilize small pieces of recycled tire ("recycled tire crumb rubber") either as infill exclusively or in mixture with sand or alternative infills [STC et al. 2016]. Tires are manufactured with a range of chemicals; additionally, tires may also pick up and absorb chemicals over their lifetime of use and serve as a sorbent for chemicals in the air and dust [EPA and CDC/ATSDR 2019]. Users of synthetic turf fields with recycled tire crumb rubber infill can potentially be exposed to chemicals such as polycyclic aromatic hydrocarbons (PAHs) in a variety of ways, including while breathing (i.e., inhalation exposure), contacting the material with their skin (i.e., dermal exposure), and by ingesting the material (i.e., ingestion exposure) [EPA, CDC/ATSDR, and CPSC 2016; EPA and CDC/ATSDR 2019].

Parents, athletes, schools, and communities have raised concerns about the safety of recycled tire crumb rubber used as infill for playing fields and playgrounds. To fill important data gaps and address key environmental and human health questions regarding the use of recycled tire crumb rubber, the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (CDC/ATSDR), U.S. Environmental Protection Agency (U.S. EPA), and U.S. Consumer Products and Safety Commission (CPSC) launched the Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds (FRAP) in 2016.[1] Key activities since performed as part of the FRAP included: a literature review and data gaps analysis [EPA, CDC/ATSDR, and CPSC 2016]; a tire crumb rubber characterization study [EPA and CDC/ATSDR 2019]; and a human exposure characterization study [EPA and CDC/ATSDR 2016 Research Protocol; EPA and CDC/ATSDR 2024].

The primary aims of the human exposure characterization research study were to (1) collect human activity data for synthetic turf field users that will reduce the reliance of default exposure factor assumptions in exposure and risk assessment; and (2) conduct an exposure measurement sub-study for people using synthetic turf fields with tire crumb rubber infill, in

[1]Additional information describing the FRAP are available at: https://www.atsdr.cdc.gov/frap/index.html.

what are likely to be among the higher exposure scenarios to improve understanding of potential exposures, particularly for the dermal and ingestion exposure pathways [EPA and CDC/ATSDR 2016 Research]. To meet these objectives, CDC/ATSDR and U.S. EPA collaborated on a pilot-scale human exposure characterization study among field users of synthetic turf with tire crumb rubber infill (Office of Management and Budget [OMB] Control No. 0923–0058). The pilot-scale study involved questionnaire administration and several types of sample collection, including field environment samples, personal samples (air, dermal wipes), and biological samples (urine and blood pre-activity and post-activity). The sample size for biological measurements was small: 14 participants provided urine samples and 13 provided blood samples. Urine samples were analyzed for 7 PAHs and serum samples derived from the blood samples were analyzed for metals. Results from the pilot-scale study are detailed in the FRAP Part 2 Report [EPA and CDC/ATSDR 2024].

In spring and summer 2022, CDC/ATSDR conducted participant recruitment for a supplemental biomonitoring measurements study regarding exposure during activities performed on synthetic turf fields with tire crumb rubber infill (OMB Control Number: 0923–0062). The design of this study addressed limitations of the 2017 pilot study, including the small sample size of participants and a lack of a comparison population. CDC/ATSDR's supplemental biomonitoring study included questionnaire administration and pre- and post-activity urine sampling among users of synthetic turf fields with tire crumb rubber infill and users of natural grass fields. Data collection efforts were consistent with those in the original pilot study's research protocol [EPA and CDC/ATSDR 2016 Research Protocol] but modified in scope (i.e., questionnaire administration and urine sampling but no blood sampling) to achieve the following objectives:

Objective 1: Expand upon the pilot-scale study by including a larger sample size of synthetic turf with recycled tire crumb rubber users and a comparison group of natural grass field users.

Objective 2: Examine potential associations in pre- and post-activity urinary PAH metabolite concentrations with field type (i.e., synthetic turf fields with recycled tire crumb rubber infill and natural grass fields).

Objective 3: Compare study participants' urinary PAH concentrations to those found in the noninstitutionalized general U.S. population using National Health and Nutrition Examination Survey (NHANES) data.

3. Methods

3.1. Recruitment

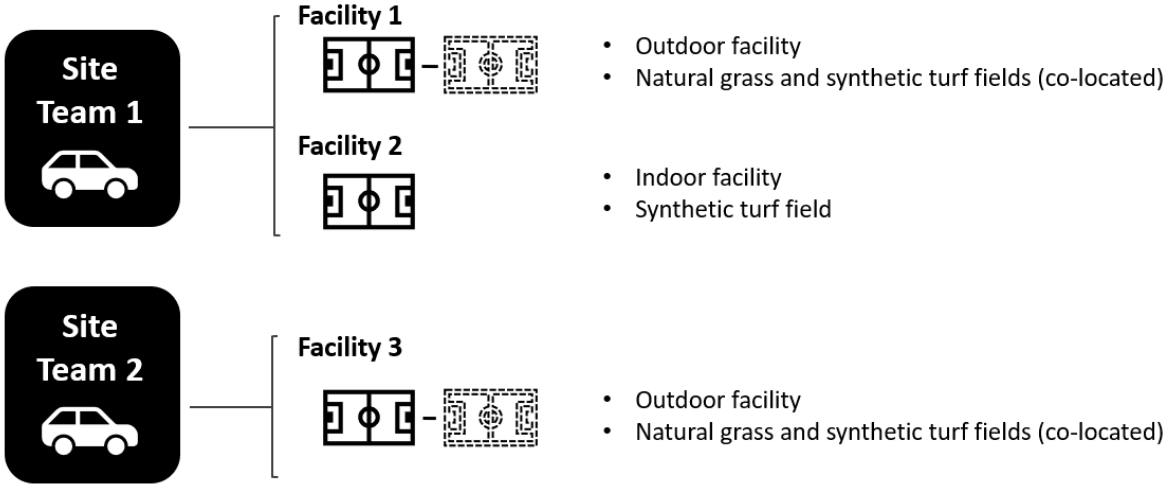
Study participants were recruited from 3 athletic facilities (2 outdoor and 1 indoor, as shown in Figure 1) in two U.S. census regions[2] during April–September 2022. The two outdoor facilities

[2]The U.S. census regions are four geographic groupings of states that subdivide the United States, including: Midwest (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin); Northeast (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York,

consisted of synthetic turf fields with tire crumb rubber infill co-located with natural grass fields. The single indoor facility consisted of a synthetic turf field with tire crumb rubber infill. At the time of study recruitment, the indoor field with recycled tire crumb rubber infill was approximately 4 years old, while the 2 outdoor synthetic turf fields with recycled tire crumb rubber infill were approximately 8 and 9 years old. Natural grass field users served as a comparison group for examining the potential association between pre- and post-activity PAH concentration differences with field type. The synthetic turf fields were previously included in the tire crumb characterization research activity of the FRAP (OMB Control No. 0923-0054).

Eligible study participants included a convenience sample of field users ages 7 years and older who engaged in physical activity on the synthetic turf fields with recycled tire crumb rubber or natural grass fields. Exclusion criteria included those who self-reported smoking or living in a household with someone who smokes. Additionally, natural grass participants were excluded if they indicated playing on synthetic turf with tire crumb rubber infill in the past 24 hours. Field users were asked to complete a study questionnaire and provide both pre- and post-activity urine samples.

Figure 1. Overview of Three Facilities for Recruitment of Study Participants



The study was performed in accordance with all required human subjects reviews and protections specified in the Code of Federal Regulations (45 CFR part 46 for the U.S. Department of Health and Human Services [HHS]) and in other applicable policies on human subjects at CDC/ATSDR. The data collection components of the tire crumb rubber exposure study went through the Office of Management and Budget (OMB) Information Collection Request (ICR) review process (OMB Control Number: 0923-0062). As a waiver of documentation

Pennsylvania, Rhode Island, and Vermont); South (Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia); and West (Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming).

of informed consent was in place, the only record linking the participants to the research was a consent document and study ID; no names or other personally identifiable data were collected.[3] A two-year extension for the research study was granted due to delays encountered with the COVID-19 pandemic.

3.2. Data Collection

3.2.1. Questionnaire Administration

Questionnaires were administered to eligible field users on the date of urine sampling collection to obtain information on participant activity patterns that could affect the duration or frequency of their potential exposures. Participants were informed the questionnaire would take about 30 minutes to complete. Depending on the age of the participant, slightly different questionnaire versions were used for administration either directly to participants ages 13 years or older, or to a parent or guardian of participants ages 7–12 years. Questionnaire items also included non-activity-related factors that could affect PAH concentrations, such as demographics (e.g., age, gender, and race), mode of commute to the field, and consumption of grilled foods in the past 24 hours, among others.

3.2.2. Urine Sample Collection and Processing

Both pre- and post-activity urine samples were collected to control for baseline body burden levels and adjust for the effects of metabolic processes to better isolate the effects of activity and potential exposure related to recycled tire crumb rubber infill. Consistent with the FRAP pilot study protocol for urine collection, preparation, and storage procedures, participants were provided with a sealed sterile urine collection cup to provide urine samples on-site using either facility restrooms or port-a-potties. Samples were shipped on dry ice to the CDC National Center for Environmental Health (NCEH) Division of Laboratory Sciences (DLS) for measurement of PAH metabolites, specific gravity, and creatinine concentrations**.** In alignment with the CDC NHANES panel [CDC 2020], 7 PAH metabolites were quantified using online solid phase extraction high performance liquid chromatography/tandem mass spectrometry (SPE-HPLC-MS/MS) [Wang 2017]. The PAH metabolites included 1-hydroxynaphthalene (1-NAP), 2-hydroxynaphthalene (2-NAP), 2-hydroxyfluorene (2-FLU), 3-hydroxyfluorene (3-FLU), 1-hydroxyphenanthrene (1-PHE), 1-hydroxypyrene (1-PYR), and the sum of 2-hydroxyphenanthrene and 3- hydroxyphenanthrene (2 & 3-PHE).[4]

[3]Detailed information describing the consenting process, forms, and protocol including Supplemental Exposure Measurement Supplemental Study Materials and questionnaires are available at: https://www.reginfo.gov/public/do/PRAViewICR?ref_nbr=202106-0923-001.

[4]Consistent with NHANES, 2-3-PHE values are comparable to the sum of urinary levels of 2-hydroxyphenanthrene and 3-hydroxyphenanthrene. Additional information is available at: https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/PAH_H.htm.

3.3. Data Analysis

3.3.1. Specific Gravity and Creatinine Adjustments

To account for the effect of urinary dilution and facilitate comparisons with data produced from other studies, all urinary PAH metabolites were adjusted for specific gravity and creatinine. The outcome variables in this analysis were urinary PAH metabolite concentrations, their specific gravity and creatinine adjusted values, and ln-transformed versions of each.

Urine specific gravity (SG) was measured and used to adjust PAH concentrations for urine dilution according to the formula,

$$C_{SG} = C_{measured} \times (1.0194 - 1)/(\rho - 1),$$

where $C_{measured}$ was the individual observed urine PAH concentration, ρ was the individual observed SG, and 1.0194 was the average SG of all urine samples in this study [Alhamdow 2017].

Creatinine (CRE) was used to adjust PAH concentrations using the ratio method according to the formula,

$$C_{Ratio} = C_{measured} / CRE$$

where $C_{measured}$ was the individual observed urine PAH concentration and CRE was the corresponding creatinine concentration.

3.3.2. Covariates

Covariate data was collected from a questionnaire administered at the time of sample collection. Categorical variables used in this analysis were age category (children [7–9 years], youth [10–12 years], adolescent [13–17 years], adult [18+ years]); sex (male, female); Hispanic ethnicity (Hispanic, Non-Hispanic); race (Asian, White, Other [Non-Hispanic participants who indicated Black, Native Hawaiian/Other Pacific Islander, multiple races, or did not report race]), field type (natural grass or synthetic turf), field environment (indoor or outdoor); consumption of grilled, barbequed, smoked, or deep fried food in the last 24 hours (yes or no); facility location by U.S. census region (i.e., South or West); activity (e.g., lacrosse, soccer, other); BMI categories (underweight, normal, overweight, and obese; with cut points at <18.5, <25, <30, and >=30, respectively, for adults).[5]

[5]CDC growth charts were used to calculate BMI for participants <20 years old with cut points defined at BMI percentages (0,5), [5,85), [85,95), and 95+. Because age was only collected for whole years and age-in-months is required to properly use growth charts, BMI calculations for participants under 20 years old were subject to misclassification due to rounding. See SAS Program for CDC Growth Charts. See: https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm.

Continuous variables used in the analysis included PAH concentrations, specific gravity concentrations, creatinine concentrations, age (rounded to whole years), height, weight, number of months that a participant reported having used a field, and time (minutes) spent commuting to the field on a typical day.

Various measures were calculated to assess the probability distributions of PAH concentrations; this included distribution plots, Q-Q plots, a statistical test of normality (Shapiro-Wilk test), calculated distribution measures such as kurtosis (a measure of the heaviness of a distribution's tail) and skewness (a measure of the asymmetry of a distribution). Regression of non-transformed concentrations can result in statistical associations solely attributable to outliers and generally do not meet the assumptions of common statistical methods, such as normality. Ln-transformation serves to lessen the influence of large concentrations that are typical of log-normally distributed biomarker data. Distribution plots and tests of normality (data not shown) indicated that the PAH concentrations were approximately log-normally distributed. Based on these assessments, PAH concentrations were ln-transformed for regression analyses. Specifically, the difference of the individual ln-transformed pre- and post-activity data were examined, which is equivalent to the ln of the ratio of concentrations on the original scale:

$$\ln(post) - \ln(pre) = \ln(post/pre)$$

3.3.3. Statistical Analysis

Analyses were conducted in SAS software, version 9.4 LTS Level 1M7 of the SAS System for Windows. Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA. Proc SGPLOT and SGPANEL were used to create plots for data visualization; Procs TTEST and UNIVARIATE were used to assess pre- and post-activity changes in PAH concentration; and Proc GLM was used to investigate statistical associations between pre- and post-activity differences in PAH concentration with covariates that were selected a priori based on their potential association with PAH concentrations.

Regression modeling focused on three dependent variables: pre-activity, post-activity, and differences between pre- and post-activity concentrations. The key independent variable was field type (synthetic turf vs. natural grass), and models were adjusted for covariates. General linear models were used to test whether variables such as field type were associated with observed pre- and post-activity differences in concentrations (defined as post concentration minus pre concentration). In these models, the intercept tested whether there was an overall difference between pre- and post-activity mean concentrations. Additionally, the beta coefficient for a covariable measured whether there was a difference in pre- and post-activity differences between levels of the covariate. The focus of this investigation was the potential association between field type (synthetic turf or natural grass) and increased PAH concentration, post-activity.

3.3.4. PAH Metabolite Comparisons to the General U.S. Population

Study participants' urinary PAH metabolite concentrations were compared to those among the non-institutionalized U.S. population by age group using NHANES data from 2015–2016 and

2007–2008. The 2015–2016 NHANES cycle was the most recent cycle to contain PAH concentrations. Because specific gravity was not measured in the 2015–2016 cycle, only unadjusted and creatinine-adjusted concentrations could be compared. Pre-activity concentrations from this study were used for comparison, as NHANES participants did not engage in physical activity prior to specimen collection, and post-activity concentrations among this present study's participants were likely affected by hydration changes. The 2007–2008 cycle was used to compare the present study's specific gravity adjusted PAH metabolite concentrations; this was the only cycle that measured both creatinine and specific gravity.

4. Results

4.1. Study Population and Urine Sample Properties

A total of 172 participants were recruited for the study, among whom all provided questionnaires and 171 provided pre- and/or post-activity urine samples. Among the 171 participants, a total of 10 participants were excluded from the analysis due to at least one of the following factors: missing post-activity urine sampling (n=4), shipping-related delays of urine samples which might have compromised sample integrity (n=3), or flagged upon sample processing (n=3). Thus, 10 participants were excluded, leaving a total of 161 participants who were included in this analysis. Finally, one participant had an interfering substance code for 1-NAP, but valid measurements for all other analytes, and so the analysis sample size for 1-NAP specifically was reduced to 160 participants. All participants were recruited in the evening or afternoon due to activity schedules. Participants spent an average of 1 hour and 39 minutes (range: 40 minutes to 2 hours and 58 minutes) between pre- and post-urine collections.

In total, 6% of PAH measurements were below the limit of detection (LOD). Specifically, 134 of 2252 PAH measurements (160 participants x 7 analytes x 2 measurements + 1 participant x 6 analytes x 2 measurements) were below the LOD. Four of these PAH measurements had a zero value and were replaced by the limit of detection (LOD) divided by $\sqrt{2}$; four creatinine measurements that had missing values due to being less than the lowest standard were also replaced by the LOD divided by $\sqrt{2}$. Non-zero values below LOD were unchanged.

4.2. Study Population Description

Table 1 presents the characteristics of the study participants. As shown, among the 161 study participants, a total of 82% (n=132) played on synthetic turf with tire crumb rubber infill and the remaining 18% (n=29) on natural grass. Moreover, 25% of study participants used an indoor field of synthetic turf with tire crumb rubber infill, and all other participants utilized outdoor fields. Across the three facilities and two U.S. census regions, 68% of the study participants were recruited from the South and 32% from the West. The study population for this analysis comprised 27% female and 73% male, with an age range of 7 to 77 years and a median age of 14 years at the time of specimen collection. Ages were distributed as approximately 15% children (7-9 years), 17% youth (10-12 years), 32% adolescent (13-17 years), and 37% adult (18+

years).[6] By race, 63% of participants identified as White persons; 14% as Asian persons; and 23% as Black persons, Native Hawaiian or Pacific Islander persons, persons of multiple races, or did not report race. By ethnicity, approximately 28% of participants identified as Hispanic or Latino persons. The most common field activity was soccer (74%), followed by lacrosse (17%), with various other activities (e.g., football, flag football, field hockey) making up the remaining 9%. By BMI, 60% of participants had a normal BMI, 20% had an overweight BMI, 10% had an obese BMI, and 5% had an underweight BMI. As noted in the footnote above, because BMI calculations for those under age 20 years depend on age-in-months, and because age was only collected to the nearest whole year, these BMI results may be subject to misclassification. All study participants reported having travelled to the facilities by car, with a median commute time of 20 minutes. Responses to the question, "how long have you been coming to this facility?" ranged from 0 to 120 months. Moreover, 33% of study participants reported having eaten grilled, barbequed, smoked, or deep-fried food within the 24 hours prior to sample collection.

Table 1. Study Participant Characteristics

Variable	Group	Value
Field Type, n(%)	Synthetic Turf with Tire Crumb Infill	132 (82)
Field Type, n(%)	Natural Grass	29 (18)
Field Environment, n(%)	Outdoor	120 (75)
Field Environment, n(%)	Indoor	41 (25)
Facility's US Census Region, n(%)	South	109 (68)
Facility's US Census Region, n(%)	West	52 (32)
Sex, n(%)	Male	118 (73)
Sex, n(%)	Female	43 (27)
Age Category, n(%) [range: 7-77 years]	Child (7-9 years)	24 (15)
Age Category, n(%) [range: 7-77 years]	Youth (10-12 years)	27 (17)
Age Category, n(%) [range: 7-77 years]	Adolescent (13-17 years)	51 (32)

[6]Percentages may not total to 100% due to rounding.

Variable	Group	Value
Age Category, n(%) [range: 7-77 years]	Adult (18+ years)	59 (37)
Race, n(%)	White	102 (63)
Race, n(%)	Asian	22 (14)
Race, n(%)	Mixed/Other: Black, Native Hawaiian/Other Pacific Islander, multiple races, or did not report race	37 (23)
Hispanic Ethnicity, n(%)	No	113 (72)
Hispanic Ethnicity, n(%)	Yes	44 (28)
Hispanic Ethnicity, n(%)	Refused	1 (1)
Field Activity, n(%)	Soccer	119 (74)
Field Activity, n(%)	Lacrosse	27 (17)
Field Activity, n(%)	Other	15 (9)
Past 24-Hour Grilled Food Consumption, n(%)	No	106 (67)
Past 24-Hour Grilled Food Consumption, n(%)	Yes	53 (33)
Weight (lbs), mean(med)[range]	N/A	131 (125) [50-131]
Height (inches), mean(med)[range]	N/A	64 (65) [48-76]
BMI, mean(med)[range]	N/A	22 (20) [11-44]
Length of time coming to facility (months), mean(med)[range]	N/A	30 (14) [0-120]
Length of commute to field (minutes), mean(med)[range]	N/A	22 (20) [2-120]

Note: N/A = not applicable

Percentages may not total to 100% due to rounding.

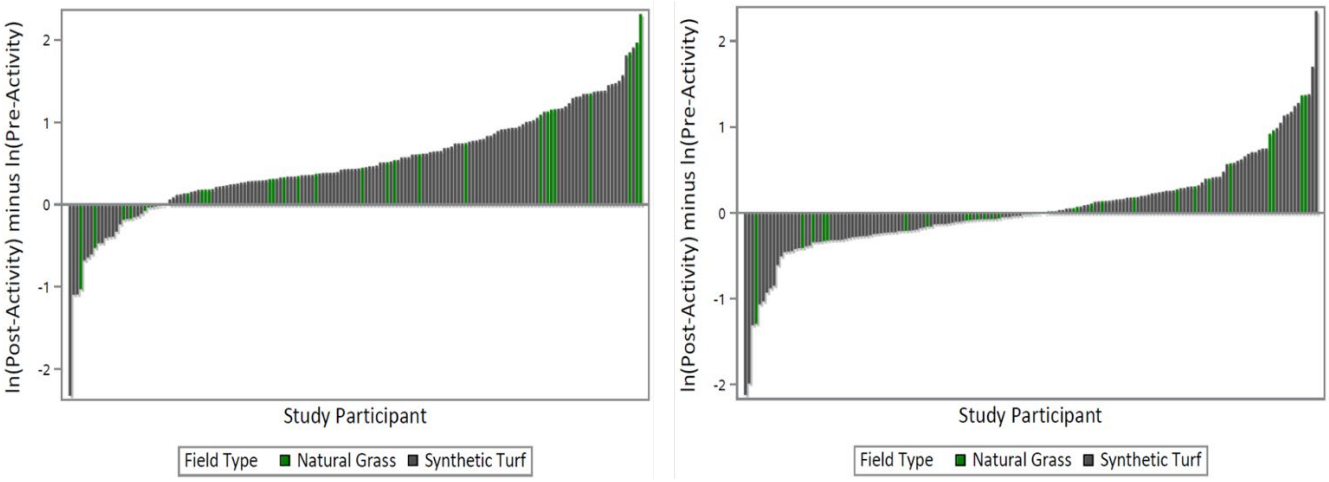
Data for race is presented in these categories due to small cell sizes.

Length of time coming to facility was assessed by the question, "How long have you been coming to this facility?" and length of commute to the field was assessed by the question, "How many minutes did it take you to get to practice today?"

4.3. Pre- and Post-Activity PAH Concentration Differences

As shown in the representative figures for 1-NAP (Figure 2), most study participants' PAH concentrations increased post-activity after adjustment for specific gravity (left). However, the equivalent result for creatinine ratio adjustment demonstrated a more even distribution between those whose concentrations increased compared to those whose concentrations decreased (right). Moreover, the identification of the participants who had the largest post-minus pre-activity degree of change in concentrations was dependent upon the adjustment method. The participants identified as having the biggest increases (when based on specific gravity adjustment) played on natural grass, whereas those identified based on creatinine adjustment played on synthetic turf (see Supplemental Figures S1-1 through S1-6 for additional plots). As shown later in this report, these differences in post- and pre-activity PAH concentrations were not associated with field type.

Figure 2. Difference in post- and pre-activity concentration for 1-hydroxynapthalene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).



Statistical differences in pre- and post-activity body burden levels for urinary PAHs are presented for specific gravity-adjusted concentration differences by all participants (Table 2) and by field type (Table 3). Additionally, statistical differences in pre- and post-activity body burden levels for urinary PAHs are presented for creatinine-adjusted concentration differences by all participants (Table 4) and by field type (Table 5). For specific-gravity adjusted and ln-transformed PAH concentrations, there was a statistically significant difference in mean concentrations when comparing pre- and post-activity differences for all 7 PAHs (p-values <0.05). However, this was irrespective of whether participants played on synthetic turf with tire crumb rubber infill or natural grass fields. Fewer differences were observed when examining creatine-adjusted and ln-transformed PAH concentrations; pre- and post-activity concentration differences were statistically significant for 1-PYR (mean: -1.76 µg/L, $p<0.05$) and 2-NAP (mean:

0.163 µg/L, $p<0.05$). As with the specific gravity-adjusted results, differences observed with creatinine-adjusted data were irrespective of whether participants played on synthetic turf with tire crumb rubber infill or natural grass fields. Statistical analysis of differences by additional measures are displayed in Supplemental Tables S1-1 through S1-7 (specific gravity-adjusted) and Supplemental Tables S2-1 through S2-7 (creatinine-adjusted).

Generally, pre- and post-activity concentrations were correlated. Specifically, the pre- and post-activity correlations of the urinary PAH metabolites ranged from 0.81 to 0.85 for specific gravity-adjusted concentrations, and 0.76 to 0.86 for creatinine-adjusted concentrations. This was consistent overall and by age group.

Table 2. Properties of Differences in Ln-Transformed Pre- and Post-Activity Burden Levels for Specific Gravity-Adjusted Urinary PAHs, by All Participants

Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
1-Hydroxynaphthalene (µg/L)	160	-2.32	2.32	.436	.506	.633	<.0001	<.0001
1-Hydroxyphenanthrene (µg/L)	161	-1.91	3.02	.437	.473	.523	<.0001	<.0001
1-Hydroxypyrene (µg/L)	161	-1.86	2.61	.231	.268	.538	<.0001	<.0001
2 & 3-Hydroxyphenanthrene (µg/L)	161	-1.55	2.55	.375	.410	.464	<.0001	<.0001
2-Hydroxyfluorene (µg/L)	161	-2.04	2.20	.395	.439	.489	<.0001	<.0001
2-Hydroxynaphthalene (µg/L)	161	-2.45	3.13	.548	.607	.646	<.0001	<.0001
3-Hydroxyfluorene (µg/L)	161	-1.66	2.52	.417	.451	.519	<.0001	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table 3. Properties of Differences in Ln-Transformed Pre- and Post-Activity Burden Levels for Specific Gravity-Adjusted Urinary PAHs, by Field Type

Field Type	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Synthetic Turf	1-Hydroxynaphthalene (µg/L)	132	-2.32	1.91	.446	.497	.609	<.0001	<.0001
Synthetic Turf	1-Hydroxyphenanthrene (µg/L)	132	-.831	1.69	.437	.471	.428	<.0001	<.0001
Synthetic Turf	1-Hydroxypyrene (µg/L)	132	-1.86	1.45	.232	.260	.490	<.0001	<.0001
Synthetic Turf	2 & 3-Hydroxyphenanthrene (µg/L)	132	-1.55	1.54	.381	.414	.428	<.0001	<.0001
Synthetic Turf	2-Hydroxyfluorene (µg/L)	132	-2.04	1.81	.414	.442	.481	<.0001	<.0001
Synthetic Turf	2-Hydroxynaphthalene (µg/L)	132	-2.45	3.13	.558	.605	.621	<.0001	<.0001
Synthetic Turf	3-Hydroxyfluorene (µg/L)	132	-1.66	2.02	.421	.440	.484	<.0001	<.0001
Natural Grass	1-Hydroxynaphthalene (µg/L)	28	-1.02	2.32	.359	.545	.747	0.0006	<.0001
Natural Grass	1-Hydroxyphenanthrene (µg/L)	29	-1.91	3.02	.389	.482	.839	0.0044	<.0001
Natural Grass	1-Hydroxypyrene (µg/L)	29	-1.15	2.61	.166	.304	.727	0.0323	0.0131

Field Type	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Natural Grass	2 & 3-Hydroxyphenanthrene (µg/L)	29	-.433	2.55	.291	.390	.612	0.0019	0.0003
Natural Grass	2-Hydroxyfluorene (µg/L)	29	-.223	2.20	.311	.426	.533	0.0002	<.0001
Natural Grass	2-Hydroxynaphthalene (µg/L)	29	-.729	2.76	.432	.619	.762	0.0002	<.0001
Natural Grass	3-Hydroxyfluorene (µg/L)	29	-.661	2.52	.374	.502	.663	0.0003	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table 4. Properties of Differences in Ln-Transformed Pre- and Post-Activity Body Burden Levels for Creatinine-Adjusted Urinary PAHs, by All Participants

Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
1-Hydroxynaphthalene (µg/gCRE)	160	-2.11	2.35	-.011	.061	.577	0.1866	0.4666
1-Hydroxyphenanthrene (µg/gCRE)	161	-2.17	2.42	-.028	.029	.490	0.4560	0.9324
1-Hydroxypyrene (µg/gCRE)	161	-3.47	1.72	-.154	-.176	.619	0.0004	0.0002
2 & 3-Hydroxyphenanthrene (µg/gCRE)	161	-1.69	1.90	-.073	-.034	.477	0.3622	0.0866
2-Hydroxyfluorene (µg/gCRE)	161	-1.25	2.18	-.020	-.005	.458	0.8950	0.1987
2-Hydroxynaphthalene (µg/gCRE)	161	-1.43	2.75	.088	.163	.581	0.0005	0.0002
3-Hydroxyfluorene (µg/gCRE)	161	-1.37	2.24	-.019	.007	.482	0.8575	0.5660

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table 5. Properties of Differences in Ln-Transformed Pre- and Post-Activity Body Burden Levels for Creatinine-Adjusted Urinary PAHs, by Field Type

Field Type	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Synthetic Turf	1-Hydroxynaphthalene (µg/gCRE)	132	-2.11	2.35	-.012	.049	.585	0.3419	0.6207
Synthetic Turf	1-Hydroxyphenanthrene (µg/gCRE)	132	-1.30	2.42	-.034	.022	.429	0.5539	0.9550
Synthetic Turf	1-Hydroxypyrene (µg/gCRE)	132	-3.47	1.22	-.162	-.189	.591	0.0003	0.0002
Synthetic Turf	2 & 3-Hydroxyphenanthrene (µg/gCRE)	132	-1.69	1.90	-.066	-.035	.472	0.3993	0.1857
Synthetic Turf	2-Hydroxyfluorene (µg/gCRE)	132	-1.25	2.18	-.028	-.007	.482	0.8762	0.2427
Synthetic Turf	2-Hydroxynaphthalene (µg/gCRE)	132	-1.43	2.75	.095	.156	.592	0.0029	0.0009
Synthetic Turf	3-Hydroxyfluorene (µg/gCRE)	132	-1.37	2.24	-.008	-.009	.484	0.8308	0.4891
Natural Grass	1-Hydroxynaphthalene (µg/gCRE)	28	-1.29	1.37	.003	.117	.547	0.2673	0.5484
Natural Grass	1-Hydroxyphenanthrene (µg/gCRE)	29	-2.17	2.07	-.005	.059	.712	0.6578	0.8911
Natural Grass	1-Hydroxypyrene (µg/gCRE)	29	-1.42	1.72	-.024	-.119	.742	0.3956	0.4025
Natural Grass	2 & 3-Hydroxyphenanthrene (µg/gCRE)	29	-.802	1.66	-.096	-.033	.509	0.7320	0.2365

Field Type	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Natural Grass	2-Hydroxyfluorene (µg/gCRE)	29	-.518	1.31	.000	.003	.338	0.9588	0.7562
Natural Grass	2-Hydroxynaphthalene (µg/gCRE)	29	-.545	1.81	.011	.196	.535	0.0582	0.1413
Natural Grass	3-Hydroxyfluorene (µg/gCRE)	29	-.924	1.63	-.065	.079	.473	0.3764	0.9245

Note: PAH = Polycyclic Aromatic Hydrocarbon.

4.4. Univariate Regression Modelling

Univariate general linear model (GLM) regression parameter estimates for differences in pre- and post-activity concentrations by field type were examined using specific gravity-adjusted (Table 6) and creatinine-adjusted (Table 7) data among participants with non-missing covariate data (n=156). For all urinary PAHs, differences in pre- and post-activity concentrations could not be explained by field type. Overall, mean differences between pre- and post-activity concentration were statistically significant for specific gravity-adjusted results, but not statistically significant for creatinine adjusted-results. Regardless of adjustment method, however, these differences were not associated with field type in the univariate analyses.

Table 6. Univariate GLM models for Field Type, by Specific Gravity-Adjusted Urinary PAHs

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxynaphthalene (µg/L)	Difference	Intercept	0.60	<.001
1-Hydroxynaphthalene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.11	0.430
1-Hydroxyphenanthrene (µg/L)	Difference	Intercept	0.57	<.001
1-Hydroxyphenanthrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.10	0.356
1-Hydroxypyrene (µg/L)	Difference	Intercept	0.36	<.001
1-Hydroxypyrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.10	0.357
2 & 3-Hydroxyphenanthrene (µg/L)	Difference	Intercept	0.42	<.001
2 & 3-Hydroxyphenanthrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.01	0.941
2-Hydroxyfluorene (µg/L)	Difference	Intercept	0.45	<.001
2-Hydroxyfluorene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.00	0.996
2-Hydroxynaphthalene (µg/L)	Difference	Intercept	0.62	<.001
2-Hydroxynaphthalene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.00	0.971
3-Hydroxyfluorene (µg/L)	Difference	Intercept	0.54	<.001
3-Hydroxyfluorene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.10	0.357

Table 7. Univariate GLM models for Field Type, by Creatinine-Adjusted Urinary PAHs

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxynaphthalene (µg/gCRE)	Difference	Intercept	0.17	0.127
1-Hydroxynaphthalene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.12	0.324
1-Hydroxyphenanthrene (µg/gCRE)	Difference	Intercept	0.14	0.115
1-Hydroxyphenanthrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.12	0.235
1-Hydroxypyrene (µg/gCRE)	Difference	Intercept	-0.07	0.536
1-Hydroxypyrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.12	0.346
2 & 3-Hydroxyphenanthrene (µg/gCRE)	Difference	Intercept	-0.01	0.922
2 & 3-Hydroxyphenanthrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.03	0.790
2-Hydroxyfluorene (µg/gCRE)	Difference	Intercept	0.02	0.836
2-Hydroxyfluorene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.02	0.836
2-Hydroxynaphthalene (µg/gCRE)	Difference	Intercept	0.19	0.091
2-Hydroxynaphthalene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.02	0.842
3-Hydroxyfluorene (µg/gCRE)	Difference	Intercept	0.11	0.210
3-Hydroxyfluorene (µg/gCRE)	Difference	Field Type (Synthetic Turf	-0.12	0.236

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
		versus Natural Grass)		

Note: PAH = Polycyclic Aromatic Hydrocarbon.

4.5. Full Model Multivariable GLM Regression

The first step taken prior to conducting multivariable regression was to address concerns about the potential for collinearity and data separation. For this study, all natural grass fields were located outdoors and co-located with outdoor synthetic fields; thus the field environment variable (indoor vs. outdoor) was also a measure of field type (synthetic turf vs. natural grass). All indoor participants were recruited from the same field, and thus the same location (U.S. Census Region; South or West). All volleyball players were female and recruited from the same outdoor facility, and thus the same region. For these reasons, a new variable containing mutually exclusive categories was generated and tested against models with individual variables; models with individual variables performed best.

As an additional exploratory step, auto-selection algorithms were used to identify variables associated with pre-activity, post-activity, and differences of concentrations as the dependent variable in multivariable models for specific gravity-adjusted and creatinine-adjusted data (data not shown). Field type was not statistically significant in any of the models of pre-and post-activity differences in concentration. For both specific gravity- and creatinine-adjusted results, the best predictor of post-activity concentration was the study participant's pre-activity concentration; this was consistent with the earlier finding that pre- and post-activity concentrations were highly correlated.

Multivariable GLM regression parameter estimates for differences in pre- and post-activity concentrations by field type, adjusted for all other previously described variables are described in Table 8 (specific-gravity adjusted) and Table 9 (creatinine-adjusted). As with the univariate analysis, the difference in pre- and post- concentrations were not associated with field type.

Table 8. Multivariable GLM models for Field Type, by Specific Gravity-Adjusted Urinary PAHs

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxynaphthalene (µg/L)	Difference	Intercept	0.61	0.032
1-Hydroxynaphthalene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.03	0.867
1-Hydroxyphenanthrene (µg/L)	Difference	Intercept	0.53	0.014

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxyphenanthrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.23	0.108
1-Hydroxypyrene (µg/L)	Difference	Intercept	0.16	0.493
1-Hydroxypyrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.2	0.204
2 & 3-Hydroxyphenanthrene (µg/L)	Difference	Intercept	0.34	0.101
2 & 3-Hydroxyphenanthrene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.16	0.234
2-Hydroxyfluorene (µg/L)	Difference	Intercept	0.44	0.049
2-Hydroxyfluorene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.13	0.363
2-Hydroxynaphthalene (µg/L)	Difference	Intercept	0.51	0.082
2-Hydroxynaphthalene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	0.2	0.299
3-Hydroxyfluorene (µg/L)	Difference	Intercept	0.45	0.055
3-Hydroxyfluorene (µg/L)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.24	0.107

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table 9. Multivariable GLM models for Field Type, by Creatinine Gravity-Adjusted Urinary PAHs

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxynaphthalene (µg/gCRE)	Difference	Intercept	-0.10	0.696

Urinary PAH	Dependent Variable	Parameter	Estimate	P-Value of t Statistic
1-Hydroxynaphthalene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.01	0.939
1-Hydroxyphenanthrene (µg/gCRE)	Difference	Intercept	-0.17	0.386
1-Hydroxyphenanthrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.21	0.108
1-Hydroxypyrene (µg/gCRE)	Difference	Intercept	-0.54	0.046
1-Hydroxypyrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.18	0.311
2 & 3-Hydroxyphenanthrene (µg/gCRE)	Difference	Intercept	-0.37	0.080
2 & 3-Hydroxyphenanthrene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.14	0.298
2-Hydroxyfluorene (µg/gCRE)	Difference	Intercept	-0.27	0.198
2-Hydroxyfluorene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.11	0.401
2-Hydroxynaphthalene (µg/gCRE)	Difference	Intercept	-0.20	0.444
2-Hydroxynaphthalene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	0.21	0.206
3-Hydroxyfluorene (µg/gCRE)	Difference	Intercept	-0.26	0.230
3-Hydroxyfluorene (µg/gCRE)	Difference	Field Type (Synthetic Turf versus Natural Grass)	-0.23	0.105

Note: PAH = Polycyclic Aromatic Hydrocarbon.

4.6. Comparison to NHANES PAH Concentrations

Pre-activity PAH concentrations among study participants were compared to values in the general U.S. population using secondary analysis of publicly available NHANES data. To facilitate these comparisons, PAH concentrations were adjusted for specific gravity or creatinine concentration. Specific gravity was only collected in the 2007–2008 NHANES cycle, whereas creatine was collected in all NHANES cycles including the most recent cycle available (2015–2016) at the time of data analysis. 2-3 PHE was not available in 2007–2008 NHANES.

Comparison of this study's specific gravity-adjusted results to those of the 2007–2008 NHANES cycle may not be entirely comparable due to the observed trend of decreasing concentrations over time for most PAHs. In NHANES, PAH concentrations markedly decreased over time, except for 2-NAP where creatinine-adjusted geometric mean concentrations were 3.88 (5.45-4.26) µg/gCRE in 2007-2008 and 5.35 (4.86-5.90) µg/gCRE in 2015-2016 (Supplemental Table S3). Additionally, pre-activity PAH geometric mean concentrations of study participants were lower than corresponding NHANES values except for adjusted 2-NAP concentrations (Figure 3). Additional stratifications by age category and NHANES 2015-2016 PAH comparison values are displayed in Table 10. This observation was consistent by age category except youth, where concentrations were 4.97 (3.49-7.09) µg/gCRE among youth in the study compared to 5.22 (4.24-6.44) µg/gCRE among youth in NHANES 2015-2016, indicating no significant difference.

Figure 3. Comparison of Urinary PAH Concentrations Across Biomonitoring Study Participants and NHANES 2007–2008 and 2016–2017 Participants Overall, by Adjustment Method

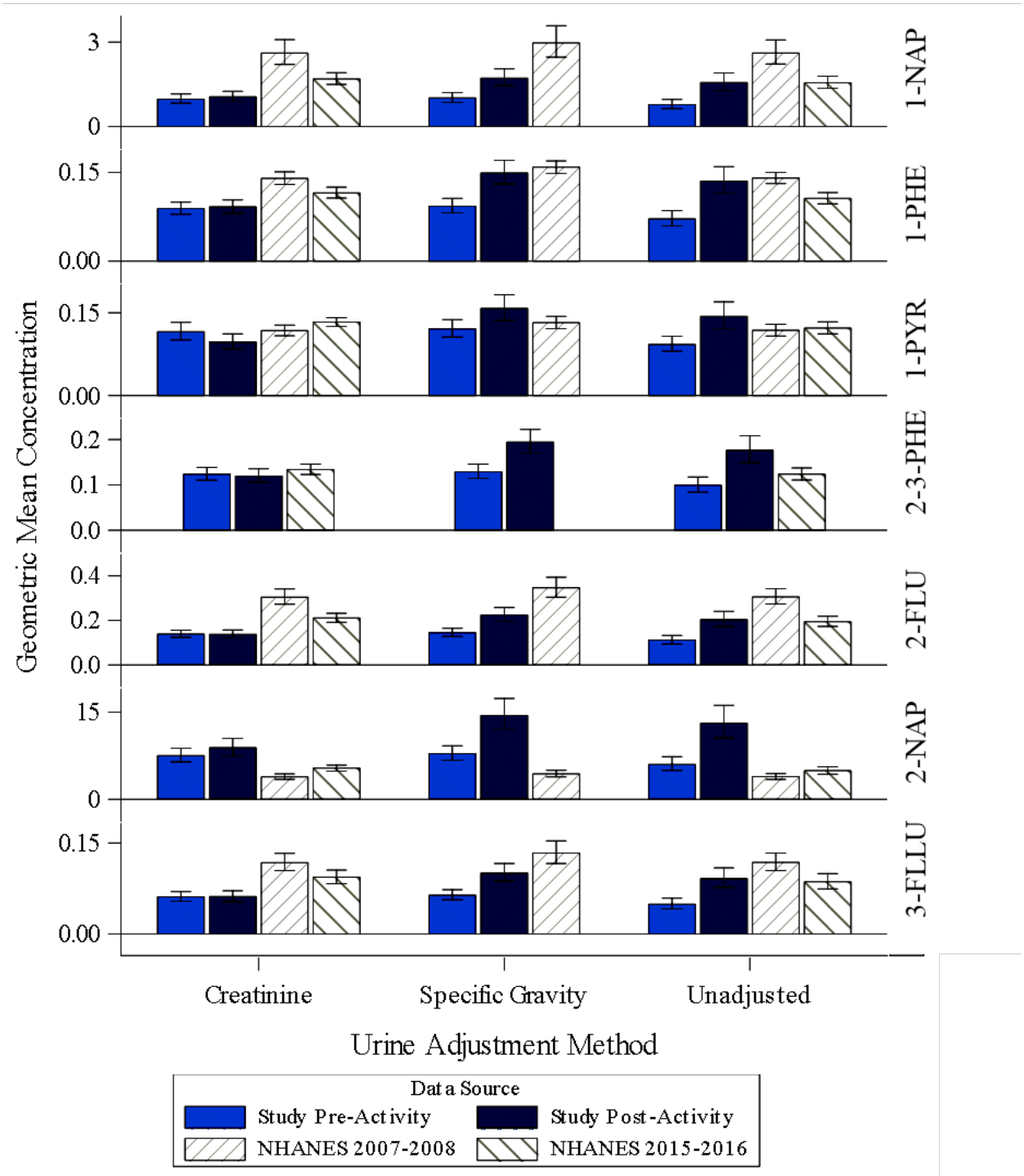


Table 10. Comparison of Geometric Means (95% CI) by Age and Urinary PAH, Pre-Activity Biomonitoring Study Concentrations versus NHANES 2015-2016

Age Category	Urinary PAH	Study n	NHANES n	Study Participants Pre-Activity Geometric Mean, Unadjusted (µg/L)	NHANES 2015-2016 Geometric Mean, Unadjusted (µg/L)	Study Participants Pre-Activity Geometric Mean, Creatinine-Adjusted (µg/gCRE)	NHANES 2015-2016 Geometric Mean, Creatinine-Adjusted (µg/gCRE)
Children	1-Hydroxynaphthalene	24	111	.997 (.557-1.79)	.817 (.625-1.07)	1.47 (.842-2.57)	1.17 (.908-1.50)
Children	1-Hydroxyphenanthrene	24	112	.052 (.029-.090)	.083 (.068-.100)	.076 (.049-.119)	.119 (.097-.146)
Children	1-Hydroxypyrene	24	112	.077 (.048-.124)	.134 (.104-.172)	.114 (.071-.183)	.193 (.145-.257)
Children	2 & 3-Hydroxyphenanthrene	24	112	.082 (.046-.144)	.098 (.078-.122)	.121 (.075-.194)	.140 (.112-.176)
Children	2-Hydroxyfluorene	24	112	.096 (.053-.173)	.132 (.101-.171)	.142 (.089-.225)	.189 (.140-.256)
Children	2-Hydroxynaphthalene	24	112	7.52 (4.31-13.1)	3.31 (2.49-4.40)	11.1 (6.41-19.2)	4.76 (3.84-5.89)
Children	3-Hydroxyfluorene	24	112	.055 (.030-.099)	.063 (.048-.082)	.081 (.049-.133)	.090 (.067-.122)
Youth	1-Hydroxynaphthalene	27	175	.709 (.499-1.01)	.864 (.695-1.07)	.728 (.522-1.02)	1.03 (.847-1.25)
Youth	1-Hydroxyphenanthrene	27	176	.069 (.054-.089)	.085 (.074-.097)	.071 (.059-.087)	.101 (.089-.115)
Youth	1-Hydroxypyrene	27	176	.084 (.066-.108)	.124 (.111-.140)	.086 (.072-.104)	.148 (.132-.166)

Age Category	Urinary PAH	Study n	NHANES n	Study Participants Pre-Activity Geometric Mean, Unadjusted (µg/L)	NHANES 2015-2016 Geometric Mean, Unadjusted (µg/L)	Study Participants Pre-Activity Geometric Mean, Creatinine-Adjusted (µg/gCRE)	NHANES 2015-2016 Geometric Mean, Creatinine-Adjusted (µg/gCRE)
Youth	2 & 3-Hydroxyphenanthrene	27	175	.094 (.073-.121)	.106 (.093-.120)	.096 (.081-.114)	.126 (.113-.139)
Youth	2-Hydroxyfluorene	27	176	.100 (.075-.135)	.129 (.112-.149)	.103 (.088-.121)	.154 (.135-.174)
Youth	2-Hydroxynaphthalene	27	176	4.84 (3.14-7.45)	4.39 (3.50-5.51)	4.97 (3.49-7.09)	5.22 (4.24-6.44)
Youth	3-Hydroxyfluorene	27	176	.047 (.034-.065)	.061 (.050-.073)	.049 (.039-.060)	.072 (.060-.086)
Adolescents	1-Hydroxynaphthalene	51	267	.603 (.450-.808)	.860 (.745-.994)	.945 (.724-1.23)	.795 (.697-.907)
Adolescents	1-Hydroxyphenanthrene	51	272	.049 (.035-.069)	.094 (.080-.110)	.077 (.066-.091)	.086 (.077-.097)
Adolescents	1-Hydroxypyrene	51	272	.078 (.065-.094)	.124 (.107-.144)	.123 (.097-.156)	.115 (.103-.128)
Adolescents	2 & 3-Hydroxyphenanthrene	51	272	.079 (.058-.107)	.114 (.098-.133)	.123 (.103-.148)	.105 (.095-.115)
Adolescents	2-Hydroxyfluorene	51	272	.090 (.066-.123)	.151 (.130-.175)	.142 (.118-.170)	.139 (.126-.154)
Adolescents	2-Hydroxynaphthalene	51	269	4.92 (3.36-7.19)	5.11 (4.15-6.29)	7.71 (5.82-10.2)	4.72 (3.91-5.70)
Adolescents	3-Hydroxyfluorene	58	272	.039 (.029-.051)	.067 (.057-.080)	.060 (.050-.074)	.062 (.055-.070)
Adults	1-Hydroxynaphthalene	59	1767	.929 (.611-1.41)	1.75 (1.50-2.04)	.970 (.710-1.33)	1.90 (1.65-2.19)

Age Category	Urinary PAH	Study n	NHANES n	Study Participants Pre-Activity Geometric Mean, Unadjusted (µg/L)	NHANES 2015-2016 Geometric Mean, Unadjusted (µg/L)	Study Participants Pre-Activity Geometric Mean, Creatinine-Adjusted (µg/gCRE)	NHANES 2015-2016 Geometric Mean, Creatinine-Adjusted (µg/gCRE)
Adults	1-Hydroxyphenanthrene	59	1868	.113 (.085-.150)	.109 (.099-.120)	.118 (.097-.143)	.119 (.109-.130)
Adults	1-Hydroxypyrene	59	1866	.121 (.090-.163)	.122 (.111-.134)	.127 (.099-.162)	.133 (.124-.142)
Adults	2 & 3-Hydroxyphenanthrene	59	1867	.135 (.102-.178)	.126 (.113-.141)	.141 (.117-.169)	.137 (.125-.151)
Adults	2-Hydroxyfluorene	59	1867	.146 (.109-.195)	.205 (.180-.233)	.152 (.126-.184)	.224 (.201-.249)
Adults	2-Hydroxynaphthalene	59	1819	7.31 (5.34-10.0)	4.99 (4.36-5.71)	7.64 (6.12-9.54)	5.45 (4.92-6.03)
Adults	3-Hydroxyfluorene	59	1863	.059 (.043-.081)	.090 (.077-.106)	.062 (.050-.077)	.099 (.086-.113)
All	1-Hydroxynaphthalene	161	2320	.782 (.638-.959)	1.56 (1.36-1.79)	.976 (.822-1.16)	1.69 (1.50-1.91)
All	1-Hydroxyphenanthrene	161	2428	.071 (.060-.085)	.106 (.097-.116)	.089 (.079-.100)	.115 (.106-.125)
All	1-Hydroxypyrene	161	2426	.093 (.080-.108)	.123 (.112-.134)	.116 (.101-.133)	.133 (.125-.141)
All	2 & 3-Hydroxyphenanthrene	161	2426	.099 (.084-.117)	.123 (.111-.137)	.124 (.110-.139)	.134 (.123-.146)
All	2-Hydroxyfluorene	161	2427	.111 (.093-.131)	.194 (.172-.218)	.138 (.123-.155)	.211 (.191-.232)
All	2-Hydroxynaphthalene	161	2376	6.04 (4.97-7.35)	4.92 (4.33-5.59)	7.54 (6.45-8.80)	5.35 (4.86-5.90)

Age Category	Urinary PAH	Study n	NHANES n	Study Participants Pre-Activity Geometric Mean, Unadjusted (µg/L)	NHANES 2015-2016 Geometric Mean, Unadjusted (µg/L)	Study Participants Pre-Activity Geometric Mean, Creatinine-Adjusted (µg/gCRE)	NHANES 2015-2016 Geometric Mean, Creatinine-Adjusted (µg/gCRE)
All	3-Hydroxyfluorene	161	2423	.049 (.041-.059)	.086 (.074-.099)	.061 (.054-.070)	.093 (.083-.105)

Note: Age Category includes children (ages 7-9 years), youth (ages 10-12 years), adolescents (13-17 years), and adults (18 and older).

PAH = Polycyclic Aromatic Hydrocarbon.

"NHANES n" corresponds to number of NHANES Participants with unadjusted urinary concentrations.

5. Discussion

Synthetic turf with recycled tire crumb rubber infill contains many substances, including PAHs [Armada 2021; Gomes 2021; Marsili 2014; Murphy 2022; US EPA and ATSDR 2019]. PAHs are a large class of widespread chemicals generally produced during the incomplete burning of organic substances, such as coal, oil and gas, garbage tobacco, and charbroiled meat [ATSDR 1995]. Routes of PAH exposure include ingestion, inhalation, and dermal contact both in occupational and non-occupational settings [ATSDR 1995]. Once inside the body, PAHs are metabolized by the liver into monohydroxylated metabolites and excreted rapidly in urine and feces with half-life in the human body of less than 30 hours (Hudson-Hanley). Therefore, urinary PAH metabolites represent useful non-invasive biomarkers for assessing recent PAH uptake from all exposure routes [Ganzleben et al. 2017; Gunnier 2006; Srogi 2007].

Supplementing the pilot-scale human exposure measurements collected under the FRAP [EPA and ATSDR 2024], this biomonitoring study examined urinary PAHs to assess the exposure potential for individuals who performed activities on turf fields with recycled tire crumb rubber infill. The same 7 urinary metabolites assessed in both studies are regularly monitored in the general U.S. population [CDC 2020], and the four parent PAHs (anthracene, fluorene, phenanthrene, pyrene) are among EPA's 16 Priority Pollutants [US EPA 2014]. This supplemental study distinguishes itself in several ways. First, it featured a larger convenience sample of study participants compared to the pilot study conducted under the FRAP; urine samples from 161 participants with age ranging from 7–77 years were analyzed, compared to urine samples from 14 participants in the pilot study with an age range of 11–21 years. This larger sample size allowed for the examination of potential differences by select demographic characteristics and behaviors. Additionally, this supplemental study also included a group of participants whose activities took place on natural grass fields. Although the sample size of natural grass participants was relatively small, this comparison group provided an important examination of pre- and post-activity differences by field type.

In this study, pre- and post-activity urinary PAH concentration differences were not associated with field type, regardless of urine adjustment method or statistical method applied. Consistent with the pilot-scale study [EPA and CDC/ATSDR 2024], most participants demonstrated an increase in specific gravity-adjusted PAH concentrations after performing field activities. However, the increase occurred irrespective of field type, among both natural grass participants as well as synthetic turf with recycled tire crumb rubber participants. Also consistent with the pilot-scale study, our findings indicated fewer significant pre- and post-activity differences when utilizing creatinine-adjusted results, underscoring the influence of urine-dilution methods. These differences may potentially be attributed to how the degree of correlation of specific gravity and creatinine with the true hydration status of an individual may vary due to endogenous and exogenous factors [Kuiper 2021]. In the context of PAHs, for example, children are generally a vulnerable group with potential for higher exposure to PAHs compared to adults due in part to their less efficient detoxification system, lower body weight, and higher inhalation rates [Oliveira et al. 2019]. Though age was not associated with post-activity PAH concentrations, it was associated with some pre-activity concentrations. Additionally, potential

sex-specific effects or differential responses to PAH exposure continue to be areas of exploration [Farzan 2016; Xing 2023; Yang 2021], and differences in BMI or body composition may contribute to differential PAH metabolism [Stallings 2018; Wang 2022]. However, in this study, neither sex nor BMI were statistically associated with pre- and post-activity differences in PAH concentrations in univariate or multivariate regression models.

In the absence of other measures of urinary dilution such as osmolality and urine flow rate [Middleton 2016], the comprehensive approach taken in this present study facilitated examination of other datasets, including NHANES 2007-2008 and 2015-2016. NHANES 2007–2008 data, which collected both specific gravity and creatinine, also revealed large differences between specific gravity- and creatinine-adjusted PAH concentrations. Our comparison of creatinine-adjusted PAH concentrations available in both NHANES cycles indicated that urinary PAH concentrations markedly decreased over time, except for 2-NAP. Differences in creatinine-adjusted urinary 2-NAP were detected in pre- and post-activity samples from this biomonitoring study as well as the previous pilot-scale study [EPA and CDC/ATSDR 2024]. In both the studies, the creatinine adjusted 2-NAP concentration (geometric mean) was higher pre- and post-activity when compared to the general US population based on available NHANES data. In our biomonitoring study, this observation held for all age groups except for youth (participants aged 10-12 years). Previous urinary biomarker investigations utilizing NHANES have also indicated naphthalene, the parent PAH of 2-NAP with widespread presence in ambient and indoor air, as the dominant PAH in the U.S. population [Li 2008]. Data from the pilot-study, however, indicated low levels of naphthalene in tire crumb rubber infill, field air, field dust, field wipe, and drag sled samples [U.S. EPA and CDC/ATSDR 2024].

Overall, measurable amounts of urinary hydroxylated metabolites of PAHs were found in users of both synthetic turf fields and natural grass fields. However, finding a measurable amount of urinary hydroxylated metabolites of PAHs does not indicate they cause an adverse health effect [ATSDR PAH Clinical Assessment 2023]. Previous biomonitoring studies have demonstrated that nearly 100% of the general U.S. population have detectable levels of urinary PAH metabolites [Grainger 2006]. Exposure to PAHs in the general population occurs mostly through inhalation of polluted air and cigarette smoke, and ingestion of food containing PAHs [ATSDR 1995]. Others have demonstrated that an exposure-free period of 24–48 hours is often required for PAH biomarkers to reach pre-exposure baseline [Zheng 2012; Brzeznicki 1997; Chien 2010; Viau 1995]. More recently, data from Choi et al. 2023 demonstrated that the fractional urinary excretion of urinary PAHs ranged from 0.07 % to 11.3% and that most were excreted within 24 hours after exposure, though the obtained fractional urinary excretion values only reflected oral intake [Choi 2023]. Accordingly, this study excluded participants who were smokers, excluded natural grass participants who had played on synthetic turf within the past 24 hours, and accounted for the ingestion of grilled foods within the past 24 hours. Outdoor synthetic turf fields were also co-located with natural grass fields to help account for potential PAHs in ambient air. Nevertheless, this study could not fully control for potential exposures to chemicals from other sources and environmental media that could occur off the field. As described previously [EPA and CDC/ATSDR 2019], while there is concern about chemical exposures resulting from the use of recycled tire in synthetic turf fields, it is important to consider that some PAHs and other chemicals are also found in surface soil and may be present

in other types of fields, including natural grass fields. For example, metals (including lead) and PAHs (including benzo[a]pyrene) of potential concern at synthetic turf fields with tire crumb rubber infill are also often found in surface soil present at natural grass playing fields [EPA and CDC/ATSDR 2019].

5.1. Limitations and Recommendations

Like the pilot-scale human exposure measurement study conducted as part of the FRAP, this current study's examination of participant activity on synthetic turf fields was limited in design to assess exposure to recycled tire crumb rubber (infill alternatives were not included) and did not investigate exposure to synthetic turf field materials or components such as synthetic grass blades and backing material. Also consistent with the constraints of the research activities in the FRAP, there was no way to determine the specific tire sources for tire crumb rubber at the participating fields. While the facility and indoor or outdoor field environment was considered, this study did not examine the potential of dust transfer or effects of water-runoff between the co-located natural grass fields and synthetic turf fields. Future research activities may also choose to expand the scope of exposure measurement studies to incorporate synthetic turf fields that use alternative types of infill as another means of comparison further allowing communities to make more informed choices.

Though some statistical models indicated a difference between pre- and post-activity PAH concentrations, these differences were dependent on choices such as ln-transformation, urine dilution adjustment method, and model specification. In all cases, observed pre- and post-activity differences were not explained by field type (exposure to synthetic turf). Observed differences could be due to unmeasured variables, such as changes in hydration level through perspiration. However, it is unknown whether a participant drank fluids between the two specimen collections, nor how much fluid they lost due to intensity of activity. For the urine collection, participants were directed to not touch the inside of the urine specimen container to prevent or minimize chances of sample contamination. However, the study recruitment team cannot confirm that all participants followed the specified procedures.

For future related studies, additional information obtained at the time of specimen collection might help provide insights on factors affecting PAH concentrations. For example, asking about fluid consumption during the activity (between pre- and post-specimen collections), and taking pre- and post-body-weight measurements (to measure potential fluid loss or gain) could help tease out factors driving pre- and post-activity concentration differences. As previous studies have indicated the potential effect of vaporization and weathering on organic chemical level concentrations [Marsili et al. 2014; EPA and CDC/ATSDR 2019], additional efforts could examine or account for the role of fluctuations in weather or field temperatures over time. Moreover, although information including the time recorded between samplings and general patterns of activity were captured in this study, these data may not directly correspond to the level of activity each participant exerted on the field; additional measures could be included to account for behaviors specific to the day-of specimen collection.

6. Conclusions

Although this study's findings cannot be generalized to the universe of synthetic field users with exposures to tire crumb rubber infill in the United States, this study provides valuable information to better understand and identify the potential chemical exposures. Notably, pre- and post-activity differences in urinary PAH concentrations were not associated with field type (i.e., synthetic turf with recycled tire crumb rubber infill compared to natural grass). This was consistent regardless of the urine dilution adjustment method applied or the statistical regression technique employed. When examining pre- and post-activity urinary PAH concentration differences overall, results varied by statistical method; methods that mitigated the effect of extreme observations were more likely to yield results that were not statistically significant, indicating that outliers could be driving some of the results. After exploring the role of different variables associated with pre- and post-activity concentrations, the best predictor of post-activity urinary PAH concentration was pre-activity concentration. Except for 2-NAP, pre-activity PAH concentrations were lower than those in the general U.S. population using 2015–2016 NHANES comparison data. It is important to note that this report is not a risk assessment. However, this study's findings supplement the pilot-scale human exposure measurements collected under the FRAP and contribute to the overarching portfolio of research activities needed to understand the potential for human exposure to chemicals found in recycled tire crumb rubber used on synthetic turf fields.

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Acknowledgements

We sincerely thank those who participated in the study. We appreciate the CDC/ATSDR staff who facilitated the recruitment and data collection efforts, including Emily Adler, Tyra Barrett, Carol Cusack, Michelle Dittrich, John Marr, Miranda Mitchell, Christopher Mugford, Chris Poulet, Nykiconia Preacely, Tara Serio, and Timothy Turner. We also thank the CDC/NCEH/DLS laboratory staff who performed the high-quality laboratory analyses, including Britnee Bailey, Julianne Botelho, Antonia Calafat, Joaudimir (Joy) Castro Georgi, Alfonsina (Sina) De Leon Salazar, Christopher Hamilton, Rebecca Hunt, Erin (Nikki) Pittman, Debra Trinidad, Denise Tevis, Hubert Vesper, Neelam Zahoor, Enada Archibold, Yuesong Wang, and Cynthia D. Ward.

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Appendices

Appendix A: Supplemental Figures

Figure S1-1. Difference in post- and pre-activity concentration for 1-hydroxyphenanthrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-2. Difference in post- and pre-activity concentration for 1-hydroxypyrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-3. Difference in post- and pre-activity concentration for 2 & 3-hydroxyphenanthrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-4. Difference in post- and pre-activity concentration for 2-hydroxyfluorene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-5. Difference in post- and pre-activity concentration for 2-hydroxynapthalene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-6. Difference in post- and pre-activity concentration for 3-hydroxyfluorene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

Figure S1-1. Difference in post- and pre-activity concentration for 1-hydroxyphenanthrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

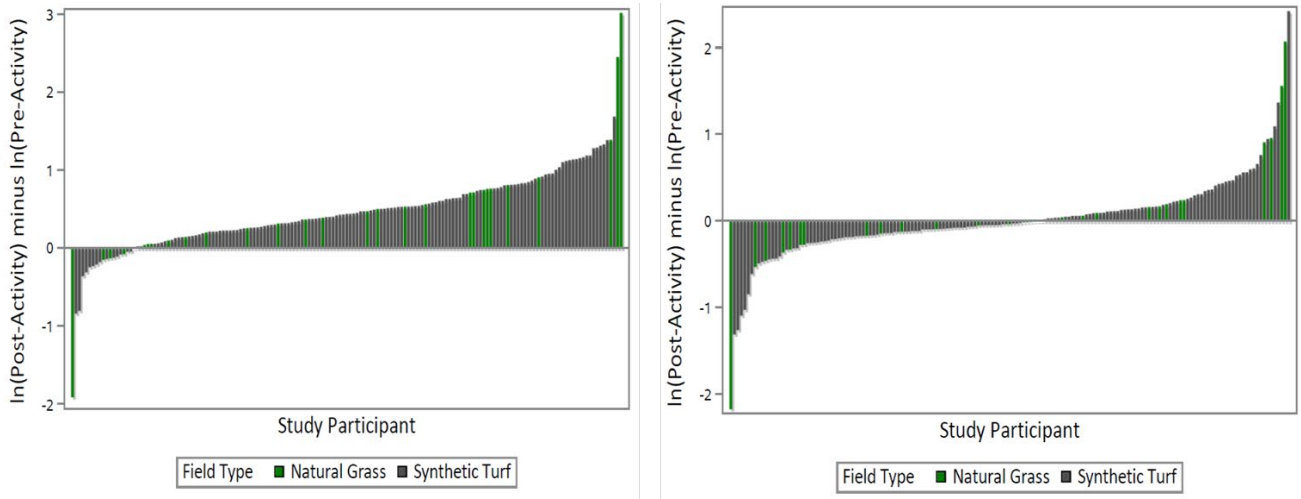


Figure S1-2. Difference in post- and pre-activity concentration for 1-hydroxypyrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

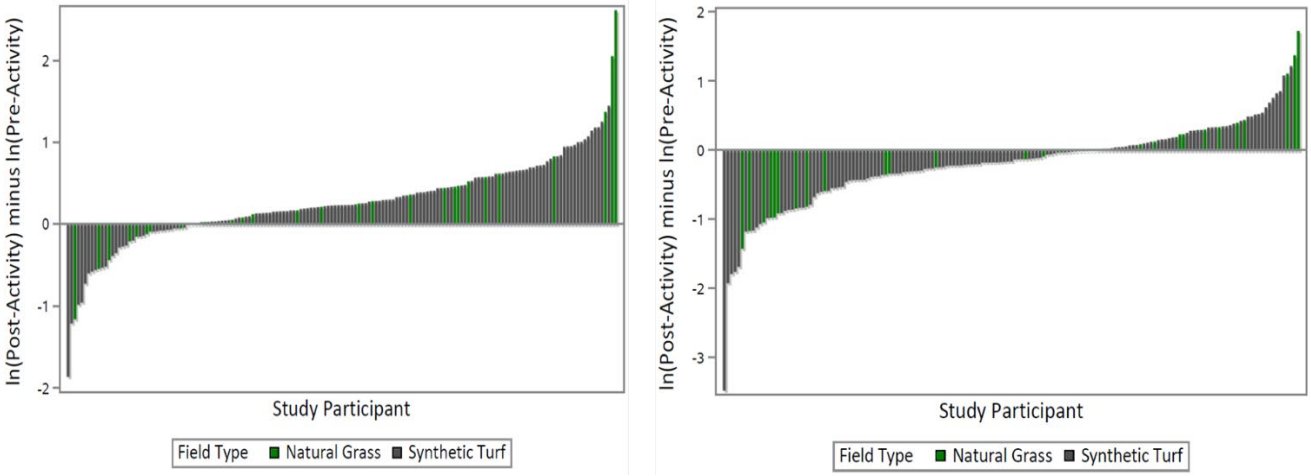


Figure S1-3. Difference in post- and pre-activity concentration for 2 & 3-hydroxyphenanthrene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

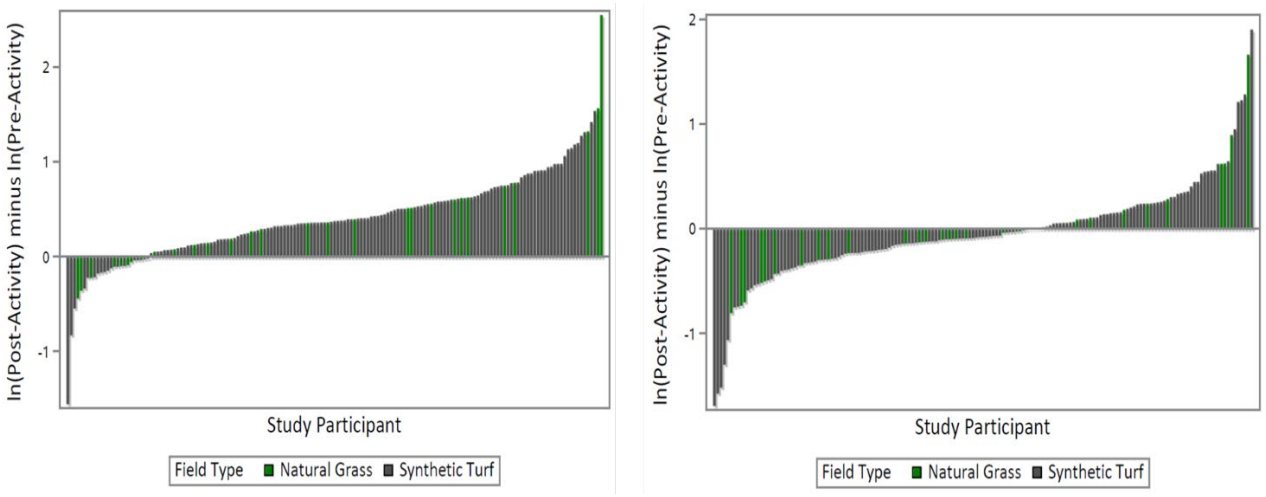


Figure S1-4. Difference in post- and pre-activity concentration for 2-hydroxyfluorene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

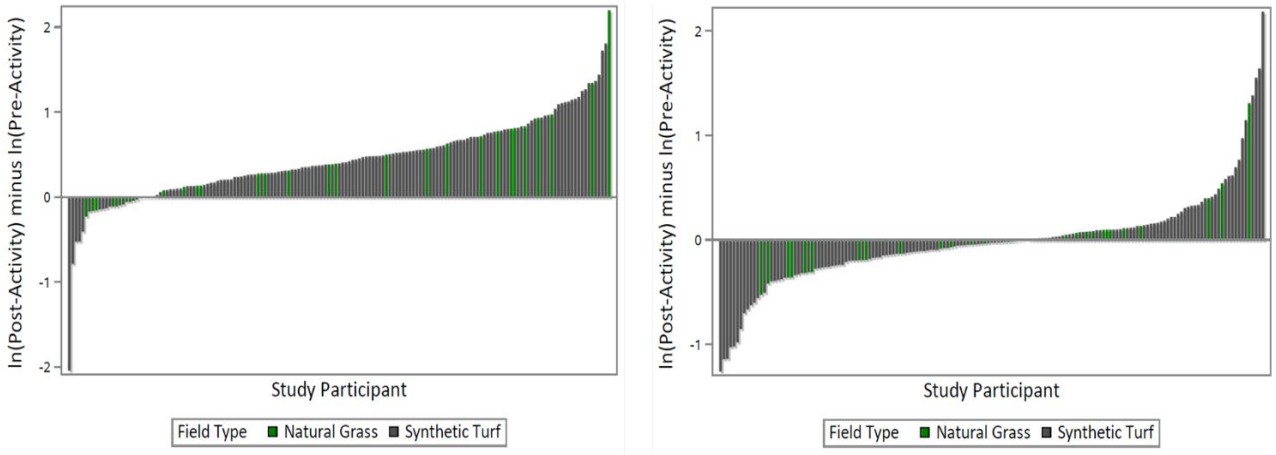


Figure S1-5. Difference in post- and pre-activity concentration for 2-hydroxynapthalene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).

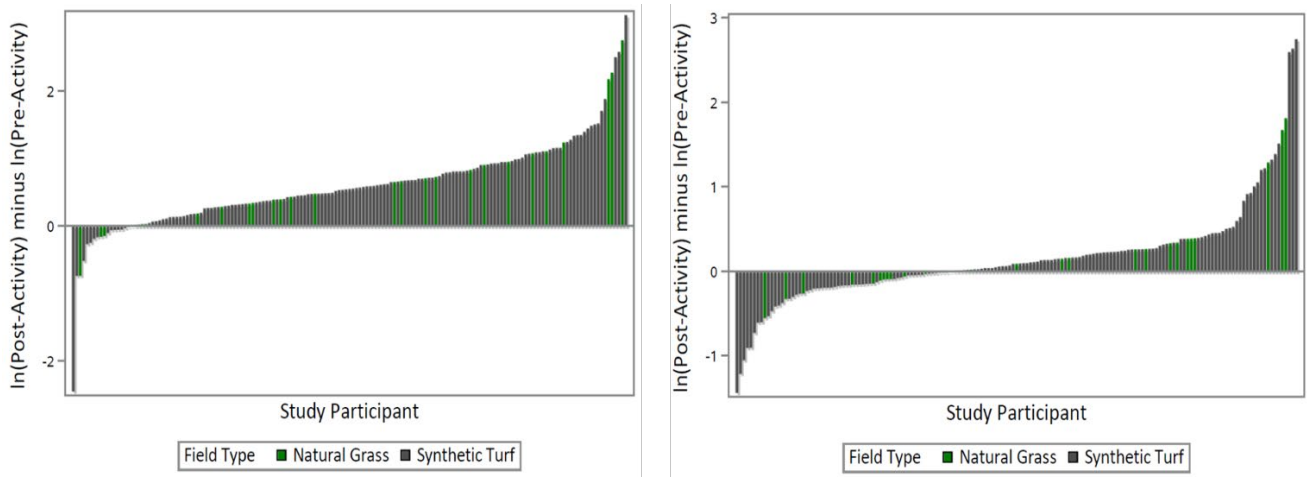
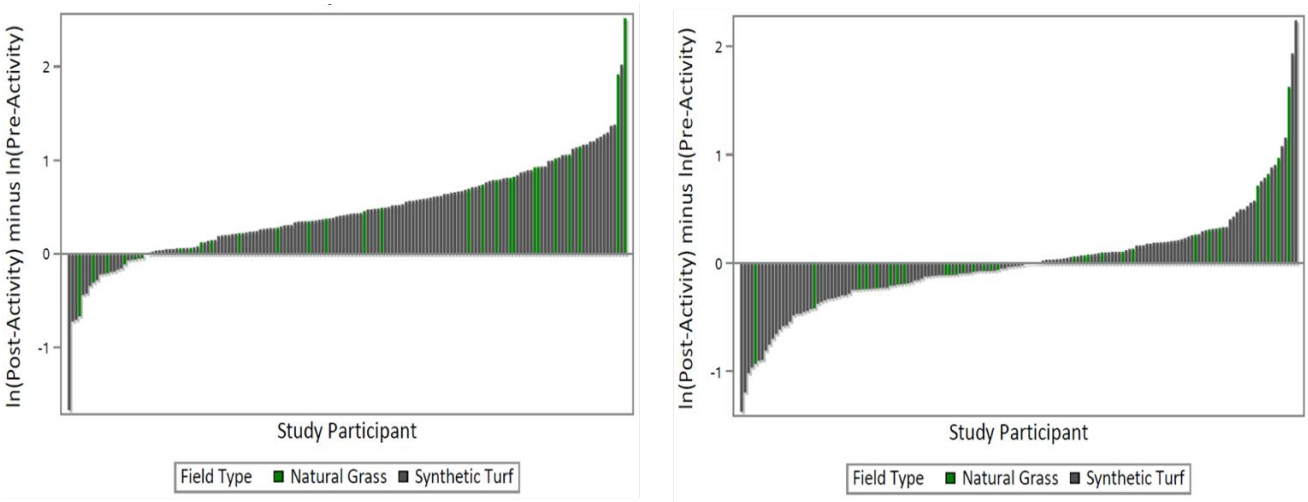


Figure S1-6. Difference in post- and pre-activity concentration for 3-hydroxyfluorene measurements, ln-transformed, by participant and field type. Specific gravity-adjusted (left) and creatinine-adjusted (right).



Appendix B: Supplemental Tables

Table S1-1. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by field environment

Table S1-2. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by age category

Table S1-3. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by sex

Table S1-4. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by race

Table S1-5. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by field location

Table S1-6. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by facility

Table S1-7. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by BMI category

Table S1-8. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by activity

Table S2-1. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by field environment

Table S2-2. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by age category

Table S2-3. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by sex

Table S2-4. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by race

Table S2-5. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by field location

Table S2-6. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by facility

Table S2-7. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by BMI category

Table S2-8. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by activity

Table S3. Comparison of creatinine-adjusted urinary PAH concentrations, NHANES 2015—2016 and 2007—2008

Table S1-1. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by field environment

Field Environment	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Outdoor	1-Hydroxynaphthalene (µg/L)	119	-1.09	2.32	.434	.514	.638	<.0001	<.0001
Outdoor	1-Hydroxyphenanthrene (µg/L)	120	-1.91	3.02	.433	.476	.545	<.0001	<.0001
Outdoor	1-Hydroxypyrene (µg/L)	120	-1.20	2.61	.230	.284	.534	<.0001	<.0001
Outdoor	2 & 3-Hydroxyphenanthrene (µg/L)	120	-.822	2.55	.359	.414	.466	<.0001	<.0001
Outdoor	2-Hydroxyfluorene (µg/L)	120	-.780	2.20	.384	.440	.464	<.0001	<.0001
Outdoor	2-Hydroxynaphthalene (µg/L)	120	-.732	3.13	.539	.637	.646	<.0001	<.0001
Outdoor	3-Hydroxyfluorene (µg/L)	120	-.716	2.52	.390	.441	.511	<.0001	<.0001
Indoor	1-Hydroxynaphthalene (µg/L)	41	-2.32	1.50	.453	.482	.624	<.0001	<.0001
Indoor	1-Hydroxyphenanthrene (µg/L)	41	-.796	1.69	.437	.464	.459	<.0001	<.0001
Indoor	1-Hydroxypyrene (µg/L)	41	-1.86	1.14	.233	.221	.551	0.0139	0.0004

Field Environment	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Indoor	2 & 3-Hydroxyphenanthrene (µg/L)	41	-1.55	1.42	.395	.396	.465	<.0001	<.0001
Indoor	2-Hydroxyfluorene (µg/L)	41	-2.04	1.72	.452	.437	.563	<.0001	<.0001
Indoor	2-Hydroxynaphthalene (µg/L)	41	-2.45	1.51	.586	.522	.646	<.0001	<.0001
Indoor	3-Hydroxyfluorene (µg/L)	41	-1.66	2.02	.475	.481	.547	<.0001	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S1-2. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by age category

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Adolescent	1-Hydroxynaphthalene (µg/L)	51	-.385	1.91	.510	.671	.549	<.0001	<.0001
Adolescent	1-Hydroxyphenanthrene (µg/L)	51	-.171	2.45	.539	.647	.491	<.0001	<.0001
Adolescent	1-Hydroxypyrene (µg/L)	51	-1.86	2.61	.219	.270	.670	0.0059	0.0011
Adolescent	2 & 3-Hydroxyphenanthrene (µg/L)	51	-.160	2.55	.424	.535	.475	<.0001	<.0001
Adolescent	2-Hydroxyfluorene (µg/L)	51	-.518	2.20	.476	.535	.488	<.0001	<.0001
Adolescent	2-Hydroxynaphthalene (µg/L)	51	-.239	2.58	.700	.793	.632	<.0001	<.0001
Adolescent	3-Hydroxyfluorene (µg/L)	51	-.698	2.52	.519	.547	.531	<.0001	<.0001
Adult	1-Hydroxynaphthalene (µg/L)	58	-2.32	2.32	.378	.419	.713	<.0001	<.0001
Adult	1-Hydroxyphenanthrene (µg/L)	59	-1.91	3.02	.344	.319	.601	0.0001	<.0001
Adult	1-Hydroxypyrene (µg/L)	59	-1.20	2.05	.207	.187	.519	0.0074	0.0005
Adult	2 & 3-Hydroxyphenanthrene (µg/L)	59	-1.55	1.57	.306	.272	.454	<.0001	<.0001

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Adult	2-Hydroxyfluorene (µg/L)	59	-2.04	1.72	.351	.335	.523	<.0001	<.0001
Adult	2-Hydroxynaphthalene (µg/L)	59	-2.45	3.13	.435	.517	.792	<.0001	<.0001
Adult	3-Hydroxyfluorene (µg/L)	59	-1.66	2.02	.374	.376	.568	<.0001	<.0001
Child	1-Hydroxynaphthalene (µg/L)	24	-1.09	1.35	.358	.271	.583	0.0322	0.0338
Child	1-Hydroxyphenanthrene (µg/L)	24	-.223	1.39	.460	.474	.397	<.0001	<.0001
Child	1-Hydroxypyrene (µg/L)	24	-.720	1.45	.299	.372	.450	0.0005	0.0002
Child	2 & 3-Hydroxyphenanthrene (µg/L)	24	-.539	1.54	.398	.438	.472	0.0001	<.0001
Child	2-Hydroxyfluorene (µg/L)	24	-.517	1.81	.355	.399	.466	0.0004	<.0001
Child	2-Hydroxynaphthalene (µg/L)	24	-.044	1.15	.525	.500	.306	<.0001	<.0001
Child	3-Hydroxyfluorene (µg/L)	24	-.433	1.16	.287	.346	.421	0.0005	0.0002
Youth	1-Hydroxynaphthalene (µg/L)	27	-.596	1.48	.636	.587	.569	<.0001	<.0001
Youth	1-Hydroxyphenanthrene (µg/L)	27	-.236	1.31	.401	.479	.400	<.0001	<.0001
Youth	1-Hydroxypyrene (µg/L)	27	-.144	1.00	.284	.348	.324	<.0001	<.0001

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Youth	2 & 3-Hydroxyphenanthrene (µg/L)	27	-.330	1.20	.382	.449	.398	<.0001	<.0001
Youth	2-Hydroxyfluorene (µg/L)	27	-.400	1.44	.511	.523	.401	<.0001	<.0001
Youth	2-Hydroxynaphthalene (µg/L)	27	-.260	1.52	.484	.549	.466	<.0001	<.0001
Youth	3-Hydroxyfluorene (µg/L)	27	-.214	1.38	.498	.527	.439	<.0001	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S1-3. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by sex

Sex	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Male	1-Hydroxynaphthalene (µg/L)	117	-2.32	2.32	.394	.479	.605	<.0001	<.0001
Male	1-Hydroxyphenanthrene (µg/L)	118	-.796	3.02	.437	.509	.503	<.0001	<.0001
Male	1-Hydroxypyrene (µg/L)	118	-1.86	2.61	.228	.288	.546	<.0001	<.0001
Male	2 & 3-Hydroxyphenanthrene (µg/L)	118	-1.55	2.55	.376	.422	.470	<.0001	<.0001
Male	2-Hydroxyfluorene (µg/L)	118	-2.04	2.20	.394	.446	.502	<.0001	<.0001
Male	2-Hydroxynaphthalene (µg/L)	118	-2.45	3.13	.525	.569	.608	<.0001	<.0001
Male	3-Hydroxyfluorene (µg/L)	118	-1.66	2.52	.408	.451	.514	<.0001	<.0001
Female	1-Hydroxynaphthalene (µg/L)	43	-1.08	1.97	.648	.578	.706	<.0001	<.0001
Female	1-Hydroxyphenanthrene (µg/L)	43	-1.91	1.38	.401	.374	.568	<.0001	<.0001
Female	1-Hydroxypyrene (µg/L)	43	-1.20	1.37	.256	.212	.517	0.0103	0.0016
Female	2 & 3-Hydroxyphenanthrene (µg/L)	43	-.822	1.28	.368	.377	.451	<.0001	<.0001

Sex	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Female	2-Hydroxyfluorene (µg/L)	43	-.780	1.27	.395	.422	.456	<.0001	<.0001
Female	2-Hydroxynaphthalene (µg/L)	43	-.732	2.58	.700	.712	.738	<.0001	<.0001
Female	3-Hydroxyfluorene (µg/L)	43	-.716	1.38	.515	.451	.538	<.0001	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S1-4. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by race

Race	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
White	1-Hydroxynaphthalene (µg/L)	101	-1.09	1.97	.429	.505	.603	<.0001	<.0001
White	1-Hydroxyphenanthrene (µg/L)	102	-.236	2.45	.399	.489	.446	<.0001	<.0001
White	1-Hydroxypyrene (µg/L)	102	-1.86	2.61	.227	.248	.502	<.0001	<.0001
White	2 & 3-Hydroxyphenanthrene (µg/L)	102	-.539	2.55	.359	.424	.443	<.0001	<.0001
White	2-Hydroxyfluorene (µg/L)	102	-.518	2.20	.369	.452	.445	<.0001	<.0001
White	2-Hydroxynaphthalene (µg/L)	102	-.729	3.13	.539	.638	.629	<.0001	<.0001
White	3-Hydroxyfluorene (µg/L)	102	-.698	2.52	.365	.455	.474	<.0001	<.0001
Mixed,Other,Unknown	1-Hydroxynaphthalene (µg/L)	37	-.596	2.32	.510	.573	.597	<.0001	<.0001
Mixed,Other,Unknown	1-Hydroxyphenanthrene (µg/L)	37	-.351	3.02	.426	.463	.578	<.0001	<.0001
Mixed,Other,Unknown	1-Hydroxypyrene (µg/L)	37	-.948	2.05	.293	.360	.543	0.0003	<.0001
Mixed,Other,Unknown	2 & 3-Hydroxyphenanthrene (µg/L)	37	-.330	1.57	.407	.412	.395	<.0001	<.0001

Race	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Mixed,Other,Unknown	2-Hydroxyfluorene (µg/L)	37	-.400	1.72	.370	.425	.448	<.0001	<.0001
Mixed,Other,Unknown	2-Hydroxynaphthalene (µg/L)	37	-.509	2.76	.435	.525	.589	<.0001	<.0001
Mixed,Other,Unknown	3-Hydroxyfluorene (µg/L)	37	-.419	2.02	.405	.456	.540	<.0001	<.0001
Asian	1-Hydroxynaphthalene (µg/L)	22	-2.32	1.16	.628	.395	.819	0.0344	0.0083
Asian	1-Hydroxyphenanthrene (µg/L)	22	-1.91	1.28	.596	.414	.742	0.0160	0.0147
Asian	1-Hydroxypyrene (µg/L)	22	-1.20	1.45	.219	.207	.681	0.1683	0.0950
Asian	2 & 3-Hydroxyphenanthrene (µg/L)	22	-1.55	1.14	.469	.341	.651	0.0228	0.0118
Asian	2-Hydroxyfluorene (µg/L)	22	-2.04	1.18	.648	.404	.725	0.0163	0.0034
Asian	2-Hydroxynaphthalene (µg/L)	22	-2.45	1.49	.800	.604	.813	0.0022	0.0006
Asian	3-Hydroxyfluorene (µg/L)	22	-1.66	1.29	.551	.422	.687	0.0089	0.0057

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S1-5. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by field location

U.S. Census Region	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
South	1-Hydroxynaphthalene (µg/L)	109	-2.32	1.91	.432	.484	.609	<.0001	<.0001
South	1-Hydroxyphenanthrene (µg/L)	109	-.831	2.45	.442	.492	.479	<.0001	<.0001
South	1-Hydroxypyrene (µg/L)	109	-1.86	2.61	.232	.257	.550	<.0001	<.0001
South	2 & 3-Hydroxyphenanthrene (µg/L)	109	-1.55	2.55	.378	.418	.472	<.0001	<.0001
South	2-Hydroxyfluorene (µg/L)	109	-2.04	2.20	.408	.446	.503	<.0001	<.0001
South	2-Hydroxynaphthalene (µg/L)	109	-2.45	3.13	.536	.561	.615	<.0001	<.0001
South	3-Hydroxyfluorene (µg/L)	109	-1.66	2.52	.472	.466	.516	<.0001	<.0001
West	1-Hydroxynaphthalene (µg/L)	51	-1.08	2.32	.448	.553	.685	<.0001	<.0001
West	1-Hydroxyphenanthrene (µg/L)	52	-1.91	3.02	.399	.433	.608	<.0001	<.0001
West	1-Hydroxypyrene (µg/L)	52	-1.15	2.05	.230	.291	.515	0.0002	<.0001

U.S. Census Region	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
West	2 & 3-Hydroxyphenanthrene (µg/L)	52	-.539	1.57	.365	.393	.452	<.0001	<.0001
West	2-Hydroxyfluorene (µg/L)	52	-.518	1.81	.368	.425	.463	<.0001	<.0001
West	2-Hydroxynaphthalene (µg/L)	52	-.729	2.76	.573	.704	.702	<.0001	<.0001
West	3-Hydroxyfluorene (µg/L)	52	-.698	1.92	.360	.419	.529	<.0001	<.0001

Note: PAH = Polycyclic Aromatic Hydrocarbon.

The U.S. census regions are four geographic groupings of states that subdivide the United States, including: Midwest (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin); Northeast (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont); South (Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia); and West (Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming).

Table S1-6. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by facility

Facility	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Facility 1	1-Hydroxynaphthalene (µg/L)	68	-1.09	1.91	.431	.485	.604	<.0001	<.0001
Facility 1	1-Hydroxyphenanthrene (µg/L)	68	-.831	2.45	.455	.509	.493	<.0001	<.0001
Facility 1	1-Hydroxypyrene (µg/L)	68	-1.20	2.61	.228	.279	.553	<.0001	<.0001
Facility 1	2 & 3-Hydroxyphenanthrene (µg/L)	68	-.822	2.55	.353	.431	.479	<.0001	<.0001
Facility 1	2-Hydroxyfluorene (µg/L)	68	-.780	2.20	.389	.452	.467	<.0001	<.0001
Facility 1	2-Hydroxynaphthalene (µg/L)	68	-.732	3.13	.487	.585	.599	<.0001	<.0001
Facility 1	3-Hydroxyfluorene (µg/L)	68	-.716	2.52	.430	.457	.500	<.0001	<.0001
Facility 2	1-Hydroxynaphthalene (µg/L)	41	-2.32	1.50	.453	.482	.624	<.0001	<.0001
Facility 2	1-Hydroxyphenanthrene (µg/L)	41	-.796	1.69	.437	.464	.459	<.0001	<.0001
Facility 2	1-Hydroxypyrene (µg/L)	41	-1.86	1.14	.233	.221	.551	0.0139	0.0004
Facility 2	2 & 3-Hydroxyphenanthrene (µg/L)	41	-1.55	1.42	.395	.396	.465	<.0001	<.0001

Facility	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Facility 2	2-Hydroxyfluorene (µg/L)	41	-2.04	1.72	.452	.437	.563	<.0001	<.0001
Facility 2	2-Hydroxynaphthalene (µg/L)	41	-2.45	1.51	.586	.522	.646	<.0001	<.0001
Facility 2	3-Hydroxyfluorene (µg/L)	41	-1.66	2.02	.475	.481	.547	<.0001	<.0001
Facility 3	1-Hydroxynaphthalene (µg/L)	51	-1.08	2.32	.448	.553	.685	<.0001	<.0001
Facility 3	1-Hydroxyphenanthrene (µg/L)	52	-1.91	3.02	.399	.433	.608	<.0001	<.0001
Facility 3	1-Hydroxypyrene (µg/L)	52	-1.15	2.05	.230	.291	.515	0.0002	<.0001
Facility 3	2 & 3-Hydroxyphenanthrene (µg/L)	52	-.539	1.57	.365	.393	.452	<.0001	<.0001
Facility 3	2-Hydroxyfluorene (µg/L)	52	-.518	1.81	.368	.425	.463	<.0001	<.0001
Facility 3	2-Hydroxynaphthalene (µg/L)	52	-.729	2.76	.573	.704	.702	<.0001	<.0001
Facility 3	3-Hydroxyfluorene (µg/L)	52	-.698	1.92	.360	.419	.529	<.0001	<.0001

Note: Facility 1 and Facility 3 consisted of outdoor, co-located fields (natural grass, synthetic turf with tire crumb rubber infill). Facility 2 consisted of an indoor, synthetic turf field with tire crumb rubber infill.
PAH = Polycyclic Aromatic Hydrocarbon.

Table S1-7. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by BMI category

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Normal	1-Hydroxynaphthalene (µg/L)	101	-2.32	1.97	.453	.519	.687	<.0001	<.0001
Normal	1-Hydroxyphenanthrene (µg/L)	102	-1.91	2.45	.440	.462	.542	<.0001	<.0001
Normal	1-Hydroxypyrene (µg/L)	102	-1.86	2.61	.225	.227	.575	0.0001	<.0001
Normal	2 & 3-Hydroxyphenanthrene (µg/L)	102	-1.55	2.55	.371	.394	.497	<.0001	<.0001
Normal	2-Hydroxyfluorene (µg/L)	102	-2.04	2.20	.402	.421	.514	<.0001	<.0001
Normal	2-Hydroxynaphthalene (µg/L)	102	-2.45	3.13	.582	.645	.696	<.0001	<.0001
Normal	3-Hydroxyfluorene (µg/L)	102	-1.66	2.52	.421	.427	.544	<.0001	<.0001
Obese	1-Hydroxynaphthalene (µg/L)	17	-.519	1.09	.346	.302	.465	0.0164	0.0305
Obese	1-Hydroxyphenanthrene (µg/L)	17	.020	1.29	.365	.440	.371	0.0002	<.0001
Obese	1-Hydroxypyrene (µg/L)	17	-.192	.952	.241	.272	.324	0.0032	0.0021
Obese	2 & 3-Hydroxyphenanthrene (µg/L)	17	-.049	1.42	.362	.404	.365	0.0003	<.0001

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Obese	2-Hydroxyfluorene (µg/L)	17	-.089	1.34	.370	.437	.381	0.0002	<.0001
Obese	2-Hydroxynaphthalene (µg/L)	17	-.729	1.40	.473	.510	.507	0.0008	0.0011
Obese	3-Hydroxyfluorene (µg/L)	17	-.162	1.28	.405	.436	.426	0.0006	0.0007
Overweight	1-Hydroxynaphthalene (µg/L)	33	-.634	2.32	.574	.560	.573	<.0001	<.0001
Overweight	1-Hydroxyphenanthrene (µg/L)	33	-.351	3.02	.491	.547	.585	<.0001	<.0001
Overweight	1-Hydroxypyrene (µg/L)	33	-.571	2.05	.234	.405	.524	0.0001	<.0001
Overweight	2 & 3-Hydroxyphenanthrene (µg/L)	33	-.351	1.57	.407	.459	.404	<.0001	<.0001
Overweight	2-Hydroxyfluorene (µg/L)	33	-.157	1.72	.406	.495	.440	<.0001	<.0001
Overweight	2-Hydroxynaphthalene (µg/L)	33	-.509	2.76	.477	.590	.614	<.0001	<.0001
Overweight	3-Hydroxyfluorene (µg/L)	33	-.419	2.02	.472	.527	.521	<.0001	<.0001
Underweight	1-Hydroxynaphthalene (µg/L)	8	-.026	1.48	.501	.573	.468	0.0105	0.0156
Underweight	1-Hydroxyphenanthrene (µg/L)	8	.054	.862	.328	.410	.301	0.0063	0.0078
Underweight	1-Hydroxypyrene (µg/L)	8	-.593	.944	.173	.207	.472	0.2548	0.1953

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Underweight	2 & 3-Hydroxyphenanthrene (µg/L)	8	-.090	1.54	.273	.461	.533	0.0443	0.0234
Underweight	2-Hydroxyfluorene (µg/L)	8	-.103	1.81	.347	.522	.608	0.0455	0.0156
Underweight	2-Hydroxynaphthalene (µg/L)	8	.070	1.13	.360	.468	.354	0.0073	0.0078
Underweight	3-Hydroxyfluorene (µg/L)	8	.048	1.16	.434	.503	.428	0.0127	0.0078
Unknown	1-Hydroxynaphthalene (µg/L)	1	.266	.266	.266	.266	N/A	N/A	1.0000
Unknown	1-Hydroxyphenanthrene (µg/L)	1	.210	.210	.210	.210	N/A	N/A	1.0000
Unknown	1-Hydroxypyrene (µg/L)	1	.330	.330	.330	.330	N/A	N/A	1.0000
Unknown	2 & 3-Hydroxyphenanthrene (µg/L)	1	.057	.057	.057	.057	N/A	N/A	1.0000
Unknown	2-Hydroxyfluorene (µg/L)	1	-.139	-.139	-.139	-.139	N/A	N/A	1.0000
Unknown	2-Hydroxynaphthalene (µg/L)	1	.160	.160	.160	.160	N/A	N/A	1.0000
Unknown	3-Hydroxyfluorene (µg/L)	1	.226	.226	.226	.226	N/A	N/A	1.0000

Note: N/A = not applicable.

PAH = Polycyclic Aromatic Hydrocarbon.

BMI categories (underweight, normal, overweight, and obese) corresponded to cut points at <18.5, <25, <30, and >=30, respectively, for adults 20 years and older. CDC growth charts were used to calculate BMI for participants <20 years old with cut points defined at BMI percentages (0,5), [5,85), [85,95), and 95+. Because age was only collected for

whole years and age-in-months is required to properly use growth charts, BMI calculations for participants under 20 years old were subject to misclassification due to rounding. See SAS Program for CDC Growth Charts. See: https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm.

Table S1-8. Properties of differences in ln-transformed pre- and post-activity body burden levels for Specific Gravity-adjusted urinary PAHs, by activity

Main Activity	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Soccer	1-Hydroxynaphthalene (µg/L)	118	-2.32	1.85	.494	.517	.599	<.0001	<.0001
Soccer	1-Hydroxyphenanthrene (µg/L)	119	-.831	2.45	.442	.449	.445	<.0001	<.0001
Soccer	1-Hydroxypyrene (µg/L)	119	-1.20	2.61	.234	.292	.494	<.0001	<.0001
Soccer	2 & 3-Hydroxyphenanthrene (µg/L)	119	-1.55	2.55	.395	.412	.469	<.0001	<.0001
Soccer	2-Hydroxyfluorene (µg/L)	119	-2.04	2.20	.441	.454	.513	<.0001	<.0001
Soccer	2-Hydroxynaphthalene (µg/L)	119	-2.45	3.13	.569	.624	.654	<.0001	<.0001
Soccer	3-Hydroxyfluorene (µg/L)	119	-1.66	2.52	.411	.453	.520	<.0001	<.0001
Lacrosse	1-Hydroxynaphthalene (µg/L)	27	-1.09	1.91	.385	.503	.636	0.0004	0.0001
Lacrosse	1-Hydroxyphenanthrene (µg/L)	27	-.072	1.69	.468	.588	.455	<.0001	<.0001
Lacrosse	1-Hydroxypyrene (µg/L)	27	-1.86	1.14	.137	.119	.565	0.2819	0.0929
Lacrosse	2 & 3-Hydroxyphenanthrene (µg/L)	27	-.030	1.42	.350	.423	.393	<.0001	<.0001

Main Activity	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Lacrosse	2-Hydroxyfluorene (µg/L)	27	-.134	1.37	.310	.423	.400	<.0001	<.0001
Lacrosse	2-Hydroxynaphthalene (µg/L)	27	-.239	1.51	.483	.555	.435	<.0001	<.0001
Lacrosse	3-Hydroxyfluorene (µg/L)	27	-.276	1.29	.377	.436	.455	<.0001	<.0001
Other	1-Hydroxynaphthalene (µg/L)	15	-1.02	2.32	.182	.422	.887	0.0864	0.0946
Other	1-Hydroxyphenanthrene (µg/L)	15	-1.91	3.02	.365	.454	1.02	0.1060	0.0181
Other	1-Hydroxypyrene (µg/L)	15	-1.15	2.05	.251	.346	.776	0.1060	0.0833
Other	2 & 3-Hydroxyphenanthrene (µg/L)	15	-.433	1.57	.291	.364	.567	0.0261	0.0302
Other	2-Hydroxyfluorene (µg/L)	15	-.223	1.34	.385	.352	.457	0.0098	0.0151
Other	2-Hydroxynaphthalene (µg/L)	15	-.729	2.76	.470	.568	.894	0.0275	0.0215
Other	3-Hydroxyfluorene (µg/L)	15	-.661	1.92	.455	.462	.642	0.0146	0.0181

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-1. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by field environment

Field Environment	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Outdoor	1-Hydroxynaphthalene (µg/gCRE)	119	-2.11	2.35	-.006	.061	.593	0.2626	0.4553
Outdoor	1-Hydroxyphenanthrene (µg/gCRE)	120	-2.17	2.42	-.029	.025	.521	0.6038	0.8473
Outdoor	1-Hydroxypyrene (µg/gCRE)	120	-1.91	1.72	-.122	-.168	.596	0.0026	0.0020
Outdoor	2 & 3-Hydroxyphenanthrene (µg/gCRE)	120	-1.69	1.90	-.087	-.037	.481	0.4029	0.1202
Outdoor	2-Hydroxyfluorene (µg/gCRE)	120	-1.25	2.18	-.025	-.011	.427	0.7764	0.2220
Outdoor	2-Hydroxynaphthalene (µg/gCRE)	120	-1.21	2.75	.064	.185	.596	0.0009	0.0015
Outdoor	3-Hydroxyfluorene (µg/gCRE)	120	-1.37	2.24	-.043	-.011	.467	0.8014	0.3588
Indoor	1-Hydroxynaphthalene (µg/gCRE)	41	-1.30	1.38	-.066	.059	.538	0.4879	0.8040
Indoor	1-Hydroxyphenanthrene (µg/gCRE)	41	-1.02	1.37	-.017	.041	.390	0.5062	0.9949
Indoor	1-Hydroxypyrene (µg/gCRE)	41	-3.47	1.22	-.169	-.202	.689	0.0681	0.0335
Indoor	2 & 3-Hydroxyphenanthrene (µg/gCRE)	41	-1.51	1.23	-.031	-.027	.473	0.7149	0.4868

Field Environment	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Indoor	2-Hydroxyfluorene (µg/gCRE)	41	-1.13	1.64	-.020	.014	.545	0.8727	0.6329
Indoor	2-Hydroxynaphthalene (µg/gCRE)	41	-1.43	1.51	.114	.098	.533	0.2440	0.0722
Indoor	3-Hydroxyfluorene (µg/gCRE)	41	-.889	1.93	.031	.058	.527	0.4831	0.7455

Note: N/A = not applicable

Table S2-2. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by age category

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Adolescent	1-Hydroxynaphthalene (µg/gCRE)	51	-1.06	2.35	.033	.177	.582	0.0348	0.1076
Adolescent	1-Hydroxyphenanthrene (µg/gCRE)	51	-1.09	2.42	.029	.152	.541	0.0502	0.1590
Adolescent	1-Hydroxypyrene (µg/gCRE)	51	-3.47	1.72	-.154	-.225	.802	0.0506	0.0379
Adolescent	2 & 3-Hydroxyphenanthrene (µg/gCRE)	51	-1.69	1.90	-.061	.040	.601	0.6330	0.8898
Adolescent	2-Hydroxyfluorene (µg/gCRE)	51	-1.13	2.18	-.020	.040	.543	0.5996	0.8607
Adolescent	2-Hydroxynaphthalene (µg/gCRE)	51	-.897	2.75	.091	.299	.697	0.0035	0.0012
Adolescent	3-Hydroxyfluorene (µg/gCRE)	51	-1.01	2.24	.023	.052	.575	0.5183	0.7959
Adult	1-Hydroxynaphthalene (µg/gCRE)	58	-1.30	1.38	-.067	.041	.589	0.6013	0.9178
Adult	1-Hydroxyphenanthrene (µg/gCRE)	59	-2.17	2.07	-.045	-.058	.531	0.4083	0.1599
Adult	1-Hydroxypyrene (µg/gCRE)	59	-1.68	1.22	-.168	-.189	.549	0.0104	0.0126
Adult	2 & 3-Hydroxyphenanthrene (µg/gCRE)	59	-1.30	1.21	-.100	-.105	.387	0.0411	0.0180

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Adult	2-Hydroxyfluorene (µg/gCRE)	59	-1.25	1.64	-.025	-.042	.438	0.4640	0.1395
Adult	2-Hydroxynaphthalene (µg/gCRE)	59	-1.43	2.59	.114	.140	.634	0.0953	0.0420
Adult	3-Hydroxyfluorene (µg/gCRE)	59	-1.19	1.93	-.014	-.001	.478	0.9854	0.7149
Child	1-Hydroxynaphthalene (µg/gCRE)	24	-2.11	.919	.006	-.156	.653	0.2525	0.6573
Child	1-Hydroxyphenanthrene (µg/gCRE)	24	-1.25	.956	-.058	.047	.428	0.5986	0.6573
Child	1-Hydroxypyrene (µg/gCRE)	24	-1.75	1.08	-.074	-.056	.583	0.6420	0.5597
Child	2 & 3-Hydroxyphenanthrene (µg/gCRE)	24	-1.57	1.28	-.050	.010	.543	0.9270	0.8464
Child	2-Hydroxyfluorene (µg/gCRE)	24	-1.14	1.55	-.031	-.029	.494	0.7756	0.5597
Child	2-Hydroxynaphthalene (µg/gCRE)	24	-.900	.596	.053	.073	.330	0.2922	0.2156
Child	3-Hydroxyfluorene (µg/gCRE)	24	-1.37	.906	-.106	-.082	.432	0.3636	0.2050
Youth	1-Hydroxynaphthalene (µg/gCRE)	27	-.500	1.05	-.030	.077	.426	0.3572	0.5581
Youth	1-Hydroxyphenanthrene (µg/gCRE)	27	-.605	.452	-.041	-.031	.268	0.5567	0.5268
Youth	1-Hydroxypyrene (µg/gCRE)	27	-.964	.484	-.154	-.162	.357	0.0260	0.0276

Age Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Youth	2 & 3-Hydroxyphenanthrene (µg/gCRE)	27	-.730	.525	-.068	-.061	.295	0.2939	0.3841
Youth	2-Hydroxyfluorene (µg/gCRE)	27	-.518	.765	-.020	.013	.274	0.8023	0.7613
Youth	2-Hydroxynaphthalene (µg/gCRE)	27	-.464	.925	-.033	.039	.292	0.4939	0.7792
Youth	3-Hydroxyfluorene (µg/gCRE)	27	-.476	.788	-.022	.017	.329	0.7902	0.9070

Table S2-3. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by sex

Sex	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Male	1-Hydroxynaphthalene (µg/gCRE)	117	-1.98	1.38	-.040	.031	.515	0.5163	0.9860
Male	1-Hydroxyphenanthrene (µg/gCRE)	118	-1.09	2.07	-.004	.062	.418	0.1073	0.4189
Male	1-Hydroxypyrene (µg/gCRE)	118	-3.47	1.72	-.123	-.158	.622	0.0066	0.0023
Male	2 & 3-Hydroxyphenanthrene (µg/gCRE)	118	-1.69	1.66	-.068	-.025	.449	0.5516	0.1982
Male	2-Hydroxyfluorene (µg/gCRE)	118	-1.13	1.64	-.020	-.001	.444	0.9819	0.3271
Male	2-Hydroxynaphthalene (µg/gCRE)	118	-1.43	2.59	.064	.123	.493	0.0078	0.0028
Male	3-Hydroxyfluorene (µg/gCRE)	118	-1.01	1.93	-.027	.004	.452	0.9190	0.3670
Female	1-Hydroxynaphthalene (µg/gCRE)	43	-2.11	2.35	.068	.141	.721	0.2071	0.1873
Female	1-Hydroxyphenanthrene (µg/gCRE)	43	-2.17	2.42	-.070	-.063	.644	0.5219	0.1360
Female	1-Hydroxypyrene (µg/gCRE)	43	-1.75	1.37	-.192	-.226	.616	0.0206	0.0270
Female	2 & 3-Hydroxyphenanthrene (µg/gCRE)	43	-1.57	1.90	-.090	-.061	.551	0.4729	0.2460

Sex	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Female	2-Hydroxyfluorene (µg/gCRE)	43	-1.25	2.18	-.025	-.015	.502	0.8422	0.3913
Female	2-Hydroxynaphthalene (µg/gCRE)	43	-1.21	2.75	.207	.274	.768	0.0240	0.0262
Female	3-Hydroxyfluorene (µg/gCRE)	43	-1.37	2.24	.070	.014	.563	0.8716	0.7576

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-4. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by race

Race	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
White	1-Hydroxynaphthalene (µg/L)	101	-2.11	2.35	-.011	.056	.580	0.3384	0.5391
White	1-Hydroxyphenanthrene (µg/L)	102	-1.25	2.42	-.026	.041	.432	0.3354	0.9775
White	1-Hydroxypyrene (µg/L)	102	-3.47	1.72	-.157	-.200	.601	0.0011	0.0004
White	2 & 3-Hydroxyphenanthrene (µg/L)	102	-1.57	1.90	-.080	-.024	.480	0.6134	0.1316
White	2-Hydroxyfluorene (µg/L)	102	-1.14	2.18	-.016	.004	.448	0.9247	0.3533
White	2-Hydroxynaphthalene (µg/L)	102	-1.04	2.75	.089	.190	.611	0.0022	0.0020
White	3-Hydroxyfluorene (µg/L)	102	-1.37	2.24	-.024	.007	.459	0.8734	0.6295
Mixed,Other,Unknown	1-Hydroxynaphthalene (µg/L)	37	-.445	1.38	.130	.249	.540	0.0080	0.0407
Mixed,Other,Unknown	1-Hydroxyphenanthrene (µg/L)	37	-.840	2.07	-.008	.139	.521	0.1142	0.3528
Mixed,Other,Unknown	1-Hydroxypyrene (µg/L)	37	-1.91	1.37	-.010	.036	.597	0.7154	0.7844
Mixed,Other,Unknown	2 & 3-Hydroxyphenanthrene (µg/L)	37	-.747	1.21	.003	.088	.376	0.1642	0.4184

Race	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Mixed,Other,Unknown	2-Hydroxyfluorene (µg/L)	37	-.618	1.64	-.008	.101	.458	0.1868	0.7700
Mixed,Other,Unknown	2-Hydroxynaphthalene (µg/L)	37	-.594	1.81	.104	.201	.511	0.0222	0.0361
Mixed,Other,Unknown	3-Hydroxyfluorene (µg/L)	37	-.958	1.93	.047	.132	.542	0.1457	0.2539
Asian	1-Hydroxynaphthalene (µg/L)	22	-1.30	.686	-.102	-.234	.519	0.0471	0.0359
Asian	1-Hydroxyphenanthrene (µg/L)	22	-2.17	.559	-.077	-.214	.617	0.1182	0.1951
Asian	1-Hydroxypyrene (µg/L)	22	-1.78	.852	-.293	-.421	.653	0.0064	0.0034
Asian	2 & 3-Hydroxyphenanthrene (µg/L)	22	-1.69	.541	-.202	-.287	.538	0.0205	0.0164
Asian	2-Hydroxyfluorene (µg/L)	22	-1.25	.489	-.082	-.225	.452	0.0295	0.0703
Asian	2-Hydroxynaphthalene (µg/L)	22	-1.43	1.05	-.010	-.024	.534	0.8338	0.7897
Asian	3-Hydroxyfluorene (µg/L)	22	-1.19	.496	-.106	-.206	.422	0.0322	0.0428

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-5. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by field location

U.S. Census Region	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
South	1-Hydroxynaphthalene (µg/gCRE)	109	-1.98	2.35	-.041	.041	.571	0.4507	0.9749
South	1-Hydroxyphenanthrene (µg/gCRE)	109	-1.30	2.42	-.005	.050	.471	0.2720	0.8160
South	1-Hydroxypyrene (µg/gCRE)	109	-3.47	1.72	-.167	-.185	.627	0.0026	0.0009
South	2 & 3-Hydroxyphenanthrene (µg/gCRE)	109	-1.69	1.90	-.072	-.025	.497	0.6062	0.1429
South	2-Hydroxyfluorene (µg/gCRE)	109	-1.25	2.18	-.031	.004	.501	0.9315	0.2432
South	2-Hydroxynaphthalene (µg/gCRE)	109	-1.43	2.75	.056	.119	.576	0.0333	0.0289
South	3-Hydroxyfluorene (µg/gCRE)	109	-1.19	2.24	-.003	.024	.515	0.6259	0.9509
West	1-Hydroxynaphthalene (µg/gCRE)	51	-2.11	1.70	.053	.101	.594	0.2283	0.1706
West	1-Hydroxyphenanthrene (µg/gCRE)	52	-2.17	2.07	-.041	-.015	.529	0.8380	0.5651
West	1-Hydroxypyrene (µg/gCRE)	52	-1.75	1.37	-.112	-.157	.607	0.0672	0.0586

U.S. Census Region	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
West	2 & 3-Hydroxyphenanthrene (µg/gCRE)	52	-1.57	1.28	-.092	-.055	.437	0.3696	0.3212
West	2-Hydroxyfluorene (µg/gCRE)	52	-1.14	1.55	-.005	-.023	.356	0.6367	0.5292
West	2-Hydroxynaphthalene (µg/gCRE)	52	-.900	2.63	.139	.256	.585	0.0027	0.0008
West	3-Hydroxyfluorene (µg/gCRE)	52	-1.37	.971	-.066	-.029	.408	0.6057	0.3439

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-6. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by facility

Facility	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Facility 1	1-Hydroxynaphthalene (µg/gCRE)	68	-1.98	2.35	-.041	.031	.594	0.6692	0.8895
Facility 1	1-Hydroxyphenanthrene (µg/gCRE)	68	-1.30	2.42	.000	.055	.516	0.3811	0.8514
Facility 1	1-Hydroxypyrene (µg/gCRE)	68	-1.91	1.72	-.154	-.175	.592	0.0172	0.0132
Facility 1	2 & 3-Hydroxyphenanthrene (µg/gCRE)	68	-1.69	1.90	-.083	-.023	.515	0.7126	0.1869
Facility 1	2-Hydroxyfluorene (µg/gCRE)	68	-1.25	2.18	-.038	-.002	.477	0.9774	0.2511
Facility 1	2-Hydroxynaphthalene (µg/gCRE)	68	-1.21	2.75	.020	.131	.604	0.0774	0.2150
Facility 1	3-Hydroxyfluorene (µg/gCRE)	68	-1.19	2.24	-.024	.003	.510	0.9551	0.6943
Facility 2	1-Hydroxynaphthalene (µg/gCRE)	41	-1.30	1.38	-.066	.059	.538	0.4879	0.8040
Facility 2	1-Hydroxyphenanthrene (µg/gCRE)	41	-1.02	1.37	-.017	.041	.390	0.5062	0.9949
Facility 2	1-Hydroxypyrene (µg/gCRE)	41	-3.47	1.22	-.169	-.202	.689	0.0681	0.0335
Facility 2	2 & 3-Hydroxyphenanthrene (µg/gCRE)	41	-1.51	1.23	-.031	-.027	.473	0.7149	0.4868

Facility	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Facility 2	2-Hydroxyfluorene (µg/gCRE)	41	-1.13	1.64	-.020	.014	.545	0.8727	0.6329
Facility 2	2-Hydroxynaphthalene (µg/gCRE)	41	-1.43	1.51	.114	.098	.533	0.2440	0.0722
Facility 2	3-Hydroxyfluorene (µg/gCRE)	41	-.889	1.93	.031	.058	.527	0.4831	0.7455
Facility 3	1-Hydroxynaphthalene (µg/gCRE)	51	-2.11	1.70	.053	.101	.594	0.2283	0.1706
Facility 3	1-Hydroxyphenanthrene (µg/gCRE)	52	-2.17	2.07	-.041	-.015	.529	0.8380	0.5651
Facility 3	1-Hydroxypyrene (µg/gCRE)	52	-1.75	1.37	-.112	-.157	.607	0.0672	0.0586
Facility 3	2 & 3-Hydroxyphenanthrene (µg/gCRE)	52	-1.57	1.28	-.092	-.055	.437	0.3696	0.3212
Facility 3	2-Hydroxyfluorene (µg/gCRE)	52	-1.14	1.55	-.005	-.023	.356	0.6367	0.5292
Facility 3	2-Hydroxynaphthalene (µg/gCRE)	52	-.900	2.63	.139	.256	.585	0.0027	0.0008
Facility 3	3-Hydroxyfluorene (µg/gCRE)	52	-1.37	.971	-.066	-.029	.408	0.6057	0.3439

Note: Facility 1 and Facility 3 consisted of outdoor, co-located fields (natural grass, synthetic turf with tire crumb rubber infill). Facility 2 consisted of an indoor, synthetic turf field with tire crumb rubber infill.
PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-7. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by BMI category

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Normal	1-Hydroxynaphthalene (µg/gCRE)	101	-2.11	2.35	.017	.067	.628	0.2847	0.3111
Normal	1-Hydroxyphenanthrene (µg/gCRE)	102	-2.17	2.42	-.020	.012	.508	0.8159	0.8489
Normal	1-Hydroxypyrene (µg/gCRE)	102	-3.47	1.72	-.138	-.223	.665	0.0010	0.0010
Normal	2 & 3-Hydroxyphenanthrene (µg/gCRE)	102	-1.69	1.90	-.072	-.056	.503	0.2648	0.1248
Normal	2-Hydroxyfluorene (µg/gCRE)	102	-1.25	2.18	-.017	-.029	.451	0.5140	0.3309
Normal	2-Hydroxynaphthalene (µg/gCRE)	102	-1.43	2.75	.120	.195	.643	0.0029	0.0001
Normal	3-Hydroxyfluorene (µg/gCRE)	102	-1.37	2.24	.007	-.023	.479	0.6247	0.5831
Obese	1-Hydroxynaphthalene (µg/gCRE)	17	-1.06	1.15	-.205	-.074	.502	0.5509	0.3289
Obese	1-Hydroxyphenanthrene (µg/gCRE)	17	-.453	1.09	-.003	.063	.360	0.4800	1.0000
Obese	1-Hydroxypyrene (µg/gCRE)	17	-1.16	.756	-.168	-.104	.527	0.4266	0.6112
Obese	2 & 3-Hydroxyphenanthrene (µg/gCRE)	17	-.802	1.23	-.115	.027	.435	0.8018	0.8900

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Obese	2-Hydroxyfluorene (µg/gCRE)	17	-.354	1.15	-.020	.060	.393	0.5358	0.8176
Obese	2-Hydroxynaphthalene (µg/gCRE)	17	-.545	1.20	.022	.133	.434	0.2237	0.4874
Obese	3-Hydroxyfluorene (µg/gCRE)	17	-.461	1.08	-.026	.060	.393	0.5409	0.7819
Overweight	1-Hydroxynaphthalene (µg/gCRE)	33	-.921	1.38	-.028	.101	.507	0.2618	0.7202
Overweight	1-Hydroxyphenanthrene (µg/gCRE)	33	-.840	2.07	-.030	.088	.542	0.3596	0.9930
Overweight	1-Hydroxypyrene (µg/gCRE)	33	-1.11	1.37	-.119	-.055	.552	0.5724	0.1873
Overweight	2 & 3-Hydroxyphenanthrene (µg/gCRE)	33	-.747	1.21	-.031	-.001	.416	0.9904	0.7202
Overweight	2-Hydroxyfluorene (µg/gCRE)	33	-.696	1.64	-.031	.035	.480	0.6770	0.5936
Overweight	2-Hydroxynaphthalene (µg/gCRE)	33	-.594	1.81	.030	.130	.511	0.1529	0.4144
Overweight	3-Hydroxyfluorene (µg/gCRE)	33	-.958	1.93	-.067	.067	.552	0.4882	0.9791
Underweight	1-Hydroxynaphthalene (µg/gCRE)	8	-.413	.656	.165	.128	.348	0.3335	0.4609
Underweight	1-Hydroxyphenanthrene (µg/gCRE)	8	-.239	.604	-.147	-.035	.286	0.7399	0.3828
Underweight	1-Hydroxypyrene (µg/gCRE)	8	-.974	.686	-.357	-.238	.476	0.2004	0.1953

BMI Category	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Underweight	2 & 3-Hydroxyphenanthrene (µg/gCRE)	8	-.373	1.28	-.182	.016	.543	0.9359	0.5469
Underweight	2-Hydroxyfluorene (µg/gCRE)	8	-.386	1.55	-.100	.077	.621	0.7348	0.5469
Underweight	2-Hydroxynaphthalene (µg/gCRE)	8	-.214	.314	-.046	.023	.200	0.7596	0.8438
Underweight	3-Hydroxyfluorene (µg/gCRE)	8	-.445	.906	.068	.058	.447	0.7245	1.0000
Unknown	1-Hydroxynaphthalene (µg/gCRE)	1	-.188	-.188	-.188	.188	N/A	N/A	1.0000
Unknown	1-Hydroxyphenanthrene (µg/gCRE)	1	-.244	-.244	-.244	.244	N/A	N/A	1.0000
Unknown	1-Hydroxypyrene (µg/gCRE)	1	-.124	-.124	-.124	.124	N/A	N/A	1.0000
Unknown	2 & 3-Hydroxyphenanthrene (µg/gCRE)	1	-.398	-.398	-.398	.398	N/A	N/A	1.0000
Unknown	2-Hydroxyfluorene (µg/gCRE)	1	-.594	-.594	-.594	.594	N/A	N/A	1.0000
Unknown	2-Hydroxynaphthalene (µg/gCRE)	1	-.295	-.295	-.295	.295	N/A	N/A	1.0000
Unknown	3-Hydroxyfluorene (µg/gCRE)	1	-.229	-.229	-.229	.229	N/A	N/A	1.0000

Note: N/A = not applicable.

PAH = Polycyclic Aromatic Hydrocarbon.

Table S2-8. Properties of differences in ln-transformed pre- and post-activity body burden levels for Creatinine-adjusted urinary PAHs, by activity

Main Activity	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Soccer	1-Hydroxynaphthalene (µg/gCRE)	118	-2.11	2.35	.006	.076	.567	0.1465	0.2606
Soccer	1-Hydroxyphenanthrene (µg/gCRE)	119	-1.30	2.42	-.029	.010	.449	0.8081	0.6655
Soccer	1-Hydroxypyrene (µg/gCRE)	119	-1.91	1.72	-.121	-.148	.551	0.0042	0.0027
Soccer	2 & 3-Hydroxyphenanthrene (µg/gCRE)	119	-1.69	1.90	-.068	-.027	.484	0.5462	0.2660
Soccer	2-Hydroxyfluorene (µg/gCRE)	119	-1.25	2.18	-.019	.015	.480	0.7373	0.4569
Soccer	2-Hydroxynaphthalene (µg/gCRE)	119	-1.43	2.75	.104	.185	.604	0.0011	0.0002
Soccer	3-Hydroxyfluorene (µg/gCRE)	119	-1.37	2.24	-.026	.014	.489	0.7635	0.5372
Lacrosse	1-Hydroxynaphthalene (µg/gCRE)	27	-1.98	1.18	-.107	-.024	.572	0.8267	0.7613
Lacrosse	1-Hydroxyphenanthrene (µg/gCRE)	27	-.464	1.09	-.005	.060	.355	0.3872	0.7972
Lacrosse	1-Hydroxypyrene (µg/gCRE)	27	-3.47	.756	-.345	-.408	.763	0.0100	0.0016

Main Activity	Urinary PAH	n	Minimum	Maximum	Median	Mean	Standard Deviation	P-Value of t Statistic	P-Value of Signed Rank Test
Lacrosse	2 & 3-Hydroxyphenanthrene (µg/gCRE)	27	-1.51	1.23	-.062	-.104	.469	0.2588	0.0630
Lacrosse	2-Hydroxyfluorene (µg/gCRE)	27	-1.13	1.15	-.088	-.105	.440	0.2269	0.1087
Lacrosse	2-Hydroxynaphthalene (µg/gCRE)	27	-.897	1.20	.056	.028	.401	0.7209	0.7972
Lacrosse	3-Hydroxyfluorene (µg/gCRE)	27	-.895	1.08	-.066	-.091	.438	0.2905	0.3340
Other	1-Hydroxynaphthalene (µg/gCRE)	15	-1.29	1.37	-.068	.090	.692	0.6223	0.9341
Other	1-Hydroxyphenanthrene (µg/gCRE)	15	-2.17	2.07	.005	.122	.893	0.6048	0.7197
Other	1-Hydroxypyrene (µg/gCRE)	15	-1.42	1.37	-.024	.014	.766	0.9450	0.9780
Other	2 & 3-Hydroxyphenanthrene (µg/gCRE)	15	-.697	.892	-.117	.032	.453	0.7911	0.9780
Other	2-Hydroxyfluorene (µg/gCRE)	15	-.413	.541	.009	.020	.279	0.7864	0.9341
Other	2-Hydroxynaphthalene (µg/gCRE)	15	-.545	1.81	.011	.236	.661	0.1891	0.4543
Other	3-Hydroxyfluorene (µg/gCRE)	15	-.924	.971	.099	.130	.497	0.3288	0.3591

Note: PAH = Polycyclic Aromatic Hydrocarbon.

Table S3. Comparison of creatinine-adjusted urinary PAH concentrations, NHANES 2015—2016 and 2007—2008

Urinary PAH	NHANES 2007-2008 n	NHANES 2007-2008 geometric mean (95% CI)	NHANES 2015-2016 n	NHANES 2015-2016 geometric mean (95% CI)
1-Hydroxynaphthalene (µg/gCRE)	2395	2.61 (2.20-3.08)	2318	1.69 (1.50-1.91)
1-Hydroxyphenanthrene (µg/gCRE)	2503	.140 (.130-151)	2426	.115 (.106-.125)
1-Hydroxypyrene (µg/gCRE)	2476	.118 (.108-.128)	2424	.133 (.125-.141)
2 & 3-Hydroxyphenanthrene (µg/gCRE)	N/A	N/A	2424	.134 (.123-.146)
2-Hydroxyfluorene (µg/gCRE)	2477	.304 (.272-.341)	2425	.211 (.191-.232)
2-Hydroxynaphthalene (µg/gCRE)	2431	3.88 (3.45-4.36)	2374	5.350 (4.86-5.90)
3-Hydroxyfluorene (µg/gCRE)	2479	.118 (.104- .133)	2421	.093 (.083-.105)

Note: N/A = not applicable.
PAH = Polycyclic Aromatic Hydrocarbon.
2&3-Hydroxyphenanthrene was not assessed in NHANES 2007-2008.

Appendix B

Quality Assurance and Quality Control

B.1 Quality Overview and Planning

Appendix B does not describe quality assurance/quality control (QA/QC) activities and results for the biomonitoring portions of the exposure characterization pilot study. Appendix B describes only those QA/QC documentation, procedures, and results for EPA-led activities. Biomonitoring QA/QC procedures and results are described in Appendix A.

The U.S. Environmental Protection Agency (EPA) requires that all data collected for the characterization of environmental processes and conditions are of the appropriate type and quality for their intended use. This is accomplished through an EPA-wide quality system for environmental data. Components of the EPA quality system can be found at http://www.epa.gov/quality/. EPA policy is based on ANSI/ASQ E4-2004 (an American National Standard). This standard recommends a tiered approach that includes the development and use of Quality Management Plans (QMPs). The organizational units in EPA that generate and/or use environmental data are required to have EPA-approved QMPs. Programmatic QMPs may also be written when program managers and their QA staff decide a program is of sufficient complexity to benefit from a QMP.

A programmatic QMP was developed for the research conducted under the Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds, described here as the Tire Crumb Research Study (TCRS). The TCRS QMP describes the program's organizational structure, defines and assigns QA and QC responsibilities, and describes the processes and procedures used to plan, implement and assess the effectiveness of the quality system. The TCRS QMP is supported by project-specific QA project plans (QAPPs).

The TCRS QAPPs provide the technical details and associated QA/QC procedures for the research activities that address TCRS objectives as described in the TCRS Research Protocol, "Collections Related to Synthetic Turf Fields with Crumb Rubber Infill." Written sample collection and analysis research-level standard operating procedures (SOPs) were also prepared to support the QAPPs, when appropriate.

The following elements were critical for producing high-quality research results:

- Research projects comply with Agency requirements and guidance for QAPPs, including the use of systematic planning;
- Technical system audits (TSAs) and data quality reviews, as described in the QMP or project-specific QAPPs;
- QA review of all products that include environmental data; and
- Inclusion of a QA/QC section in the final study report.

This research was supported by a Program QA Manager (PQAM) who was independent of the technical work and who assisted the QA staff in the implementation of the TCRS quality program and QMP requirements. Requirements specified in the TCRS QMP and QAPPs were intended to ensure consistency in the QA approach for all participating organizations.

B.2 Quality Assurance Activities and Results

B.2.1 Quality Assurance Project Plans

As part of the QA processes implemented in this research study, QAPPs were prepared by research staff for several components of the TCRS, including the Literature Review/Gaps Analysis and the tire crumb rubber characterization, and the exposure characterization. QAPPs were reviewed and approved by the respective research staff supervisors and QAMs. QAPPs (or QAPP addendums) prepared for the tire crumb characterization portion of the study were described in the Part 1 Report (EPA/600/R-19/051). One QAPP and two QAPP addendums were prepared for the TCRS exposure characterization pilot study (Table B-1) and related activity assessment research.

Table B-1. Quality Assurance Project Plans (QAPPs) and Addendums for Exposure Characterization

#	QAPP Title	Approval Date
1	Activity Characterization for the Tire Crumb Research Study	June 2016
2	QAPP Addendum for the Tire Crumb Research Study -Exposure Characterization Pilot Study	August 2017
3	QAPP Addendum for the Tire Crumb Research Study Exposure Characterization Pilot Study Procedures for Exposure Pathway Modeling	March 2018

B.2.2 Standard Operating Procedures

Research-level SOPs were developed for all sample collection, data collection and sample analysis activities. Prior to undertaking the activities covered by a SOP, the SOP was reviewed and approved by the respective research staff supervisors and QAMs. Research-level SOPs developed or applied in the exposure characterization pilot study are provided in Appendix C.

B.2.3 Technical Systems Audits

The EPA Office of Research and Development (ORD) quality program requires at least one audit be conducted per project, at a minimum. However, due to the high visibility and multi-component nature of the TCRS, a robust quality review process (including technical system audits and data quality reviews) was implemented to identify and correct issues immediately. Several audits for tire crumb sample collection and sample analysis activities were previously described in the Part 1 Report (U.S. EPA & CDC/ATSDR, 2019). Additional technical system audits (TSAs) were conducted on exposure pilot study field sampling and for the publicly-available videography data compilation. The purpose of each audit was to ensure that the research tasks prescribed within the QAPPs or SOPs were verified and documented. These audits are summarized in Table B-2. **No significant findings were identified during the audits, and minor findings that were identified did not directly affect the integrity or quality of the data.**

B.2.4 Deviations from the QAPPs or SOPs

There were no significant deviations from the QAPP addendums listed in Table B-1. Deviations from SOPs identified during field or laboratory activities were documented and confirmed, if applicable, during field or laboratory audits. All SOPs unique to this project that deviated from the original procedure were amended and, if needed, reviewed by the QAM and approved by the analyst's supervisor. Minor changes such as mislabeled sampling containers, contaminated sampling tools or issues identified in the field related to specific samples or information collection were documented on

the TCRS field forms and chain of custody.

Table B-2. Tire Crumb Research Study Exposure Characterization Technical System Audits[a,b]

Date	Target	Description	Interviewed	Auditor
09/25 – 27/2017	Exposure Characterization Field Sampling and Monitoring	TSA of field data collection activities and adherence to planned activities was conducted at a one exposure pilot study location. In addition, data extraction activities taken from videos recorded during the field collection at this location was conducted upon return to the RTP EPA NERL laboratory by JTI. This TSA was extended to assess if JTI followed the approved SOP for Videography of Activity Characterization Study Participants. No corrective actions were deemed necessary and no findings were identified.	Kent Thomas, Matt Allen, team lead for JTI and team members which included Denise Popeo-Murphy, Patrick Lawler, and Guy Fazzio	Christine Alvarez
12/06/2016	Videography and Surveys	Onsite TSA was conducted at the NERL laboratory, RTP office to assess QA/QC procedures specified in the SOP for Collecting and Using Extant Publicly Available Video. Coding of the extant videography data had not been performed prior or during the audit, therefore, only those activities involving collection and handling of videography files could be assessed. Findings included lack of research notebooks to document daily activities. Corrective action was issued to the group coding the collected data and to the task lead.	Marsha Morgan	Brittany Stuart, Christine Alvarez reviewed the research notebook after completion of audit

[a] All documentation associated with these audits including audit reports, corrective actions and email correspondence is documented and saved in the TCRS QA SharePoint, https://usepa.sharepoint.com/sites/ORD_Work/TCRS%20QA/SitePages/Home.aspx

[b] TSA = technical system audit; TCRS = Tire Crumb Research Study; QAPP = quality assurance project plan; SOP = standard operating procedure; RTP = Research Triangle Park; EPA = U.S. Environmental Protection Agency; NERL = National Exposure Research Laboratory; JTI = Jacobs Technology, Incorporated; QA = quality assurance; QC = quality control

B.2.5 Data Quality Reviews

Reviews of data quality were performed at several stages throughout the course of the research study (Table B-3). Data produced through field sample collection, data collection and sample analysis received data quality reviews by QAMs and/or secondary technical expert reviewers. Reviews were performed after data were produced and before they were submitted for data processing or included in data analysis.

Much of the analytical chemistry measurement data for the exposure characterization pilot study was compiled, standardized and processed by data managers to prepare data analysis files. Data quality reviews were performed to verify that the data in the data analysis files were correct and complete and that all processing calculations were performed correctly.

Using the data analysis files, data were organized to prepare outputs for reporting, such as tables and figures. Statistical summaries of the data were prepared and in some cases, statistical testing was performed. Data quality reviews were performed to ensure that the data analysis outputs were complete and correct and that data calculations and analyses were performed correctly.

Finally, multiple data quality reviews were performed to verify that the outputs from the data analyses were correctly and completely compiled in report tables and figures. This set of data quality reviews is depicted in the Table B-3, but does not quantify the number of reviews completed for data compilation and analysis.

Table B-3. Data Quality Reviews of Tire Crumb Exposure Characterization[a,b]

Data/Information Type	Technical Lead	Reviewer	Completion Date
Field Data	Kent Thomas	Margie Vazquez	02/09/2018
Metals ICP/MS Digests	Kasey Kovalcik	Clay Nelson	02/26/2018
SVOC GC/MS/MS	Scott Clifton/Dawn Mills	Elin Ulrich	01/25/2018, 02/13/2018
SVOC LC/MS	Larry McMillan, Elin Ulrich	Jim Starr	02/14/2018, 02/27/2018
VOC TOFMS	Don Whitaker	Christine Alvarez and Rachel Porter	Final date: 03/08/2018
Filter Weighing	Chen Fu-Lin	Kent Thomas, Rachel Porter, Christine Alvarez check	01/24/2018
Characterization and Videography Summary	Jacobs Technology Incorporated	Marsha Morgan/Christine Alvarez	10/2017 and 12/2017

[a] ICP/MS = inductively coupled plasma/mass spectrometry; SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry; GC/MS = gas chromatography/mass spectrometry; LC/MS = liquid chromatography/mass spectrometry; VOC = volatile organic compound; TOFMS = time of flight mass spectrometry

[b] Errors or issues identified during data quality reviews (e.g., transcription errors) are documented on the TCRS QA SharePoint, https://usepa.sharepoint.com/sites/ORD_Work/TCRS%20QA/SitePages/Home.aspx

B.3 Quality Control Overview

Numerous quality control activities and analyses were performed over the course of the study and included, but were not limited to the following:

- Sample collection media and sample containers were pre-cleaned or purchased as certifiably clean, when appropriate;
- Whenever possible, media were evaluated prior to field deployment to ensure minimal background or interferences, and blank media were analyzed to assess potential background contamination;
- Chain of custody procedures were implemented for all samples;
- Field quality control samples, consisting of blank, spike, and duplicate samples, were taken when applicable; location-specific field blanks were taken to and handled in the field in the same manner as samples, including opening and closing of containers, where appropriate;
- Laboratory quality control samples were applied, as appropriate, for each analysis method and included one or more of the following: procedure or method blanks and spikes, matrix blanks and spikes where feasible, and replicate sample analysis;
- Reference standards were obtained from reputable and traceable sources, where available;
- Solvents used for device cleaning, media preparation, or sample extraction were HPLC-grade or better in purity;
- Appropriate methods were used to determine analytical detection or quantifiable limits and to quantify target chemical amounts in samples;
- Blank and recovery correction were applied, as appropriate;
- Research notebooks were maintained.

Key quality control measures and their results are reported in this Appendix, including:

- *Completeness:* a measure of the amount of verified data obtained from a measurement system compared to the amount of data that was expected to be obtained under normal conditions.
- *Quantification Limits*: the lowest concentration or amount of analyte that can be measured in an analytical method to a known and acceptable degree of confidence and precision. This is determined in a manner that is appropriate and applicable for each type of measurement.
- *Background:* the amount of analyte or signal present that was not associated with the sample and can interfere with or inflate measurement results. Background is assessed by using unspiked field and/or laboratory media and analyses.
- *Precision:* a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. Precision is best expressed in terms of the standard deviation.
- *Accuracy:* the degree of agreement of measurements (or an average of measurements) with an accepted reference or true value. Accuracy is a measure of the bias or systematic error in a system and was assessed by measuring recovery of target analytes through laboratory analysis and where applicable, through combined field and laboratory conditions and procedures.

Each of these general quality criteria and the process by which they were addressed were not universal throughout the study. Each analyst's task and characterization process could differ substantially; therefore, it was impossible to have a single standard operating procedure or consistent approach for addressing or validating all methods used in tire crumb rubber characterization and exposure

measurement analysis. This Appendix describes how each method addressed the general quality control measures described above.

Each analytical method had its own set of quality control measures appropriate for that method. In addition to the assessments listed above, the SOPs for the sample collection and sample analysis methods described quality control elements that were implemented for each method. Not all quality control procedures and results are reported here. For example, calibration procedures and acceptance criteria, mass spectrometer tuning check procedures, and other quality-related activities related to quantitative analysis were described in the quantitative analysis SOPs. Quality control procedures for field sample collection and analysis were also described in their respective SOPs.

B.3.1 Exposure Characterization Pilot Study

The exposure characterization pilot study included several types of quality control samples for each field and lab medium that was sampled (Tables B-4 and B-5, respectively). Field blanks were used to assess potential contamination or background. Where applicable, the field blanks (e.g., dermal wipes and field wipes) were handled at the field site in the same way the samples were handled to account for any potential contamination during handling (e.g., chemical transfer from gloves). Spiked field controls were deployed where possible to assess overall analyte recovery through the field condition, transport, storage, and analysis activities. Duplicate samples were collected when possible to assess overall measurement precision. Laboratory blanks and laboratory spiked controls were prepared to assess background and recovery for media not deployed to the field sites.

Table B-4. Number and Types of Field Quality Control Samples for Exposure Field Study[a]

Sample Type	Analytes	First Field Sampled - Field Blanks (# per field)	First Field Sampled - Spiked Field Control (# per field)	First Field Sampled - Co-located Duplicate Sample (# per field)	Remaining Fields - Field Blanks (# per field)	Remaining Fields - Spiked Field Control (# per field)	Remaining Fields – Co-located Duplicate Sample (# per field)
Personal Samples	Air[b] VOCs	0	0	0	0	0	0
Personal Samples	Dermal SVOCs	2	2	0	1	1	0
Personal Samples	Dermal Metals	2	2	0	1	1	0
Field Air	VOCs (passive)	2	2	1	1	1	1
Field Air	VOCs (active)	2	2	1	1	1	1
Field Air	SVOCs	2	2	1	1	1	1
Field Air	Particulates/Metals	2	0	1	1	0	1
Field Drag Sled	SVOCs	2	2	1	1	1	1
Field Surface Wipe	SVOCs	2	2	1	1	1	1
Field Surface Wipe	Metals	2	2	1	1	1	1
Field Dust	SVOCs	2	0	0	1	0	1
Field Dust	Metals	2	0	0	1	0	1

[a] VOC = volatile organic compound; SVOC = semivolatile organic compound

[b] QC samples for personal air VOCs are covered in the passive facility air VOC collection since they use the same sampler

Table B-5. Number and Types of Laboratory Quality Control Samples[a]

Sample Type	Analytes	First Field Sampled - Lab Blank (# per field)	First Field Sampled - Spiked Lab Control (# per field)	First Field Sampled – Lab Replicate[b] (# per field)	Remaining Fields - Lab Blank (# per field)	Remaining Fields – Spiked Lab Control (# per field)	Remaining Fields – Lab Replicate[b] (# per field)
Personal Samples	Air[c] VOCs	0	0	0	0	0	0
Personal Samples	Dermal SVOCs	2	2	2	1	1	1
Personal Samples	Dermal Metals	2	2	2	1	1	1
Field Air	VOCs (passive)	2	2	0	1	1	0
Field Air	VOCs (active)	2	2	0	1	1	0
Field Air	SVOCs	2	2	2	1	1	1
Field Air	Particulates/Metals	2	0	2	1	1	1
Field Drag Sled	SVOCs	2	2	2	1	1	1
Field Surface Wipe	SVOCs	2	2	2	1	1	1
Field Surface Wipe	Metals	2	2	2	1	1	1
Field Dust	SVOCs	2	2	1	1	1	1
Field Dust	Metals	2	2	1	1	1	1

[a] VOC = volatile organic compound; SVOC = semivolatile organic compound

[b] Replicate analysis of sample extract

[c] QC samples for personal air VOCs are covered in the passive facility air VOC collection since they use the same sampler

Overall project-level DQI are listed in Table B-6. Because there are no standard methods for sample collection and analysis procedures for measuring environmental and personal exposures at synthetic turf fields, the DQI target values developed for exposure characterization were considered to be objectives and were assessed as the work proceeded and following work completion. Additional data quality indicators were described, where applicable, in the technical SOPs for each experimental or analytical method.

Table B-6. Target Exposure Characterization Pilot Study Quantitative Data Quality Indicator Objectives[a,b]

Metric	Precision (%)	Accuracy (%)	% Completeness - Collection	% Completeness - Analysis
Metals ICP/MS	± 25	75 – 125	90	95
VOC TD/GC/TOFMS	± 25	70 – 130	90	95
SVOC GC/MS/MS	± 25	70 – 130	90	95
SVOC LC/MS	± 25	70 – 130	90	95

[a] Collection completeness is based on the number of samples attempted for collection. It is not based on the overall design goals for numbers of fields and participants

[b] VOC = volatile organic compound; TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry; SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry; LC/MS = liquid chromatography/mass spectrometry; ICP/MS = inductively coupled plasma/mass spectrometry

B.4 Exposure Characterization Pilot Study Quality Control Results

Exposure characterization pilot study quality control information, measurements, and results are reported in the following subsections for:

- Measurement of total suspended particulate in field air samples (Section B.4.1),
- Measurement of metals by ICP/MS in field air, field dust, field wipe, and dermal wipe samples (Section B.4.2),
- Measurement of VOCs by TD/GC/TOFMS in field air samples (Section B.4.3),
- Measurement of SVOCs by GC/MS/MS in field air, field dust, field wipe, drag sled, and dermal wipe samples (Section B.4.4),
- Attempted measurement of SVOCs by LC/MS in field air, field dust, field wipe, drag sled, and dermal wipe samples (Section B.4.5),
- Field user questionnaires (Section B.4.6), and,
- Video activity data analysis (Section B.4.7).

B.4.1 Total Suspended Particulate in Field Air QC Samples

Total suspended particulate was measured in field air during four sample collection events. All scheduled samples were successfully collected and analyzed. Five field blanks carried to the field sites and returned with the samples had average weight increases on the filters of 5.8 ± 4.4 µg/filter. Five lab blanks that remained in the laboratory had average weight increases on the filters of 1.6 ± 3.4 µg/filter. The average field blank result was subtracted from the total suspended particulate measurement prior to calculating concentrations in air. The average percent relative standard deviation for four duplicate sample collection and analysis measurements was 11 ± 5.0%.

B.4.2 Metals in Field Air, Field Dust, Field Wipe, and Dermal Wipe QC Samples

Completeness – All (100%) of the scheduled exposure characterization pilot study samples were successfully analyzed for metals by ICP/MS.

Quantification Limits – Table B-7 reports the minimum reportable limits for metal analytes for field wipe, dermal wipe, field dust, and field air samples.

Table B-7. Minimum Reportable Limits for Metals in Field and Dermal Wipe, Field Dust, and Field Air Filter Samples Analyzed by ICP/MS[a]

Chemical	Field Wipes Minimum Reportable Limit (ng/cm^2)	Dermal Wipes Minimum Reportable Limit (ng/cm^2)	Field Dust Samples Minimum Reportable Limit (mg/kg)	Field Air Filters Minimum Reportable Limit (ng/m^3)[b]
Aluminum	0.00686	0.05692	0.783	2.93
Antimony	0.00019	0.00155	0.035	0.03
Arsenic	0.00319	0.02646	0.039	0.50
Barium	0.00192	0.01594	1.460	1.29
Beryllium	0.00041	0.00343	0.031	0.79
Cadmium	0.00007	0.00057	0.023	0.16
Chromium	0.00090	0.00749	0.147	0.20
Cobalt	0.00008	0.00069	0.012	0.46
Copper	0.00060	0.00501	0.144	1.06
Iron	0.01037	0.08602	1.743	11.22
Lead	0.00070	0.00583	0.549	0.30
Magnesium	0.00579	0.04805	1.855	13.85
Manganese	0.00023	0.00190	0.084	0.33
Molybdenum	0.00014	0.00116	0.028	0.05
Nickel	0.00009	0.00078	0.015	0.57
Rubidium	0.00760	0.06302	0.045	1.82
Selenium	0.01153	0.09565	3.344	2.92
Strontium	0.00094	0.00783	0.295	0.22
Tin	0.00005	0.00045	0.017	0.05
Vanadium	0.00101	0.00834	0.113	0.19
Zinc	0.00677	0.05614	0.425	2.84

[a] ICP/MS = inductively coupled plasma/mass spectrometry

[b] Based on a nominal air sampling volume of 3.43 m^3

Blanks – Table B-8 reports average concentrations of metals across field and laboratory blanks for field and dermal wipes and field air filters. Relatively high background levels were observed in the wipe materials for aluminum, iron, magnesium and zinc. These were also elements with relatively high measurement results in the tire crumb rubber samples. Metals concentrations were adjusted for each sample by subtracting the field blank result obtained for each field site from the analysis result for each metal in samples collected at that field site.

Table B-8. Field and Laboratory Blank Quality Control Results for Metals in Field and Dermal Wipes and Field Air Filters Analyzed by ICP/MS[a,b]

Chemical	Field and Dermal Wipe Blanks Mean (ng/wipe)	Field and Dermal Wipe Blanks Standard Deviation (ng/wipe)	Field Air Filter Blanks Mean (ng/filter)	Field Air Filter Blanks Standard Deviation (ng/filter)
Aluminum	4120	3370	137	44
Antimony	38.5	3.8	3.87	7.13
Arsenic	74.2	11.5	< MRL	N/A
Barium	147	36	< MRL	N/A
Beryllium	6.72	1.47	< MRL	N/A
Cadmium	10.4	1.6	< MRL	N/A
Chromium	72.9	11.1	49.1	2.7
Cobalt	2.78	0.95	< MRL	N/A
Copper	728	79	25.9	20.8
Iron	3610	637	72.9	55.4
Lead	19.6	6.3	1.61	1.08
Magnesium	18,800	619	21.9	13.8
Molybdenum	39.6	3.5	0.320	0.099
Nickel	53.5	12.8	4.44	3.24
Rubidium	< MRL	N/A	< MRL	N/A
Selenium	< MRL	N/A	< MRL	N/A
Strontium	140	21	1.05	0.53
Tin	7.29	11.53	1.53	1.02
Vanadium	11.9	1.8	0.872	0.084
Zinc	81,700	6760	122	87.9

[a] ICP/MS = inductively coupled plasma/mass spectrometry; MRL = minimum reportable limit; N/A = not applicable

[b] Wipe Blanks (n=18), Field Air Sample Blanks (n=8)

Recovery – Table B-9 reports recovery results for metal analytes from the method spike solution and field and dermal wipe spiked controls across field and laboratory sampling. Average recoveries from the spiked controls ranged from 83% to 120%.

No standard synthetic turf field dust sample is available, and there are no methods for spiking an equivalent dust with metals. The National Institute of Standards and Technology (NIST) standard reference material (SRM) 1648a (urban particulate matter) was used to prepare field and laboratory controls. Recovery of metals from this material were uneven, and not all metals had certified values. Results are shown in Table B-10. Recoveries for cadmium, cobalt, lead and zinc ranged from 74% to 110%. However, the average recovery for chromium was only 20%. It is not known how well this urban particulate matter SRM represents dust collected from synthetic turf fields. No recovery adjustments were performed for the exposure characterization pilot study samples.

Table B-9. Spike Recovery Quality Control Results for Metals in Field and Dermal Wipes After Microwave Digestion by ICP/MS[a,b]

Chemical	Method Spike Mean % Recovery	Method Spike % Recovery Standard Deviation	Wipe Spiked Controls Mean % Recovery	Wipe Spiked Controls % Recovery Standard Deviation
Aluminum	97	7	85	25
Antimony	92	3	94	5
Arsenic	78	8	87	3
Barium	99	4	98	5
Beryllium	89	5	91	3
Cadmium	89	4	91	5
Chromium	96	5	98	8
Cobalt	97	5	95	4
Copper	94	4	95	5
Iron	96	5	93	7
Lead	97	4	96	5
Magnesium	94	4	93	5
Molybdenum	94	5	95	4
Nickel	94	4	95	6
Rubidium	96	4	96	5
Selenium	70	7	83	5
Strontium	98	5	94	5
Tin	98	5	91	6
Vanadium	96	5	95	4
Zinc	92	6	120	48

[a] ICP/MS = inductively coupled plasma/mass spectrometry

[b] Method Spikes (n=8), Wipe Spiked Controls (n=18); Spike = 250 microliters (µL); Spike solution from SCP Science (Champlain, NY)

Table B-10. Recovery Quality Control Results for Metals in Dust Surrogate (NIST SRM 1648a Urban Particulate Matter) Analyzed by ICP/MS[a,b]

Chemical	NIST 1648a Mean % Recovery	NIST 1648a % Recovery Standard Deviation
Aluminum	34	6
Antimony	68	13
Arsenic	116	21
Barium	*	N/A
Beryllium	*	N/A
Cadmium	94	15
Chromium	20	3
Cobalt	74	14
Copper	106	19
Iron	55	10
Lead	97	15
Magnesium	95	17

Table B-10 Continued

Chemical	NIST 1648a Mean % Recovery	NIST 1648a % Recovery Standard Deviation
Molybdenum	*	N/A
Nickel	78	13
Rubidium	30	5
Selenium	88	13
Strontium	88	15
Tin	*	N/A
Vanadium	86	15
Zinc	110	20

[a] NIST = National Institute of Standards and Technology; SRM = standard reference material; ICP/MS = inductively coupled plasma/mass spectrometry; N/A = not applicable

[b] NIST SRM 1648a (n=8); approximately 20-30 mg of sample used

* Several elements do not have certified reference values

Precision –Duplicate samples were collected for field air and for field wipe samples. Measurement precision results for metals in these duplicate samples are reported in Table B-11. Average % relative standard deviation (%RSD) values for metals in field air duplicates ranged from 3.7% to 98%. The average %RSDs for cobalt, lead, and zinc in field air duplicates were 6%, 11%, and 39%, respectively; the average %RSD for chromium was 56%. Average %RSD values for metals in field wipes ranged from 7% to 24% for all metals except cobalt, which had an average %RSD of 53%. Duplicate measurement results for field wipes may include components of both measurement precision as well as spatial heterogeneity in loading levels across sampled surfaces.

Table B-11. Measurement Precision Quality Control Results for Metals in Duplicate Field Air and Field Wipe Samples Analyzed by ICP/MS

Chemical	Field Air Duplicate Samples Average % Relative Standard Deviation	Field Wipe Duplicate Samples Average % Relative Standard Deviation
Aluminum	6.8	12
Arsenic	12	10
Barium	9.1	24
Beryllium	31	11
Cadmium	98	16
Cobalt	6.0	53
Chromium	56	13
Copper	46	11
Iron	7.0	15
Magnesium	3.7	7
Manganese	9.1	11
Molybdenum	21	9
Nickel	75	14
Lead	11	19
Rubidium	7.0	15
Antimony	12	12
Selenium	32	ND[c]
Tin	66	23
Strontium	8.1	14
Vanadium	8.5	11
Zinc	39	21

[a] ICP/MS = inductively coupled plasma/mass spectrometry

[b] Field Air Duplicate Samples (n=4 sample pairs), Field Wipe Duplicate Samples (n=3 sample pairs)

[c] Selenium was not detected in field wipe samples

DQI – Based on the quality control measurement results, DQI objectives were met for lead in all media. All metals met recovery objectives in the field air and the field and dermal wipe media; however, recoveries were uneven in the dust surrogate (NIST SRM 1648a) media. Precision was uneven in the field air media, but all metals except cobalt met the precision objective in the wipe media.

B.4.3 VOCs in Field Air QC Samples

Completeness – All (100%) of the scheduled exposure characterization pilot study active fence line monitor (FLM) field air samples were successfully collected and analyzed. None of the sample measurement results from the Radiello passive field air samples or personal air samples were reported in Volume 1 of this report due to inconsistent sample collection rates determined between laboratory and field trials and because of unacceptably low recoveries for benzothiazole and methyl isobutyl ketone. However, results for the Radiello quality control samples are reported in this Appendix.

Quantification Limits – Table B-12 reports the method detection limits for VOC analytes in FLM field air samples.

Table B-12. Minimum Detection Limits for VOCs Measured in Fence Line Monitor Air Samples Analyzed by TD/GC/TOFMS[a]

Chemical	FLM Air Samples Minimum Detection Limits (ng/m^3)[b]
Freon 12	2.94
1,3-Butadiene	8.24
trans-2-Butene	6.47
cis-2-Butene	8.24
Freon 11	5.29
1,1-Dichloroethene	14.7
Freon 113	4.12
1,1-Dichloroethane	21.2
cis-1,2-Dichloroethene	43.5
1,2-Dichoroethane	21.2
1,1,1-Trichloroethane	45.3
Benzene	49.4
Carbon tetrachloride	35.3
1,2 -Dichloropropane	29.4
Trichloroethene	81.2
Methyl isobutyl ketone	64.1
Toluene	14.7
Tetrachloroethene	2.35
Chlorobenzene	1.76
Ethylbenzene	4.71
m,p-Xylene	9.41
Styrene	14.1
o-Xylene	5.29
4-Ethyltoluene	30.6
1,3,5-Trimethylbenzene	20.0
m-Dichlorobenzene	5.88
p-Dichlorobenzene	9.41
o-Dichlorobenzene	5.29
Benzothiazole	282

[a] TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry; VOC = volatile organic compound; FLM = fence line monitor

[b] Based on a nominal air sample volume of 0.017 m^3

Blanks – Table B-13 reports average amounts of VOC analytes measured in FLM run blanks (blank tubes prepared in the analysis laboratory), laboratory blanks, and field blanks. All average VOC results for the FLM blanks were ≤ 1.02 ng/tube. Table B-14 reports average amounts of VOC analytes measured in Radiello passive field air and personal air laboratory and field blanks. All average VOC results for the Radiello blanks were ≤ 1.62 ng/tube. Air sample concentrations were adjusted by subtracting the field blank result obtained for each field site from the analysis result for each VOC in samples collected at that field site.

Table B-13. Run, Laboratory, and Field Blank Quality Control Results for VOCs in Fence Line Monitor Air Samples Analyzed by TD/GC/TOFMS[a]

Chemical	FLM Run Blank Mean (ng/tube)	FLM Run Blank Standard Deviation (ng/tube)	FLM Lab Blank Mean (ng/tube)	FLM Lab Blank Standard Deviation (ng/tube)	FLM Field Blank Mean (ng/tube)	FLM Field Blank Standard Deviation (ng/tube)
Freon 12	0.15	0.06	0.19	0.07	0.17	0.04
1,3-Butadiene	0.47	0.27	0.33	0.06	0.58	0.40
trans-2-Butene	0.14	0.07	0.11	0.03	0.16	0.07
cis-2-Butene	0.13	0.06	0.11	0.02	0.12	0.09
Freon 11	0.25	0.17	0.33	0.01	0.34	0.01
1,1-Dichloroethene	0.01	0.05	0.04	0.09	0.04	0.08
Freon 113	0.19	0.22	0.24	0.19	0.24	0.19
1,1-Dichloroethane	0.00	0.00	0.00	0.00	0.00	0.00
cis-1,2-Dichloroethene	-0.01	0.03	0.00	0.00	0.00	0.00
1,2-Dichoroethane	0.00	0.00	0.00	0.00	0.00	0.00
1,1,1-Trichloroethane	0.06	0.18	0.12	0.23	0.00	0.00
Benzene	0.60	0.16	0.50	0.07	0.57	0.19
Carbon tetrachloride	0.00	0.00	0.00	0.00	0.00	0.00
1,2 -Dichloropropane	0.00	0.00	0.00	0.00	0.00	0.00
Trichloroethene	0.31	0.21	0.32	0.16	0.32	0.16
Methyl isobutyl ketone	0.00	0.00	0.00	0.00	0.00	0.00
Toluene	0.56	0.16	0.61	0.07	0.74	0.17
Tetrachloroethene	0.31	0.21	0.32	0.16	0.32	0.16
Chlorobenzene	0.08	0.15	0.14	0.17	0.08	0.16
Ethylbenzene	0.38	0.13	0.30	0.15	0.31	0.15
m,p-Xylene	0.94	0.21	0.88	0.03	0.73	0.36
Styrene	0.41	0.39	0.26	0.32	0.40	0.33
o-Xylene	0.41	0.14	0.33	0.16	0.33	0.17
4-Ethyltoluene	0.12	0.20	0.00	0.00	0.07	0.14
1,3,5-Trimethylbenzene	0.11	0.13	0.00	0.00	0.04	0.09
m-Dichlorobenzene	0.17	0.22	0.07	0.15	0.07	0.15
p-Dichlorobenzene	0.16	0.22	0.07	0.14	0.07	0.14
o-Dichlorobenzene	0.34	0.20	0.33	0.16	0.24	0.20
Benzothiazole	0.90	0.45	1.02	0.04	1.00	0.11

[a] VOC = volatile organic compound; TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry; FLM = fence line monitor

[b] FLM Run Blanks (n=18), FLM Laboratory Blanks (n=5), FLM Field Blanks (n=5)

Table B-14. Laboratory and Field Blank Quality Control Results for VOCs in Radiello Passive Air Samples Analyzed by TD/GC/TOFMS[a,b]

Chemical	Radiello Lab Blank Mean (ng/tube)	Radiello Lab Blank Standard Deviation (ng/tube)	Radiello Field Blank Mean (ng/tube)	Radiello Field Blank Standard Deviation (ng/tube)
Freon 12	0.09	0.08	0.20	0.05
1,3-Butadiene	0.00	0.00	0.00	0.00
trans-2-Butene	0.00	0.00	0.01	0.01
cis-2-Butene	0.00	0.00	0.01	0.02
Freon 11	0.13	0.15	0.20	0.16
1,1-Dichloroethene	0.00	0.00	0.04	0.08
Freon 113	0.15	0.18	0.29	0.15
1,1-Dichloroethane	0.00	0.00	0.07	0.14
cis-1,2-Dichloroethene	0.00	0.00	-0.03	0.05
1,2-Dichoroethane	0.00	0.00	0.09	0.18
1,1,1-Trichloroethane	0.00	0.00	0.11	0.22
Benzene	1.62	0.95	1.14	0.36
Carbon tetrachloride	0.00	0.00	0.12	0.24
1,2 -Dichloropropane	0.00	0.00	0.12	0.23
Trichloroethene	0.31	0.16	0.24	0.19
Methyl isobutyl ketone	0.02	0.05	0.03	0.03
Toluene	0.40	0.03	0.48	0.14
Tetrachloroethene	0.31	0.16	0.24	0.19
Chlorobenzene	0.22	0.18	0.30	0.15
Ethylbenzene	0.22	0.18	0.22	0.18
m,p-Xylene	0.51	0.42	0.68	0.34
Styrene	0.13	0.26	0.13	0.26
o-Xylene	0.24	0.19	0.24	0.19
4-Ethyltoluene	0.07	0.13	0.07	0.13
1,3,5-Trimethylbenzene	0.05	0.09	0.04	0.08
m-Dichlorobenzene	0.00	0.00	0.14	0.18
p-Dichlorobenzene	0.00	0.00	0.07	0.14
o-Dichlorobenzene	0.25	0.20	0.41	0.01
Benzothiazole	0.20	0.40	0.40	0.50

[a] VOC = volatile organic compound; TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry

[b] Laboratory Blanks (n=5), Field Blanks (n=5)

Recovery – Table B-15 reports average recoveries of VOC analytes measured in FLM calibration checks, spiked laboratory controls, and spiked field controls. Average recoveries ranged from 60% to 139% in the calibration checks, 75% to 176% in the spiked lab controls, and 66% to 170% in the spiked field controls. Most analytes had recoveries in the range of 70% to 130%. No recovery adjustments were performed for the exposure characterization pilot study samples.

Table B-16 reports average recoveries of VOC analytes measured in Radiello passive field air and personal

air spiked laboratory controls and spiked field controls. Average recoveries ranged from 4% to 370% in the spiked field controls. Mean recovery values of 12% or less were observed for methyl isobutyl ketone and benzothiazole. Mean recovery values of 370% to 373% were observed for cis-1,2-dichloroethene.

Table B-15. Calibration Check and Spike Recovery Quality Control Results for VOCs in Fence Line Monitor Air Samples Analyzed by TD/GC/TOFMS[a,b,c]

Chemical	FLM Run Calibration Check Mean % Recovery	FLM Run Calibration Check % Recovery Standard Deviation	FLM Spiked Lab Control[d] Mean % Recovery	FLM Spiked Lab Control[d] % Recovery Standard Deviation	FLM Spiked Field Control Mean % Recovery	FLM Spiked Field Control % Recovery Standard Deviation
Freon 12	105	5	101	4	100	2
1,3-Butadiene	86	11	110	3	104	9
trans-2-Butene	96	4	103	3	97	5
cis-2-Butene	95	4	102	4	98	6
Freon 11	98	8	105	3	99	4
1,1-Dichloroethene	97	8	106	4	104	3
Freon 113	99	5	104	3	102	2
1,1-Dichloroethane	99	12	125	12	115	8
cis-1,2-Dichloroethene	133	27	150	27	134	8
1,2-Dichoroethane	123	30	132	25	115	8
1,1,1-Trichloroethane	98	12	111	10	102	4
Benzene	93	6	100	3	99	4
Carbon tetrachloride	139	42	109	19	99	6
1,2 -Dichloropropane	109	27	128	22	117	7
Trichloroethene	95	5	103	2	101	1
Methyl isobutyl ketone	60	32	176	79	170	28
Toluene	91	9	101	3	101	4
Tetrachloroethene	95	5	103	2	101	1
Chlorobenzene	99	1	101	2	104	8
Ethylbenzene	86	6	92	3	95	9
m,p-Xylene	86	7	98	2	101	10
Styrene	83	6	95	5	100	13
o-Xylene	88	7	97	2	98	8
4-Ethyltoluene	99	17	99	11	105	21
1,3,5-Trimethylbenzene	97	19	93	14	97	24
m-Dichlorobenzene	101	5	94	2	94	8
p-Dichlorobenzene	101	5	93	2	94	9
o-Dichlorobenzene	101	4	89	3	90	8
Benzothiazole	120	29	75	22	66	6

[a] VOC = volatile organic compound; TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry; FLM = fence line monitor

[b] FLM Run Calibration Check (n=14), FLM Lab Control (n=5), FLM Field Control (n=6); Spike=2.2–14.8 ng/tube

[c] Recoveries are calculated using the blank corrected tube results and the theoretical mass (ng) loaded.

[d] All Lab Spikes were prepared on Carbopack™ X tubes at nominal concentrations of either 1 or 2 ppbv

Table B-16. Field and Laboratory Spike Recovery Quality Control Results for VOCs in Radiello Passive Samples Analyzed by TD/GC/TOFMS [a,b]

Chemical	Radiello Spiked Lab Control Mean % Recovery	Radiello Spiked Lab Control % Recovery Standard Deviation	Radiello Spiked Field Control Mean % Recovery	Radiello Spiked Field Control % Recovery Standard Deviation
Freon 12	83	5	84	6
1,3-Butadiene	38	9	56	10
trans-2-Butene	86	3	90	7
cis-2-Butene	83	4	88	6
Freon 11	88	3	91	6
1,1-Dichloroethene	93	7	96	11
Freon 113	95	3	94	5
1,1-Dichloroethane	25	15	33	11
cis-1,2-Dichloroethene	373	12	370	15
1,2-Dichoroethane	130	29	155	21
1,1,1-Trichloroethane	24	12	26	3
Benzene	90	12	113	28
Carbon tetrachloride	81	36	57	29
1,2 -Dichloropropane	36	28	38	6
Trichloroethene	96	3	94	5
Methyl isobutyl ketone	3	3	4	3
Toluene	79	3	80	5
Tetrachloroethene	96	3	94	5
Chlorobenzene	95	2	94	2
Ethylbenzene	78	10	74	10
m,p-Xylene	77	12	73	11
Styrene	56	16	55	13
o-Xylene	79	11	75	9
4-Ethyltoluene	69	12	65	14
1,3,5-Trimethylbenzene	63	13	60	15
m-Dichlorobenzene	83	2	85	2
p-Dichlorobenzene	83	2	84	1
o-Dichlorobenzene	80	1	81	2
Benzothiazole	10	6	12	2

[a] VOC = volatile organic compound TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry

[b] Radiello Laboratory Control (n=5), Radiello Field Control (n=4); Spike=2.2–14.8 ng/tube

[c] Recoveries are calculated using the blank corrected tube results and the theoretical mass (ng) loaded.

Precision – Duplicate FLM field air measurement precision results are reported in Table B-17. Average %RSD values for VOCs in field air duplicates ranged from 2% to 43%, with most %RSD values < 25%.

DQI – Most DQI objectives were met for most field air VOC analytes collected using FLM tubes. Benzothiazole had an average recovery of 66% in spiked field controls. Methyl isobutyl ketone had an average 170% recovery in the spiked field controls. Trichloroethylene and o-dichlorobenzene had average relative percent standard deviations greater than 25% in duplicate samples.

Table B-17. Measurement Precision Quality Control Results for Duplicate Field Air VOC Fence Line Monitor Samples Analyzed by TD/GC/TOFMS[a,b]

Chemical	Field Air FLM Duplicate Samples Average % Relative Standard Deviation
Methyl isobutyl ketone	20
Benzothiazole	15
1,3-Butadiene	24
Styrene	20
Benzene	17
Toluene	17
Ethylbenzene	16
m/p-Xylene	17
o-Xylene	17
trans-2-Butene	18
cis-2-Butene	21
4-Ethyltoluene	11
1,3,5-Trimethylbenzene	16
1,1-Dichloroethene	2
1,1-Dichloroethane	3
cis-1,2-Dichloroethene	NM
1,2-Dichloroethane	19
1,1,1-Trichloroethane	7
Carbon Tetrachloride	33
1,2-Dichloropropane	NM
Trichloroethylene	43
Tetrachloroethylene	16
Chlorobenzene	3
m-Dichlorobenzene	5
p-Dichlorobenzene	9
o-Dichlorobenzene	35
Trichlorofluoromethane (Freon 11)	14
Dichlorodifluoromethane (Freon 12)	4
Trichlorotrifluoroethane (Freon 113)	13

[a] VOC = volatile organic compound; TD/GC/TOFMS = thermal desorption/liquid chromatography/time-of-flight mass spectrometry; FLM = fence line monitor; NM = not measured in samples

[b] Field Air Duplicate Samples (n=4 sample pairs)

B.4.4 SVOCs in Field Air, Field Dust, Field Wipe, Field Drag Sled and Dermal Wipe QC Samples

Completeness – All (100%) of the scheduled exposure characterization pilot study field air, field dust, field wipe, field drag sled, and dermal wipe samples were successfully collected and analyzed for SVOCs by GC/MS/MS.

Quantification Limits – Minimum quantifiable limits (MQLs) for SVOC analytes were determined for field air, field dust, drag sled, field wipe, and dermal wipe samples. Results are shown in Table B-18.

Table B-18. Minimum Quantifiable Limits for SVOCs in Field Air, Field Dust, Drag Sled, Field Wipe, and Dermal Wipe Sample Extracts Analyzed by GC/MS/MS[a]

Chemical	Field Air MQL (ng/m^3)[b]	Field Dust MQL (mg/kg)	Drag Sled MQL (ng/cm^2)	Field Wipe MQL (ng/cm^2)	Dermal Wipe MQL (ng/cm^2)
Aniline	0.148	0.01	0.000010	0.00108	0.0006 – 0.0089
n-Butylbenzene	0.074	0.0025	0.000005	0.00027	0.0012 – 0.0089
Naphthalene	0.148	0.025	0.000005	0.00269	0.0060 – 0.0222
Benzothiazole	0.148	0.005	0.000010	0.00054	0.0024 – 0.0222
Cyclohexylisothiocyanate	0.148	0.005	0.000020	0.00054	0.0012 – 0.0089
2-Methylnaphthalene	0.740	0.025	0.000050	0.00269	0.0060 – 0.0089
1-Methylnaphthalene	0.296	0.005	0.000020	0.00054	0.0024 – 0.0089
Dimethyl Phthalate	0.148	0.005	0.000010	0.00054	0.0002 – 0.0044
Acenaphthalene	0.148	0.005	0.000020	0.00054	0.0012 – 0.0089
2,6-Di-tert-butyl-p-cresol	0.148	0.01	0.000020	0.00108	0.0024 – 0.0222
Diethyl phthalate	0.074	0.25	0.000005	0.02691	0.0024 – 0.0089
n-Hexadecane	0.740	0.01	0.000050	0.00108	0.0060 – 0.0222
Fluorene	0.030	0.005	0.000005	0.00054	0.0012 – 0.0044
4-tert-Octylphenol	0.740	0.05	0.000100	0.00538	0.0120 – 0.0044
2-Bromomethylnaphthalene	0.740	0.01	0.000050	0.00108	0.0060 – 0.0222
2-Hydroxybenzothiazole	NA	0.005	0.000020	0.00054	0.0602 – 0.222
Dibenzothiophene	0.074	0.005	0.000020	0.00054	0.00060 – 0.0044
Phenanthrene	0.740	0.025	0.000050	0.00269	0.0024 – 0.0444
Anthracene	0.740	0.01	0.000020	0.00108	0.0060 – 0.0444
Diisobutyl phthalate	0.740	0.025	0.000020	0.00269	0.0120 – 0.222
3-Methylphenanthrene	0.740	0.025	0.000050	0.00269	0.0120 – 0.222
2-Methylphenanthrene	1.479	0.05	0.000050	0.00538	0.0120 – 0.0889
1-Methylphenanthrene	0.148	0.01	0.000010	0.00108	0.0012 – 0.0089
Dibutyl phthalate	1.479	0.1	0.000100	0.01076	0.0060 – 0.0444
Fluoranthene	0.148	0.01	0.000020	0.00108	0.0012 – 0.0044
Pyrene	2.959	0.025	0.000050	0.00269	0.0120 – 0.0444
Benzyl butyl phthalate	0.074	0.025	0.000100	0.00269	0.0006 – 0.0222
bis(2-ethylhexyl) adipate	0.740	0.01	0.000020	0.00108	0.0060 – 0.0222
Benz(a)anthracene	0.296	0.01	0.000020	0.00108	0.0024 – 0.0222
Chrysene	0.074	0.005	0.000010	0.00054	0.0024 – 0.0089
Bis(2-ethylhexyl)phthalate	0.740	0.01	0.000050	0.00108	0.0060 – 0.0222
Di-n-octyl phthalate	0.740	0.005	0.000050	0.00054	0.0006 – 0.0044

Table B-18 Continued

Chemical	Field Air MQL (ng/m^3)	Field Dust MQL (mg/kg)	Drag Sled MQL (ng/cm^2)	Field Wipe MQL (ng/cm^2)	Dermal Wipe MQL (ng/cm^2)
Benzo(b)fluoranthene	1.479	0.01	0.000050	0.00108	0.0012 – 0.0222
Benzo(k)fluoranthene	0.074	0.01	0.000002	0.00108	0.0024 – 0.0089
Benzo(e)pyrene	0.296	0.001	0.000005	0.00011	0.0012 – 0.0089
Benzo(a)pyrene	0.740	0.025	0.000050	0.00269	0.0012 – 0.0222
Bis(2,2,6,6-tetramethyl-4piperidyl) sebacate	0.148	0.25	0.000050	0.02691	0.0024 – 0.0222
DBA + ICDP[c]	0.740	0.01	0.000010	0.00108	0.0024 – 0.0222
Benzo(g,h,i)perylene	0.030	0.01	0.000010	0.00108	0.0060 – 0.0222
Coronene	2.959	0.005	0.000020	0.00054	0.0012 – 0.0222

[a] MQL = minimum quantifiable limit; SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Based on a nominal air sample volume of 3.38 m^3

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Blanks – Table B-19 reports average concentrations of SVOC analytes in air filter field and laboratory blanks. High background levels were observed for diisobutyl phthalate, with other analytes much lower.

Field dust quality control samples were prepared using solvent-cleaned and heat-baked diatomaceous earth. Table B-20 reports average concentrations of SVOC analytes in dust field and laboratory blanks. Cyclohexylisothiocyanate was found at 50 ng/sample, with other analytes much lower.

Field air and field dust sample concentrations were adjusted by subtracting the field blank result obtained for each field site from the analysis result for each SVOC in samples collected at that field site.

Table B-19. Field and Laboratory Blank Quality Control Results for SVOCs in Field Air Samples Analyzed by GC/MS/MS[a,b]

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Aniline	0	0	0	0
n-Butylbenzene	1.8	0.6	2	0.4
Naphthalene	4.9	1.1	4	2
Benzothiazole	60.8	16.5	44.1	17.1
Cyclohexylisothiocyanate	0	0	0	0
2-Methylnaphthalene	7	1.4	5.5	2.6
1-Methylnaphthalene	3.6	0.8	2.8	1.2
Dimethyl Phthalate	1.1	0.3	1	0.5
Acenaphthalene	0.4	0.1	0.5	0.1
2,6-Di-tert-butyl-p-cresol	6.5	5	1.7	3.3
Diethyl phthalate	142	53.5	69.8	61.4
n-Hexadecane	80.1	45.4	38.5	10.5

Table B-19 Continued

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Fluorene	1.1	0.3	0.6	0.2
4-tert-octylphenol	3.6	2.6	3.6	1.4
2-Bromomethylnaphthalene	0	0	0	0
Dibenzothiophene	0.1	0.1	0	0.1
Phenanthrene	5.7	2.4	4.6	4.1
Anthracene	0.4	0.1	0.5	0.1
Diisobutyl phthalate	799.5	306.3	573	137.8
3-Methylphenanthrene	1.8	0.1	1.7	0.2
2-Methylphenanthrene	2.7	0.1	2.7	0.2
1-Methylphenanthrene	0.3	0.1	0.3	0.1
Dibutyl phthalate	47.6	23.7	33.4	17.4
Fluoranthene	0.3	0	0.3	0.1
Pyrene	2.9	0.1	2.9	0.1
Benzyl butyl phthalate	48.8	34.2	30.4	35.8
bis(2-ethylhexyl) adipate	15.8	10.5	16.3	10.3
Benz(a)anthracene	0.2	0.1	0.3	0
Chrysene	0	0	0	0
Bis(2-ethylhexyl)phthalate	89.9	57.8	37.8	11.9
Di-n-octyl phthalate	6.9	8.9	1	0.5
Benzo(b)fluoranthene	0	0	0	0
Benzo(k)fluoranthene	0	0	0	0
Benzo(e)pyrene	0.4	0.2	0.5	0.1
Benzo[a]pyrene	1.1	0	1.2	0.1
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	0	0	0	0
DBA + ICDP[c]	0	0	0	0
Benzo[ghi]perylene	0	0	0	0
Coronene	0	0	0	0

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Blanks (n=5), Lab Blanks (n=4),

[c] DBA + ICDP = Sum of Dibenz[a,h]anthracene and Indeno(1,2,3-cd)pyrene

Table B-20. Field and Laboratory Blank Quality Control Results for SVOCs in Field Dust Samples Analyzed by GC/MS/MS[a,b]

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Aniline	0	0	0	0
n-Butylbenzene	0	0	0.3	0.6
Naphthalene	0	0	0	0
Benzothiazole	5.5	3.2	3.3	0.3
Cyclohexylisothiocyanate	49.6	4.8	40.8	6.4
2-Methylnaphthalene	0.3	0.2	0.2	0
1-Methylnaphthalene	0.4	0.1	0.4	0
Dimethyl Phthalate	0.2	0	0.3	0
Acenaphthalene	0	0	0	0
2,6-Di-tert-butyl-p-cresol	4.3	5	0	0
Diethyl phthalate	0	0	0	0
n-Hexadecane	20.2	4.2	16.9	3.9
Fluorene	0.5	0.1	0.5	0
4-tert-octylphenol	0	0	0	0
2-Bromomethylnaphthalene	0	0	0	0
2-Hydroxybenzothiazole	3.1	1.9	1.6	0.1
Dibenzothiophene	0.3	0	0.4	0
Phenanthrene	1	0.1	1	0.1
Anthracene	0	0	0	0
Diisobutyl phthalate	5.5	0.5	5.3	0.9
3-Methylphenanthrene	0	0	0	0
2-Methylphenanthrene	0	0	0	0
1-Methylphenanthrene	0	0	0	0
Dibutyl phthalate	4.5	0.1	4.3	0.1
Fluoranthene	0.4	0	0.4	0
Pyrene	0	0	0	0
Benzyl butyl phthalate	3.5	0.7	3.5	1.6
bis(2-Ethylhexyl) adipate	0	0	0	0
Benz(a)anthracene	0	0	0	0
Chrysene	0	0	0	0
Bis(2-ethylhexyl)phthalate	13.9	2.7	12	2.1
Di-n-octyl phthalate	0	0	0	0
Benzo(b)fluoranthene	0.2	0.5	0	0
Benzo(k)fluoranthene	0	0	0	0
Benzo(e)pyrene	0	0	0	0
Benzo(a)pyrene	0	0	0	0
Benzo(ghi)perylene	0	0	0	0
Coronene	0.6	0.1	0.7	0.2

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Blanks (n=4), Lab Blanks (n=4)

Table B-21 reports average concentrations of SVOC analytes in field wipe field and laboratory blanks. Bis(2-ethylhexyl) phthalate had the highest measured background levels (79 ng/wipe). Table B-22 reports average concentrations of SVOC analytes in drag sled field and laboratory blanks. Bis(2-ethylhexyl) phthalate had the highest measured background levels (930 ng/wipe) followed by benzothiazole (216 ng/wipe).

Table B-23 reports average concentrations of SVOC analytes in dermal wipe field and laboratory blanks. Diisobutyl phthalate had the highest measured background levels (2100 ng/wipe) followed by benzyl butyl phthalate (151 ng/wipe). The field wipe and dermal wipe field blank wipe material was handled in the field using gloved hands to mimic handling for the samples. Sample concentrations were adjusted by subtracting the field blank result obtained for each field site from the analysis result for each SVOC in samples collected at that field site.

Table B-21. Field and Laboratory Blank Quality Control Results for SVOCs in Field Wipes Analyzed by GC/MS/MS[a,b]

Chemical	Field Blank Mean (ng/wipe)	Field Blank Standard Deviation (ng/wipe)	Lab Blank Result (ng/wipe)
Aniline	0	0	0
n-Butylbenzene	2.5	1.5	1.9
Naphthalene	0	0	0.2
Benzothiazole	11.5	3.7	10.7
Cyclohexylisothiocyanate	15.3	19.8	0
2-Methylnaphthalene	0.4	0.3	1.4
1-Methylnaphthalene	0.7	0.3	1.6
Dimethyl Phthalate	0.3	0.1	0.3
Acenaphthalene	0.4	0.2	0.6
2,6-Di-tert-butyl-p-cresol	9.3	2	10.7
Diethyl phthalate	21.7	6.1	18
n-Hexadecane	42.6	11	24.8
Fluorene	0.6	0.2	0.7
4-tert-octylphenol	6.9	0.9	4.8
2-Bromomethylnaphthalene	0	0	0
2-Hydroxybenzothiazole	26.1	15.3	10.9
Dibenzothiophene	0.6	0.3	0.3
Phenanthrene	1.3	0.4	0.9
Anthracene	1	0.3	0.6
Diisobutyl phthalate	44.9	8.1	60.2
3-Methylphenanthrene	2.2	0.4	1.7
2-Methylphenanthrene	2.5	0.3	2
1-Methylphenanthrene	1.3	0.5	0.6
Dibutyl phthalate	39.8	18.6	35.2
Fluoranthene	0.9	0.2	0.5
Pyrene	2.2	0.2	1.8
Benzyl butyl phthalate	71.4	37.2	199.5
bis(2-ethylhexyl) adipate	43	20.5	6.6
Benz(a)anthracene	0.6	0.2	0.5

Table B-21 Continued

Chemical	Field Blank Mean (ng/wipe)	Field Blank Standard Deviation (ng/wipe)	Lab Blank Result (ng/wipe)
Chrysene	0.6	0.2	0.3
Bis(2-ethylhexyl)phthalate	78.6	38.7	69.8
Di-n-octyl phthalate	3.4	3.9	1
Benzo(b)fluoranthene	0.9	0.1	0.6
Benzo(k)fluoranthene	0.7	0.1	0.5
Benzo(e)pyrene	0.5	0.2	0.1
Benzo(a)pyrene	0.9	0.1	0.7
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	0	0	0
DBA + ICDP[c]	0.5	0.1	0.6
Benzo(ghi)perylene	0.7	0.1	0.4
Coronene	0.4	0	0.2

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Blanks (n=4), Lab Blanks (n=1)

[c] DBA + ICDP = Sum of Dibenz[a,h]anthracene and Indeno(1,2,3-cd)pyrene

Table B-22. Field and Laboratory Blank Quality Control Results for SVOCs in Drag Sled Wipes Analyzed by GC/MS/MS

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Aniline	0	0	0	0
n-Butylbenzene	5.2	3.3	11.1	1.4
Naphthalene	3.5	1.8	3.8	1.5
Benzothiazole	215.7	217.5	333.3	437.5
Cyclohexylisothiocyanate	0	0	0	0
2-Methylnaphthalene	3.5	1.6	3.7	1.5
1-Methylnaphthalene	2.1	1	2.2	0.9
Dimethyl Phthalate	1.5	0.7	2.3	2.4
Acenaphthalene	0.6	0.2	0.7	0.2
2,6-Di-tert-butyl-p-cresol	9.3	5.2	9.2	7.4
Diethyl phthalate	61.4	10.8	82.7	62.8
n-Hexadecane	95.5	18.2	86.7	67.5
Fluorene	1.1	0.8	1.3	1.5
4-tert-octylphenol	8	2.3	6	1.1
2-Bromomethylnaphthalene	0	0	0	0
2-Hydroxybenzothiazole	23.1	15.6	41	36.4
Dibenzothiophene	0.5	0.1	0.5	0.2
Phenanthrene	2.7	1.7	4.6	5.3
Anthracene	0.2	0.1	0.3	0.2

Table B-22 Continued

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Diisobutyl phthalate	87.2	29.5	78	14.9
3-Methylphenanthrene	1.6	0.2	1.8	0.5
2-Methylphenanthrene	1.4	0.2	1.7	0.5
1-Methylphenanthrene	0.6	0.3	1.4	1.1
Dibutyl phthalate	135.6	31.5	78.1	25.2
Fluoranthene	0.6	0.3	1.4	1.9
Pyrene	2.4	1	4.4	5.2
Benzyl butyl phthalate	148.7	141.7	136.9	100.3
bis(2-ethylhexyl) adipate	61.5	30.8	22.7	14
Benz(a)anthracene	0.2	0	0.4	0.2
Chrysene	0.2	0.1	0.8	1
Bis(2-ethylhexyl)phthalate	930.4	1004.9	1109.7	1840.4
Di-n-octyl phthalate	1.9	0.5	2.1	0.8
Benzo(b)fluoranthene	0.2	0.3	0.5	0.4
Benzo(k)fluoranthene	0.1	0.2	0.2	0.1
Benzo(e)pyrene	0.2	0.2	0.8	1.7
Benzo(a)pyrene	0.5	0.6	0.9	1.4
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	5.1	4	13.5	3.4
DBA + ICDP[c]	0	0	0	0
Benzo(ghi)perylene	0.2	0.1	0.6	0.9
Coronene	0.3	0	0.3	0.1

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Blanks (n=4), Lab Blanks (n=4)

[c] DBA + ICDP = Sum of Dibenz[a,h]anthracene and Indeno(1,2,3-cd)pyrene

Table B-23. Field and Laboratory Blank Quality Control Results for SVOCs in Dermal Wipes Analyzed by GC/MS/MS[a,b]

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Aniline	0	0	0	0
n-Butylbenzene	4.6	1.9	5.5	2.2
Naphthalene	2.2	0.4	1.9	0.2
Benzothiazole	5.4	1.4	10.2	2.2
Cyclohexylisothiocyanate	9.8	20.3	12.9	22.1
2-Methylnaphthalene	1.6	0.2	3.6	1.6
1-Methylnaphthalene	0	0	0.9	0.8
Dimethyl Phthalate	0.5	0.4	0.1	0.2

Table B-23 Continued

Chemical	Field Blank Mean (ng/sample)	Field Blank Standard Deviation (ng/sample)	Lab Blank Mean (ng/sample)	Lab Blank Standard Deviation (ng/sample)
Acenaphthalene	0.4	0.1	0.2	0.2
2,6-Di-tert-butyl-p-cresol	8.6	5.4	15.5	11.7
Diethyl phthalate	38.2	16.7	41.2	8.9
n-Hexadecane	86.1	26.2	169.4	111.3
Fluorene	0.4	0.1	0.2	0.2
4-tert-octylphenol	30.5	34.9	16.8	8.7
2-Bromomethylnaphthalene	0	0	11.3	11.8
Dibenzothiophene	0.4	0.1	0.3	0.3
Phenanthrene	0.5	0.4	1.6	1.4
Anthracene	2.1	0.3	0.8	1.2
Diisobutyl phthalate	2102.3	581.9	2146.5	643.8
3-Methylphenanthrene	3.2	0.2	3	1.7
2-Methylphenanthrene	3.5	0.3	3.7	0.2
1-Methylphenanthrene	0.7	0.2	0.9	0.3
Dibutyl phthalate	85.6	43.7	122.8	39.4
Fluoranthene	0.6	0.2	0.6	0.3
Pyrene	3.2	0.1	2.7	0.7
Benzyl butyl phthalate	151.1	115.1	224.1	160
bis(2-ethylhexyl) adipate	25.3	20.8	374.6	777.2
Benz(a)anthracene	0.3	0.2	0.3	0.4
Chrysene	0.4	0.1	0.3	0.3
Bis(2-ethylhexyl)phthalate	79.9	13.1	108.1	61.2
Di-n-octyl phthalate	8.5	7.4	2.5	2.2
Benzo(b)fluoranthene	0	0	0	0.1
Benzo(k)fluoranthene	0.3	0.2	0.2	0.4
Benzo(e)pyrene	0.1	0.1	0.2	0.3
Benzo(a)pyrene	0.2	0.3	0.3	0.5
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	3.7	1.3	1.6	0.9
DBA + ICDP[c]	0	0	0.4	0.4
Benzo(ghi)perylene	0	0	0.9	0.2
Coronene	0	0	0	0

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Blanks (n=5), Lab Blanks (n=5)

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Recovery – Table B-24 reports recovery results for SVOC analytes measured in air filter spiked field controls and spiked laboratory controls. Average recoveries ranged from 0% to 232% for controls not corrected for blank levels. Most SVOCs had average spiked field control recoveries in a range from 70% to 120%. Aniline had an average recovery of 18% in spiked field controls, while n-butylbenzene and napthalene had recoveries of 54% each. These were the three most volatile analytes and there may have been losses during the extraction solvent volume reduction step. Diisobutyl phthalate and bis(2-ethylhexyl) adipate had recoveries of 232% and 190%, respectively, in spiked field controls. The diisobutyl phthalate recovery was inflated by the relatively high background levels (reported in Table B-23).

Table B-24. Field and Laboratory Spike Quality Control Results for SVOCs in Field Air Samples Analyzed by GC/MS/MS[a,b]

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean% Recovery	Lab Spike % Recovery Standard Deviation
Aniline	18.2	9.1	20.2	3.8
n-Butylbenzene	53.6	2.3	62.4	3.1
Naphthalene	53.5	14.9	64.7	7.2
Benzothiazole	112	8.2	117.3	10.6
Cyclohexylisothiocyanate	119.6	10	127.3	18.8
2-Methylnaphthalene	75	5.1	84.6	7
1-Methylnaphthalene	78.1	4.7	87.8	8
Dimethyl Phthalate	74.8	5.2	73.4	4.1
Acenaphthalene	73.6	5	77.2	9.5
2,6-Di-tert-butyl-p-cresol	72.4	5.9	73.4	9.7
Diethyl phthalate	103.6	8.3	97.7	5.7
n-Hexadecane	76.4	5.3	92	11.9
Fluorene	91.2	3.6	96.4	2.5
4-tert-octylphenol	72	9.2	109.7	79.8
2-Bromomethylnaphthalene	1.7	0.9	6.8	2.4
Dibenzothiophene	104.3	4.6	103.1	6.1
Phenanthrene	90.2	4.8	87.3	4.9
Anthracene	97.6	4.5	94.1	4.7
Diisobutyl phthalate	232.5	63.1	220.9	11.1
3-Methylphenanthrene	107.3	3.9	104.5	4.3
2-Methylphenanthrene	101.5	6.5	100.8	6.4
1-Methylphenanthrene	110	6.5	109.2	4.7
Dibutyl phthalate	114.1	16.4	123.2	3.9
Fluoranthene	85	4.7	81.9	2.3
Pyrene	82.5	3.8	81.9	2.6
Benzyl butyl phthalate	108.3	27	92	8.8
bis(2-ethylhexyl) adipate	189.6	81.3	211.9	72.5
Benz(a)anthracene	81.8	4.1	82.4	7.3
Chrysene	111.2	6.4	106.8	4.3
Bis(2-ethylhexyl)phthalate	96.5	8.7	94	5.4
Di-n-octyl phthalate	82.9	2.8	82.6	4.9

Table B-24 Continued

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean% Recovery	Lab Spike % Recovery Standard Deviation
Benzo(b)fluoranthene	113.8	14.5	105.5	8.9
Benzo(k)fluoranthene	108.6	4.1	108.4	10.6
Benzo(e)pyrene	69.4	7.7	76.1	6.7
Benzo(a)pyrene	72.9	7.9	75.8	11.5
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	0.3	0.6	0	0.1
DBA + ICDP[c]	120.8	29.7	115.4	3.5
Benzo(ghi)perylene	95.3	1.4	100.9	11.1
Coronene	100.7	18.2	71.5	11.8

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Spikes (n=5), Lab Spikes (n=5); Spike=500 ng

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Table B-25 reports recovery results for SVOC analytes in field dust surrogate spiked field controls and spiked laboratory controls. Average recovery levels were low and ranged from 36% to 70% for most analytes. Aniline had an average recovery of 10% in spiked field controls, while n-butylbenzene and napthalene had recoveries of 19% and 29%, respectively. 2-bromomethylnaphthalene had an average recovery of 17% in the spiked field controls. The reason for the relatively low recoveries is not clear. Uniform spiking of dust surrogate material is difficult, and some portion of the spiking solution may not have been applied directly to the material. It is possible that recoveries from the diatomaceous earth are not complete using the solvent and extraction method used. It is also not clear whether the surrogate material provides a quality control measure that can accurately represent recoveries obtained from actual field dust.

Table B-25. Field and Laboratory Spike Quality Control Results for SVOCs in Field Dust Surrogate Analyzed by GC/MS/MS[a,b]

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
Aniline	9.8	1.7	10.5	1.4
n-Butylbenzene	18.7	9.8	22.8	1.9
Naphthalene	28.9	19.3	38.2	1.8
Benzothiazole	50.1	11.5	47.6	2.3
Cyclohexylisothiocyanate	58.1	14.5	65.2	4.6
2-Methylnaphthalene	44.8	17.4	51	2.5
1-Methylnaphthalene	40.5	15.9	47.9	2.2
Dimethyl Phthalate	65.9	2	67.9	3.3
Acenaphthalene	37.2	4.6	35.2	2.2
2,6-Di-tert-butyl-p-cresol	25.6	2.5	22	4
Diethyl phthalate	46.8	1.8	47.6	1.9
n-Hexadecane	44.1	3.2	45.2	3.3

Table B-25 Continued

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
Fluorene	52.4	3.1	55.1	1.9
4-tert-octylphenol	40.5	0.9	40	1.6
2-Bromomethylnaphthalene	17.2	3	14.9	1.7
2-Hydroxybenzothiazole	36.1	4.5	34.5	3.4
Dibenzothiophene	52.4	1.3	53.7	2.5
Phenanthrene	47.4	2.1	48.2	1.6
Anthracene	36.4	3.3	32.1	0.8
Diisobutyl phthalate	39.6	2.4	42.2	2.2
3-Methylphenanthrene	42.8	2.6	41.2	2.2
2-Methylphenanthrene	41.7	3.3	40	2.7
1-Methylphenanthrene	42.3	3	40.8	2.6
Dibutyl phthalate	37.3	3.2	39.5	3
Fluoranthene	43.3	1.5	44.1	1.8
Pyrene	43.4	2.2	44.6	1.2
Benzyl butyl phthalate	38.4	0.9	40.9	1.3
bis(2-ethylhexyl) adipate	56.5	21.3	87.3	25.9
Benz(a)anthracene	35.6	1	35.6	1.6
Chrysene	57.8	2.5	59.5	2.6
Bis(2-ethylhexyl)phthalate	43.3	0.9	45.6	0.5
Di-n-octyl phthalate	47	6.2	46.6	2.4
Benzo(b)fluoranthene	50.3	2.2	54.7	1.4
Benzo(k)fluoranthene	54	1.8	61.5	2.4
Benzo(e)pyrene	53.3	3	55.3	5.8
Benzo(a)pyrene	38.1	4.3	36.4	3.8
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	32.1	7.8	0	0
DBA + ICDP[c]	48	3.4	50.2	4.1
Benzo(g,h,i)perylene	47	1.9	47.7	2.0
Coronene	70.1	4.7	83.1	3.3

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Spikes (n=4), Lab Spikes (n=4); Spike=1667 ng/g (0.1 g sample)

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Table B-26 reports recovery results for SVOC analytes in field wipe spiked field controls and spiked laboratory controls. Average recovery levels above 150% were measured for 2,6-di-tert-butyl-p-cresol, 4-tert-octylphenol, 2-hydroxybenzothiazole, benzyl butyl phthalate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate in spiked field controls. Aniline had an average recovery level of 32% in spiked field controls, likely due to losses during the solvent extraction volume reduction step. Average recoveries ranged from 62% to 144% for the remainder of the SVOC analytes.

Table B-26. Field and Laboratory Spike Quality Control Results for SVOCs in Field Wipes Analyzed by GC/MS/MS[a,b]

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
Aniline	31.7	14.9	29.8	4.9
n-Butylbenzene	79.8	8.4	91.7	7.8
Naphthalene	97.2	6.3	107.9	3
Benzothiazole	97.7	17.7	104.5	24.2
Cyclohexylisothiocyanate	97.7	13.8	106.6	4.6
2-Methylnaphthalene	107.5	5.5	118.1	3.7
1-Methylnaphthalene	107.1	5.8	117.3	3.2
Dimethyl Phthalate	71.9	8.9	76.6	4.5
Acenaphthalene	72.2	26.9	77.8	19.1
2,6-Di-tert-butyl-p-cresol	229.1	129.1	201.4	60
Diethyl phthalate	89	1.1	89.9	2.4
n-Hexadecane	106.3	16.9	132.2	30.3
Fluorene	102.5	4.8	105.8	5.5
4-tert-octylphenol	200.8	26.1	219.2	69.6
2-Bromomethylnaphthalene	18.3	1.4	21.4	3.4
2-Hydroxybenzothiazole	179.2	51.4	180.4	37.9
Dibenzothiophene	100.1	1.8	102.5	2.8
Phenanthrene	95.9	3.4	99.1	3.1
Anthracene	95.9	3.4	99.1	3.1
Diisobutyl phthalate	115	12.7	111.4	12.2
3-Methylphenanthrene	103.7	8.4	109.4	5.2
2-Methylphenanthrene	94.5	16.2	92.6	3.3
1-Methylphenanthrene	100	16.3	101	4.7
Dibutyl phthalate	144.6	15.4	132.9	10.5
Fluoranthene	75.9	1.4	74.9	2.1
Pyrene	78.8	4.1	80	6.3
Benzyl butyl phthalate	167.7	115.8	69.9	4.6
bis(2-ethylhexyl) adipate	181.1	54	147.7	16.5
Benz(a)anthracene	61.7	5.9	63.3	2.7
Chrysene	102.4	1.9	104.2	4.1
Bis(2-ethylhexyl)phthalate	101.8	22.1	83.6	13.3
Di-n-octyl phthalate	93.9	21	80.1	2
Benzo(b)fluoranthene	81.8	5.6	88	9.5
Benzo(k)fluoranthene	95.1	2.3	95.9	3.3
Benzo(e)pyrene	82.2	5.8	83.2	9.2
Benzo(a)pyrene	80.5	13.5	85.6	6.3
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	298.4	97	300	39.3

Table B-26 Continued

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
DBA + ICDP[c]	82.1	0.8	87.3	6.5
Benzo(ghi)perylene	77.4	1.9	82.9	3.6
Coronene	86.3	17.7	79.5	8.1

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Spikes (n=4), Lab Spikes (n=4); Spike=500 ng

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Table B-27 reports recovery results for SVOC analytes in drag sled spiked field controls and spiked laboratory controls. Average recovery levels above 140% were measured for cyclohexylisothiocyanate, 4-tert-octylphenol, diisobutyl phthalate, dibutyl phthalate, benzo(e)pyrene, and benzo[a]pyrene in field spike controls. Aniline had average recovery levels of 7% in spiked field controls, likely as a result of losses during the solvent extraction volume reduction step. 2-bromomethylnaphthalene had an average recovery of 2% in the spiked field controls. Average recoveries ranged from 46% to 123% for the remainder of the SVOC analytes.

Table B-27. Field and Laboratory Spike Quality Control Results for SVOCs in Drag Sled Wipes Analyzed by GC/MS/MS[a,b]

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
Aniline	7	7.3	10.5	8.2
n-Butylbenzene	107.7	10.2	111.6	1.4
Naphthalene	110.8	2.8	107	3.3
Benzothiazole	122.2	3.9	118.5	3.9
Cyclohexylisothiocyanate	172.8	24.7	151.3	58.7
2-Methylnaphthalene	106	1.8	100	1.7
1-Methylnaphthalene	104.8	2	98.4	2
Dimethyl Phthalate	88.4	4.8	85.1	5.1
Acenaphthalene	83.2	7.5	78.6	2
2,6-Di-tert-butyl-p-cresol	109.5	20.6	85.2	16.1
Diethyl phthalate	104.9	4	103.5	3.1
n-Hexadecane	99.4	8.7	93.2	2.7
Fluorene	95.1	5	91.9	1.6
4-tert-octylphenol	147.7	10	150	22.4
2-Bromomethylnaphthalene	1.9	0.2	3.8	0.7
2-Hydroxybenzothiazole	108.1	21.1	96.3	7.2
Dibenzothiophene	61.9	7.1	57.2	4
Phenanthrene	103.9	4.1	105.8	4.8
Anthracene	101.7	3.3	104.1	8.9
Diisobutyl phthalate	142.3	3.8	141	16.4

Table B-27 Continued

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean % Recovery	Lab Spike % Recovery Standard Deviation
3-Methylphenanthrene	120.1	5.6	119.8	5.7
2-Methylphenanthrene	114.7	7.2	119.5	10.9
1-Methylphenanthrene	122.5	5.5	124.1	10.8
Dibutyl phthalate	174.4	7.5	163.3	16.2
Fluoranthene	77.1	4.5	73.9	2.2
Pyrene	79.7	4.2	77.1	2.5
Benzyl butyl phthalate	117.6	28.6	101.2	8.2
bis(2-ethylhexyl) adipate	88.7	31.5	65.7	1.4
Benz(a)anthracene	87	2.4	83.8	4.5
Chrysene	96.4	5	95.5	2.7
Bis(2-ethylhexyl)phthalate	105.1	10.5	104.4	9
Di-n-octyl phthalate	114.1	7.3	116.4	3.7
Benzo(b)fluoranthene	47.3	6.4	41.2	2.2
Benzo(k)fluoranthene	70.1	5.9	66.2	2.4
Benzo(e)pyrene	154.3	13	161.1	13.9
Benzo(a)pyrene	147.1	7.5	147.5	7.8
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	91.7	53.3	194.1	82.2
DBA + ICDP[c]	55.5	7.6	49.1	2.7
Benzo(ghi)perylene	78.4	5	74.9	2.8
Coronene	45.6	11.2	38.1	6.4

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Spikes (n=4), Lab Spikes (n=4); Spike=500 ng

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Table B-28 reports recovery results for SVOC analytes in dermal wipe spiked field controls and spiked laboratory controls. Average recovery levels above 150% were measured for 2,6-di-tert-butyl-p-cresol, 4-tert-octylphenol, diisobutyl phthalate, dibutyl phthalate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate in spiked field controls. The average recovery of 2-bromomethylnaphthalene was 6% in spiked field controls. Average recoveries ranged from 60% to 144% for the remainder of the SVOC analytes.

No recovery adjustments were performed for the exposure characterization pilot study samples.

Table B-28. Field and Laboratory Spike Quality Control Results for SVOCs in Dermal Wipes Analyzed by GC/MS/MS[a,b]

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean% Recovery	Lab Spike % Recovery Standard Deviation
Aniline	72.9	30.7	46.5	18.8
n-Butylbenzene	77.4	27.2	96.5	17.8
Naphthalene	95.5	2.9	109.2	10.3
Benzothiazole	86.1	31.3	81.4	16.3
Cyclohexylisothiocyanate	74.3	27.9	93.5	27.4
2-Methylnaphthalene	97.1	8.5	82.9	28.6
1-Methylnaphthalene	102.5	8.6	109.1	24.8
Dimethyl Phthalate	70.6	8.7	91.1	13.9
Acenaphthalene	60.4	14.9	70.4	19.3
2,6-Di-tert-butyl-p-cresol	169.4	35.5	166.1	31.1
Diethyl phthalate	95.4	2.3	103.6	14.2
n-Hexadecane	144.1	24.9	126.9	61.4
Fluorene	99.7	20.3	114	23.6
4-tert-octylphenol	188.8	42.1	135.5	61.9
2-Bromomethylnaphthalene	5.7	4.1	10.3	2.5
Dibenzothiophene	89.5	9.6	92.3	13
Phenanthrene	96.1	10.5	103.4	3.5
Anthracene	96.5	3.1	94.7	9.1
Diisobutyl phthalate	644.3	28.1	852.7	168.8
3-Methylphenanthrene	126.7	7.5	124.2	8.5
2-Methylphenanthrene	104.1	9	105.5	15.3
1-Methylphenanthrene	115.6	4.9	111.3	14.8
Dibutyl phthalate	237.5	14.5	310.2	70
Fluoranthene	78.8	3.7	81.4	3.2
Pyrene	76.4	3.1	78.9	2.3
Benzyl butyl phthalate	95.1	5.5	98.7	3.7
bis(2-ethylhexyl) adipate	144	22.1	145.1	17.3
Benz(a)anthracene	69.1	1.5	85.2	31.6
Chrysene	98	5.2	102.6	2.8
Bis(2-ethylhexyl)phthalate	100.9	4.7	104.2	3
Di-n-octyl phthalate	92.8	2.8	101.9	2.7
Benzo(b)fluoranthene	60.7	11.2	63.9	4
Benzo(k)fluoranthene	96.2	7.1	95.9	4.9
Benzo(e)pyrene	95.5	8.7	93.3	7.5
Benzo(a)pyrene	100.6	8.7	96.5	9.8
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	241	46.6	248.8	22.8

Table B-28 Continued

Chemical	Field Spike Mean % Recovery	Field Spike % Recovery Standard Deviation	Lab Spike Mean% Recovery	Lab Spike % Recovery Standard Deviation
DBA + ICDP[c]	71.7	15.8	74.2	8
Benzo(ghi)perylene	86.6	4.8	90	3.1
Coronene	51.1	24.4	50.3	6.7

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry

[b] Field Spikes (n=5), Lab Spikes (n=5); Spike=500 ng

[c] DBA + ICDP = Dibenz(a,h)anthracene + Indeno(1,2,3-cd)pyrene

Precision – Table B-29 shows precision results for SVOC analytes in field air, field wipe and drag sled duplicate samples when both of the measurement results in a pair were > 0. Average %RSD values for SVOCs in field air duplicates ranged from 1% to 101%. In many cases, the higher %RSDs were the result of measurements near or below the minimum quantifiable limits. Average %RSD values for SVOCs in field wipe duplicates ranged from 2% to 119%, with results for most analytes < 50 %RSD. Field wipe duplicates may reflect spatial heterogeneity in analytes on the field surface in addition to measurement precision. In many cases, the higher %RSDs were the result of measurements near or below the minimum quantifiable limits. Average %RSD values for SVOCs in drag sled duplicates ranged from 10% to 71%, with results for most analytes < 35%. Drag sled duplicates may reflect spatial heterogeneity in analytes on the field surface in addition to measurement precision. In many cases, the higher %RSDs were the result of measurements near or below the minimum quantifiable limits.

Table B-29. Measurement Precision Quality Control Results for Duplicate SVOC Field Air, Field Wipe, and Drag Sled Samples Analyzed by GC/MS/MS[a,b]

Chemical	Field Air - n[b]	Field Air - Average % Relative Standard Deviation	Field Wipe - n[b]	Field Wipe - Average % Relative Standard Deviation	Drag Sled - n[b]	Drag Sled - Average % Relative Standard Deviation
Phenanthrene	4	42	3	15	3	10
Fluoranthene	4	11	3	12	3	11
Pyrene	4	18	3	14	3	13
Benzo(a)pyrene	1	1	3	4	3	16
Benzo(ghi)perylene	1	40	3	10	3	12
Sum15PAH[c]	0	NR	3	13	3	10
Benzothiazole	2	49	3	21	3	43
2-Hydroxybenzothiazole	0	NR	3	18	3	25
Dibutyl phthalate	4	89	1	8	3	60
Bis(2-ethylhexyl) phthalate	2	30	3	34	2	35
Aniline	1	20	3	17	0	NR
4-tert-octylphenol	4	31	3	14	3	17
n-Hexadecane	1	48	0	NR	2	40
Naphthalene	2	32	0	NR	1	14
1-Methylnaphthalene	1	101	3	44	2	30

Chemical	Field Air - n[b]	Field Air - Average % Relative Standard Deviation	Field Wipe - n[b]	Field Wipe - Average % Relative Standard Deviation	Drag Sled - n[b]	Drag Sled - Average % Relative Standard Deviation
2-Methylnaphthalene	0	NR	3	49	2	33
Acenaphthylene	3	21	2	119	3	14
Fluorene	4	7	2	73	3	33
Anthracene	3	56	2	72	3	15
1-Methylphenanthrene	4	34	3	35	3	14
2-Methylphenanthrene	4	28	3	23	3	13
3-Methylphenanthrene	4	30	3	21	3	13
Benz(a)anthracene	2	14	3	4	3	18
Chrysene	3	15	3	5	3	10
Benzo(b)fluoranthene	0	NR	3	12	3	18
Benzo(k)fluoranthene	0	NR	3	27	2	33
Benzo(e)pyrene	1	9	3	2	3	13
DBA + ICDP[d]	0	NR	3	22	3	12
Coronene	1	75	3	24	3	11
Dibenzothiophene	3	18	2	25	3	17
2-Bromomethylnaphthalene	0	NR	0	NR	0	NR
n-Butylbenzene	0	NR	0	NR	0	NR
Dimethyl phthalate	4	29	2	60	3	69
Diethyl phthalate	2	46	1	77	3	71
Diisobutyl phthalate	2	19	1	10	3	51
Benzyl butyl phthalate	2	60	1	60	3	43
Di-n-octyl phthalate	2	96	1	88	3	38
2,6-Di-tert-butyl-p-cresol	1	9	2	47	3	25
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	0	NR	3	45	2	44
Cyclohexylisothiocyanate	0	NR	1	34	0	NR
bis(2-Ethylhexyl) adipate	4	65	2	60	3	56

[a] SVOC = semivolatile organic compound; GC/MS/MS = gas chromatography/tandem mass spectrometry; NR = not reported

[b] Number of duplicate sample pairs in which both measurements are >0.

[c] Sum15PAH = Sum of 15 of the 16 EPA 'priority' PAHs, including Acenaphthylene, Anthracene, Benz[a]anthracene, Benzo[a]pyrene, Benzo(b)fluoranthene, Benzo[ghi]perylene, Benzo(k)fluoranthene, Chrysene, Dibenz[a,h]anthracene, Fluoranthene, Fluorene, Indeno(1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Pyrene

[d] DBA + ICDP = Sum of Dibenz[a,h]anthracene and Indeno(1,2,3-cd)pyrene

DQI – Recovery and precision DQI objective values were not met for a portion of the SVOC analytes across the exposure characterization pilot study field air, field dust, field wipe, drag sled and dermal wipe samples. For each sample type, the quality control results were examined as a whole, and decisions were made to not report measurement results for some chemicals in Volume 1 of this report. Exclusion decisions were primarily made because of high background, high recovery or very low recovery. Analytes that were retained in the report may have not met all DQI objectives in all media.

Analyte measurement results that were not reported in Volume 1 of this report are shown below for each sample type:

- *Field air samples* – aniline, napthalene, n-butlybenzene, 2-bromomethylnaphthalene, diisobutyl phthalate, bis(2-ethylhexyl) adipate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate measurement results were not reported.
- *Field dust samples* – aniline, napthalene, n-butylbenzene, cyclohexylisothiocyanate, 2-bromomethylnaphthalene, bis(2-ethylhexyl) adipate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate measurement results were not reported.
- *Field wipe samples* – aniline, n-butylbenzene, diethyl phthalate, n-hexadecane, 2-bromomethylnaphthalene, 2-hydroxybenzothiazole, diisobutyl phthalate, dibutyl phthalate, benzyl butyl were not reported.
- *Field drag sled samples* – aniline, n-butylbenzene, cyclohexylisothiocyanate, 2-bromomethylnaphthalene, bis(2-ethylhexyl) phthalate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate measurement results were not reported.
- *Dermal wipe samples* – cyclohexylisothiocyanate, dimethyl phthalate, diethyl phthalate, 2-bromomethylnaphthalene, 2-hydroxybenzothiazole, anthracene, diisobutyl phthalate, dibutyl phthalate, and bis(2,2,6,6-tetramethyl-4piperidyl) sebacate were not reported.

Overall, the methods for SVOC collection and analysis by GC/MS/MS performed adequately, but not perfectly, for most target analytes. It is likely that better performance in field air sampling would be obtained through the use of higher flow rates or collection volumes. For this study, it was decided that portability and battery operation were necessary due to the nation-wide scope of the potential sampling territory. It was also not certain how much time would be available to set up and take down equipment at the fields, and whether suitable power supplies would be available. Relatively small amounts of some analytes were captured with the field wipes and dermal wipes, with some degradation of quality performance at very low concentrations. The much larger surface area sampled by the drag sled helped alleviate some of the problems with small analyte amounts for that method. The field dust is a unique material that may be hard to replicate for preparing suitable quality control samples. The time and difficulty in collecting dust samples also impacts the ability to collect duplicate samples at fields.

B.4.5 Attempted Measurement of SVOCs by LC/MS in Field Air, Field Dust, Field Wipe, Field Drag Sled and Dermal Wipe QC Samples

During the tire crumb rubber sample analyses, tire crumb rubber was extracted using an acetone:hexane (1:1) solvent mixture for analysis of target SVOC analytes by GC/MS/MS. Extraction was accomplished using a simple vortex mixing procedure and, due to the relatively high concentrations of most target analytes, no solvent volume reduction step was required. For tire crumb rubber extracts, a solvent exchange into methanol was successfully performed to allow analysis of the following analytes in crumb rubber by LC/MS:

- 2-Mercaptobenzothiazole,
- 2-Hydroxybenzothiazole,
- Cyclohexylamine,
- Dicyclohexylamine,
- N-Cyclohexyl-N-methylcyclohexanamine,
- Diisononylphthalate, and,
- Diisodecylphthalate.

As part of the exposure characterization pilot study, there was interest in learning whether LC/MS analysis methods could successfully be applied for analysis of SVOCs using the exposure study environmental and personal samples that were collected and analyzed by GC/MS/MS. All of the air, wipe, and dust sample media were extracted for GC/MS/MS analysis using an acetone:hexane (1:1) solvent mixture. Other than for field dust, a solvent volume reduction step was performed to concentrate the analytes which were present at relatively low levels. Following the GC/MS/MS analyses, the sample extracts were solvent exchanged into methanol to attempt LC/MS analyses.

The LC/MS quality control results for duplicate samples, field and laboratory blanks, field and laboratory spiked controls, matrix spike samples, recovery spike samples, and calibration check analyses were reviewed. Overall, the methods did not meet data quality indicator objectives for any of the target analytes. Recoveries of spiked analytes in lab and field controls and in matrix spike samples were below 30% in all test samples, and often below 10%. The duplicate samples, when target analytes were detected, often showed poor reproducibility. Therefore, no exposure characterization pilot study LC/MS measurement results are included in this report.

Due to the multiple steps needed for extraction, solvent reduction, and solvent exchange to generate extracts for GC/MS/MS and LC/MS analyses, there are several places where analyte losses could occur due to volatilization or adsorption to materials. This problem becomes more acute due to the relatively low amounts of target analytes collected in most samples. In the future, it may be necessary to collect environmental and personal samples expressly for LC/MS analysis and to develop and test suitable extraction procedures for those samples.

B.4.6 Field User Questionnaire Quality Control

Field user activity questionnaires were administered to exposure pilot research study participants by trained interviewers. Interviewers filled out the questionnaire forms during the oral interview. Questionnaires were reviewed for completeness at the field site. All questionnaires received 100% duplicate keyed entry; any discrepancies between the two entries were resolved and the data entry was finalized. Following data entry, the questionnaire results received a data quality review by an independent staff member. All result compilations for reporting were verified through report table reviews.

B.4.7 Video Activity Data Analysis Quality Control

B.4.7.1 Publicly-Available Video Data Quality Control

Quality control measures for the publicly available video data acquisition and analysis are described in Volume 1, Section 3.5.1.

B.4.7.2 Participant Video Quality Control

Quality control measures for the exposure pilot study participant video data acquisition and analysis are described in Volume 1, Section 3.5.2.

Appendix C

Standard Operating Procedures (SOPs) for Exposure Characterization Research

Tables C-1 and C-2 list the standard operating procedures (SOPs) that were prepared or used for the exposure characterization research activities by research area. The SOPs follow the tables These are research-level SOPs.

Table C-1. Summary of the Exposure Characterization Sample and Data Collection Standard Operating Procedures (SOPs)

Analytes/Sample Type	SOP Title	EPA SOP Identification Number
Field Metadata	Collection of Field and Activity Metadata During Exposure Characterization Pilot Study Field Sampling	D-SED-IEMB-030-SOP-01
Air PUF	Collection of Semi-Volatile Organic Compound (SVOC) Air Samples at Activity Fields Involving Tire Crumb Rubber	D-EMMD-SSAB-012-SOP-01
Active air sampling	Collection of Tire Crumb Active Field Ambient Air Samples for VOCs using Thermal Desorption Tubes and Low-Flow Pumps	D-EMMD-AQB-024-SOP-01
Passive Air sampling	Radiello Carbopack X Diffusive Sampler Handling: Field Deployment and Shipping for Tire Crumb Exposure Studies	D-EMMD-AQB-019-SOP-01
Surface Wipe	Collection of Surface Wipe Samples from Synthetic Turf Fields	D-SED-IEMB-026-SOP-01
Dermal Wipe	Collection of Dermal Wipe Samples	D-SED-IEMB-028-SOP-01
Field Dust	Collection of Dust Samples from Synthetic Turf Fields	D-SED-IEMB-029-SOP-01
PM filter sampling	Collection of Particulate Matter (PM) Air Samples at Activity Fields Constructed using Crumb Rubber	D-EMMD-SSAB-007-SOP-01
Human activity data collection	Standard Operating Procedure for Collecting and Using Extant Publicly- Available Video	D-SED-EHCAB-001-SOP-01
Human activity data collection	Videography of Activity Characterization Study Participants	D-SED-EHCAB-005-SOP-01
Child User Questionnaire data	Procedure for administering the facility child user questionnaire	D-SED-EHCAB-004-SOP-01
Adult User Questionnaire data	Procedure for administering the facility adult user questionnaire	D-SED-EHCAB-003-SOP-01

Table C-2. Summary of the Exposure Characterization Sample Analysis Standard Operating Procedures (SOPs)

Analytes/Sample Type	SOP Title	EPA SOP Identification Number
Air sample analysis	Determination of Volatile Organic Compounds Desorbed from Sorbent Tubes Using the Markes International Ultra/Unity Thermal Desorption System	D-EMMD-AQB-018-SOP-01 (Updated Identification Number: D-EMMD-AQB-SOP-3465-0)
SVOC Air Samples by GC/MS	Standard Operating Procedure for Preparation of Air Samples Collected on PUF Plugs for GC/MS Analysis	D-EMMD-PHCB-036-SOP-01
SVOC Air Samples by LC/MS	Determination of Selected Organic Contaminants in Tire Crumb Rubber Subsamples for Multi-Residue Characterization by Ultra Pressure Liquid Chromatography/ Tandem Mass Spectrometry (UPLC-MS/MS)	D-EMMD-PHCB-SOP-2327-0
TCR SVOC Extraction and Analysis by GC/MS	Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples	D-EMMD-PHCB-033-SOP-01
SVOC Field Dust Samples by GC/MS/MS	Preparation of Synthetic Field Dust Samples for SVOC Analysis	D-EMMD-PHCB-068-SOP-01
SVOC Field Wipe Samples and Dermal Wipe Samples by GC/MS	Preparation of Dermal and Surface Wipe Samples for SVOC Analysis	D-EMMD-PHCB-067-SOP-01
PM filter and dust metals analysis by HR-ICP/MS	Extraction of Filter Media for Ion Chromatography and High Resolution Inductively Coupled Plasma Mass Spectrometry	D-EMMD-PHCB-071-SOP-01 (Updated Identification Number: D-EMMD-AQB-SOP-3465-0)
Metals Extraction for Solid Samples	Total Nitric Acid Extractable Metals from Solid Samples by Microwave Digestion	D-EMMD-ECB-003-SOP-01
Metals Extraction for Wipe Samples	Total Nitric Acid Extractable Metals from Wipe Samples by Microwave Digestion	D-EMMD-ECB-013-SOP-01
ICP/MS analysis	Operation and Maintenance of the Element High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument	D-EMMD-PHCB-042-SOP-03

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 the Collection of Field and Activity Metadata During Exposure Characterization Pilot Study Field Sampling

Number: D-SED-IEMB-030-SOP-01	Effective Date: August 21, 2017

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Kent W. Thomas

Signature: Date:

Approval

Name: Caroline Stevens
Title: Branch Chief, NERL/SED/IEMB

Signature: Date:

Concurrence*

Name: Christine Alvarez
Title: NERL QA Manager, NERL/SED/IO

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QA Track: _____ _____
Initials Date

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

STANDARD OPERATING PROCEDURE FOR COLLECTION OF FIELD AND ACTIVITY METADATA DURING EXPOSURE CHARACTERIZATION PILOT STUDY FIELD SAMPLING

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the collection of 'meta data' to describe activities and conditions at a field during exposure characterization pilot field study activities in the tire crumb rubber research study (TCRS).

2.0 SUMMARY OF THE METHOD

Several types of information will be collected on structured forms regarding activities and conditions that may be relevant for interpreting measurement results obtained from the TCRs exposure characterization pilot field sampling events. The general categories of information include:

Meteorological information	Collected on each sampling day	Appendix A pg. 10
General field observations and information	Collected on each sampling day	Appendix A pg. 11
General activity information for the field	Collected on each sampling day	Appendix A pg. 12
Specific information for participant activities	Collected for each research participant	Appendix A pg. 13
Field sampling location record	Collected for each air and field wipe sampling day	Appendix A pg. 14
Field environment record	Collected once	Appendix A pg. 15

Field staff are responsible for recording information on the forms included in this protocol. One form allows for open-ended observational information to be collected regarding any condition or activity that might be important in understanding and interpreting results at the specific field or across fields in the study.

3.0 DEFINITIONS

SOP – Standard operating procedure

TCRS – Tire Crumb Rubber Research Study

CDC – Centers for Disease Control and Prevention

QC – Quality Control

RTP – Research Triangle Park

4.0 CAUTIONS

4.1 No photography is to be performed by any field staff member other than the planned participant video recordings. No GPS coordinate information will be collected or recorded. All information is to be based on visual observations and written information on the forms provided with this SOP.

5.0 RESPONSIBILITIES

5.1 The EPA project staff will prepare the SOP and the data collection record forms.

5.2 The field coordinator will assign data collection responsibilities for field staff members, will review the collected information for content and completeness, and will transmit completed records to the EPA WACOR.

5.3 The EPA contractor field staff will be responsible for collection and recording data and observations on these forms:

Meteorological information (pg. 10)

General field observations and information (pg. 11)

Field sampling location record (pg. 14)

Field environment record (pg. 15)

5.4 CDC/ATSDR has agreed to have their field staff record information for the General Field Activity (pg. 12) and Participant Information (pg. 13) forms. The forms will be provided by the field coordinator to CDC/ATSDR staff prior to the monitored activities and obtained from CDC staff at the end of research activities at a field.

6.0 MATERIALS AND RESOURCES

6.1 Meteorological Conditions data collection form

6.2 General Field Information/Observations information collection form

6.3 General Activity Information collection form

6.4 Participant Information collection form

6.5 Field Sampling Location Record Form

6.6 Field Environment Record Form

6.7 Kestrel 5500 or equivalent handheld meteorological measurement device

6.8 Compass

6.9 Portable wind direction vane or streamer

6.10 Ink pen with black ink

6.11 Stopwatch or watch with stopwatch function

7.0 PROCEDURES

7.1 General Data and Information Collection Information

Information regarding field conditions, field environment, and activities will be collected as part of the exposure characterization pilot field study conducted at each participating fields. Information will be

collected on structured record forms, but will allow for open-ended observations to be recorded. Data and information collection procedures are described below for each of the six categories of information.

7.2 Meteorological Conditions Data Collection

7.2.1 Select meteorological information will be measured and recorded at three time points around the monitored participant activities each day at each field. The three time points include the approximate start of monitored participant activities, the approximate middle time point of the monitored participant activity, and at the approximate end time of participant activities. If field staff must prioritize their time, the priority should be in deploying and collecting participant samples. Meteorological information can be measured and recorded shortly before or after working with participants, if necessary. Meteorological measurements should be performed on the synthetic turf field and as close to the participant activities as is practical and safe.

7.2.2 At each time point, use the Kestrel 5500 handheld meteorological measurement device.

7.2.2.1 Record the time of day the observations were begun for each time period.

7.2.2.2 Power on the device by pressing and holding the ‘on’ button.

7.2.2.3. Allow the device to equilibrate to current conditions at 1 m above the field surface for five minutes.

7.2.2.4 Press the ‘mode’ button to obtain the temperature reading in °C and record the temperature measurement at a 1-m height above the field. Note: the Kestrel 5500 will be shaded during this measurement period to avoid direct sunlight causing and incorrect reading of ambient air temperature.

7.2.2.5 Power the device off by holding the ‘on’ button. Determine the prevalent wind direction at a 1-m height using a portable vane or streamer. Aim the wind meter rotor at the prevalent wind direction at a 1-m height above the field. Turn the device on, and after 60 seconds press the ‘mode’ button until the average wind speed is displayed. Record the average 60-second wind speed. Press the ‘mode’ button again to get the peak wind speed during the 60-second period and record the peak wind speed. Record wind speeds in units of km/h.

7.2.2.6 Use a compass to determine the predominant direction the wind is coming from. Record the direction the wind is coming from in compass degrees from magnetic north.

7.2.2.7 Place the device flat on the surface of the field in an exposed location for five minutes to measure the field surface temperature. After five minutes, and while the device is still on the field, cycle through the ‘mode’ button to obtain the temperature reading. Record the field surface temperature in °C.

7.2.2.8 By observation, record whether the field surface is wet from dew (yes/no), whether the field is wet from rain or other source of water besides dew (yes/no), and the current general sky conditions (sunny, partly cloudy, cloudy, drizzle, rain).

7.2.9 7.2.9For each day of meteorological data collection, record the field ID number, date, and whether the field is an indoor or outdoor field.

7.3 General Field Information/Observations

7.3.1 Use the General Field Information/Observations collection form to record information about the field and surrounding environment that may be useful for understanding and interpreting the exposure characterization pilot study measurement results. This is an open-ended observational record form that should be completed on each day of monitored participant activities.

7.3.2 The form lists several examples of types of observations of interest as examples. However, the field staff should record information for any observations they believe should be communicated.

7.3.3 The form can be partially completed prior to or after monitored participant activities if the observations are about something that will not change or vary during the monitored activities. However, observed conditions potentially affecting or relevant to measurements performed should be recorded during the monitored participant activities. For example, the level of traffic on adjacent roads or parking areas could be relevant to the air sample collection measures.

7.3.4 Depending upon the type of observation, it may be necessary to also include the time or time interval that you observed the condition or activity. Such examples might include a car, truck or bus idling in the parking lot or road; precipitation conditions or construction activities.

7.3.5 For each day of information collection, record the field ID number and date.

7.4 General Activity Information

7.4.1 Use the General Activity Information collection form to record information about the overall activities (types and estimated numbers of people) that occur on the participant activity field and surrounding fields during the monitored participant activity. A new form should be used for each day of monitored participant activities at each field.

7.4.2 Information should be collected at three time points including the approximate start of monitored participant activities, the approximate middle time point of the monitored participant activity, and at the approximate end time of participant activities.

7.4.3 The structured form lists specific types of information to collect.

7.4.4 For each day of information collection, record the field ID number and date.

7.5 Participant Information

7.5.1 Use the Participant Information collection form to record information about each participant during their monitored activity at the synthetic turf field. A new form should be used for each participant. The form should be completed for all participants, including those that are participating in the video recording portion of the study. The research team plans to have participants wear pinnies with unique participant identifiers (1 – 8) to differentiate the participants. A maximum of four participants per day is expected.

7.5.2 Information about participant activities should be at the start, at approximately 30-minute intervals, and at the end of their monitored activity. Information about participant clothing and equipment should be collected once during the monitored activity; if the clothing or equipment changes then that should be recorded as well.

7.5.3 The structured form lists specific types of information to collect.

7.5.4 For each participant information collection record, record the field ID number, date, and participant number.

7.6 Field Sample Location Record

7.6.1 Use the Field Sample Location Record form to record the sample collection location for each type of sample collected. The form should not be used for quality control samples. Only one form should be completed for each field even if samples are collected on more than one day.

7.6.2 A single letter code should be used for type of sample:

Field SVOC wipe locations	S
Field metals wipe locations	M
Field SVOC drag sled locations	D
Air sample station locations	A

7.6.3 On the field diagram, write the sample type code at each place on the field where samples are collected. For the upwind off-field air sample station, place the code in the correct direction for the field orientation, and write down an estimate of the distance (in meters) from the center point of the field.

7.6.4 In the upper right corner box, draw a directional arrow representing magnetic north using a compass.

7.6.5 Record the field ID number and sample collection date (or the first day of sample collection if it is performed over more than one day; for example, if air samples are collected on one day and wipe samples on another).

7.7 Field Environment Record Form

7.7.1 Use the Field Environment Record form to sketch and label the built and natural structures within approximately 100 meters of the field in each direction. The form needs to be completed only one time, and can be completed during times that don't involve participant activities.

7.7.2 Of interest are parking areas, other fields, buffers, buildings, roads and other natural and built features.

7.7.3 Note the approximate off-field sampling station location.

7.7.4 In the upper right corner box, draw a directional arrow representing magnetic north using a compass.

7.7.5 Record the field ID number and sample collection date (or the first day of sample collection if it is performed over more than one day; for example, if air samples are collected on one day and wipe samples on another).

8.0 RECORDS

The data and information will be collected on the forms described above and shown in Appendix A.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

The field coordinator should collect all forms at the end of each sample collection day to verify completeness and to ensure the content meets the data and information requirements described in this SOP.

10.0 REFERENCES

No references are cited for this SOP.

Appendix A

Data and Information Collection Forms

Meteorological Conditions

TCRS Exposure Characterization Study		**Meteorological Conditions**	
Note: Complete this form for each day of monitored participant activities at each field.			**DRAFT**
		Field ID Number	
		Date	
		Outdoor or Indoor Field	

Metric/Information	Near Start of Participant Activities	Near Middle of Participant Activities	Near End of Participant Activities
Time of Day (Military time format)			
Field Air Temperature at 1 m (°C)			
Field Surface Temperature (°C)			
Wind 1-minute average speed (km/h)			
Wind 1-minute maximum speed (km/h)			
Wind direction (compass degrees, where wind is coming from)			
Dew on field (yes/no)			
Field wet from rain or watering (yes/no)			
Conditions (sunny, partly cloudy, cloudy, drizzle, rain)			

DRAFT

TCRS Exposure Characterization Study — General Field Information/Observations

Use this form to record observations that may be relevant to the research study.

Examples include, but are not limited to, condition of field, field maintenance, construction on/near field, adjacent high traffic on roads or parking areas, ventilation information for indoor fields, other relevant information.

Use a different row for each type of observation.

Field ID Number	
Date	

TCRS Exposure Characterization Study		**General Activity Information**	
Note: Complete this form for each day of monitored participant activities at each field.			**DRAFT**
		Field ID Number	
		Date	

Metric/Information	At Start of Participant Activities	Near Middle of Participant Activities	At End of Participant Activities
Approx. Number People in Active Play on Study Field			
Approx. Number People as Bystanders on Study Field			
Sport Name on Study Field (soccer, football, etc.)			
Type of Active Play on Study Field (1)			
Type of Active Play on Study Field (2)			
Type of Active Play on Study Field (3)			
Type of Active Play on Study Field (4)			
Type of Active Play on Study Field (5)			
Adjacent Synthetic Fields in Use (yes/no)			
Approx. Number People at Adjacent Synthetic Fields			
Adjacent Grass Fields in Use (yes/no)			
Approx. Number People at Adjacent Grass Fields			

TCRS Exposure Characterization Study	**Participant Information**			**DRAFT**	
Note: Complete this form for each participant at each field.					

Field ID Number	
Date	
Participant ID (from 1 to 8)	

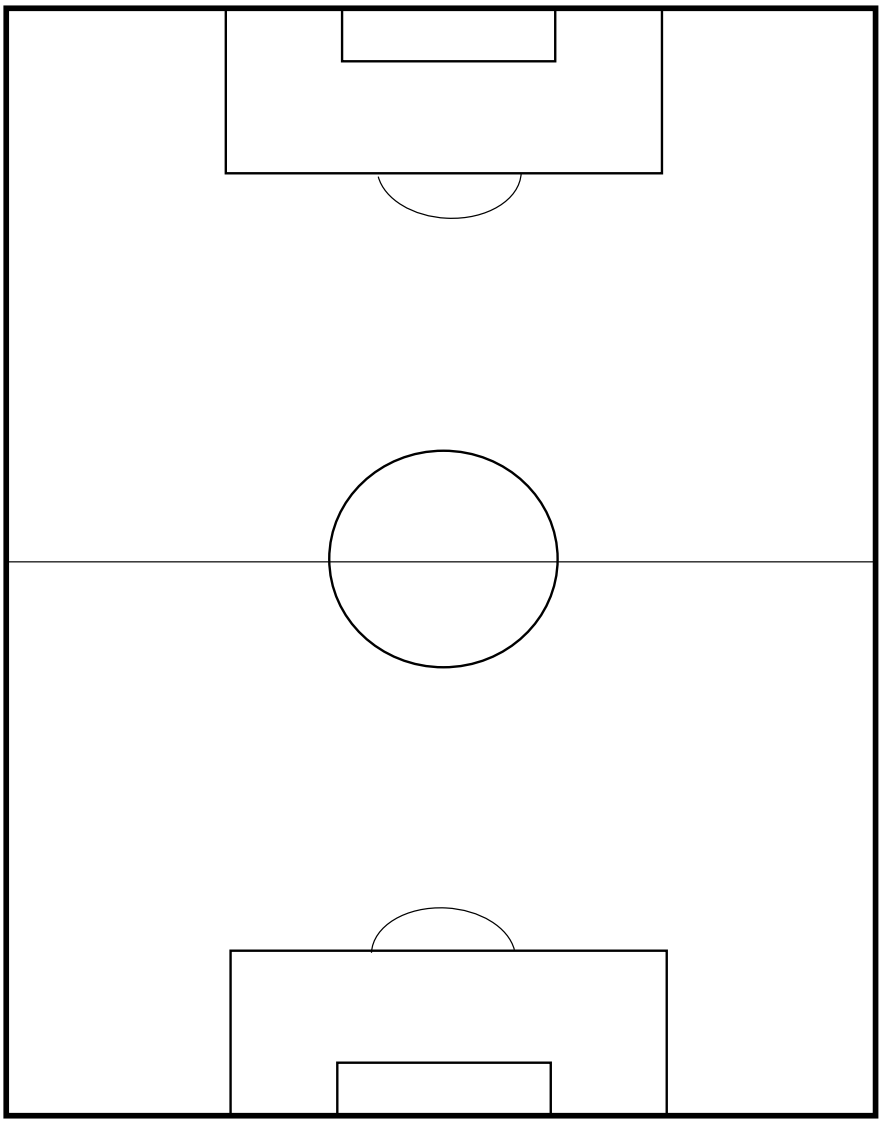
Metric/Information	At Start of Participant Activities	Approx. 30 minutes Into Participant Activities	Approx. 60 minutes Into Participant Activities	Approx. 90 minutes Into Participant Activities	Approx 120 minutes Into Participant Activities	At End of Participant Activities
Time of Day (military time format)						
Activities						
Sport Name (soccer, football, etc.)						
Sport Position (goal keeper, soccer field player, football receiver, etc)						
Type of Activities on Study Field						
Physical Activity Level (low, medium, high)						
Contacting Turf (yes/no)						
Types of Turf Contact (hands, arms, legs, face, body)						
Frequency of Turf Contact (≥ 1/min; ≥ 1/5min; < 1/5min)						
Clothing/Equipment Types (record only once - not at each time period)						
Shirt (yes/no; long/short)						
Pants (long/short)						
Socks (yes/no; high/mid/low)						
Gloves (yes/no and type)						
Head Gear (yes/no and type; hat, helmet, other)						
Mouth Guard (yes/no)						
Pads (yes/no and type; shoulder, hip, leg, other)						
Wearing sunscreen						
Wearing bug repellent						
Other Information						

Field Sampling Location Record Form

Field ID Number

Field SVOC wipe locations S
Field metals wipe locations M
Field SVOC drag sled locations D
Air sample station locations A
(For off-field upwind air sample station estimate and record distance from field center; record type of ground surface)

Draw Magnetic North Direction Arrow Here

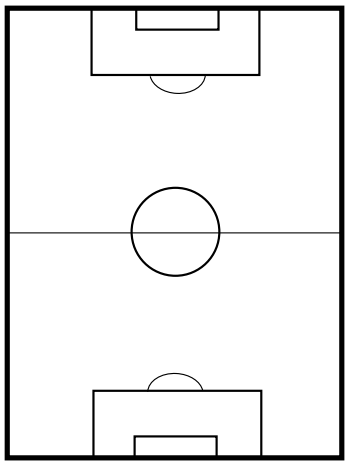


Field Environment Record Form

Field ID Number

Sketch and label features within approx.. 100 m of field
Include roads, parking areas, other fields, buffers, buildings or other natural and built features
Record approximate off-field sampling station location

Draw Magnetic North Direction Arrow Here



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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: Procedure for the Collection of Semi-Volatile Organic Compound (SVOC) Air Samples at Activity Fields Involving Tire Crumb Rubber

Number: D-EMMD-SSAB-012-SOP-01	Effective Date: August 21, 2017

SOP was Developed ☒ In-House ☐ Extramural

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Concurrence

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Title: EMMD QAM

Signature/Date:

PROCEDURE FOR THE COLLECTION OF SEMI-VOLATILE ORGANIC COMPOUND (SVOC) AIR SAMPLES AT ACTIVITY FIELDS INVOLVING TIRE CRUMB RUBBER

Table of Contents

NOTICE
This Analytical Procedure has been prepared for use by the Sensing and Spatial Analysis Branch of the U.S. Environmental Protection Agency, Research Triangle Park, North Carolina and may not be specifically applicable to the activities or objectives of other organizations. This procedure has not been fully validated and should be used for research purposes only. Adequate QA/QC measures must be implemented with this procedure to allow assessment of data quality.

1.0 Scope and Application

This method applies to the collection of semi-volatile organic compounds (SVOCs) from ambient air at activity fields that utilize crumb rubber and/or artificial turf. Samples will be analyzed for a suite of SVOCs.

2.0 Summary of Method

Portable, battery operated, air sampling pumps equipped with polyurethane foam (PUF) cartridges will be used to absorb gas-phase and particle-phase SVOCs from ambient air.

2.1 Definitions

COC - chain-of-custody

Field Blank - sampling media that travels with and is handled like regular sampling media with the exception that it is never opened and exposed to the environment for sampling

Field Control - sampling media that has been spiked with a known concentration of select compounds. This media also travels with and is handled like regular sampling media with the exception that it is never opened and exposed to the environment for sampling

PUF - polyurethane foam

QAPP - quality assurance project plan

SOP - standard operating procedure

SVOCs - semi-volatile organic compounds

VOC - volatile organic compounds

OD – outer diameter

ID – inner diameter

lpm – liters per minute

3.0 Prerequisites Prior to Field Sampling

- JTI will test sampling pumps. Use the procedure outlined in Section 5.2 to verify the sampler can achieve and maintain a flow rate of 20 lpm. EPA has provided a number of working pumps including spares. If a pump is not working, switch out the pump with one that can.
- JTI will replace the Tygon tubing (½ inch OD, 5/16 inch ID, 3/16 inch wall) if visually dirty, sticky, or occluded in order to protect the pump only. It does not need to be replaced after each field study. EPA provided new, clean tubing at the start of the study

and it should last for the duration.

- JTI will charge batteries. EPA has supplied a working 4-Bank Battery Charger (Battery Tender P/N 022-0148-DL-WH). After connecting the charger to power and connecting the batteries to the charger using the attached cords, charging will be automatic and lights will turn solid green when battery charging is complete. More charging information is available in the user's manual (Appendix II).
- JTI will test the flow meter. EPA has supplied a working BIOS DryCal DC- Lite (Model DCL-H Rev. 1.08) and JTI should only need to charge the unit using the power cord supplied. If needed, a manual including operating, charging, maintenance, calibration, and leak check procedures is available (Appendix III).
- EPA will clean and prepare PUF cartridges, including blanks.
- EPA will spike field control PUF cartridges.
- EPA will print and label resealable bags containing sample media. Section 5.4 covers the naming convention for the samples.
- JTI will pack all field supplies listed in Section 3.1. Most equipment loaned by the EPA has transportation enclosures. All equipment should be packed so as to avoid damage.

3.1 Equipment and Supplies

- Sampling Media (University Research Glassware URG- 2000-25 Personal Pesticide Sampler)
    - PUF filter media (URG-2000-25CC) – qty. 7+ per sampling day
    - Glass tubes (URG-2000-25D) – qty. 7+ per sampling day
    - Cartridge caps (URG-2000-25J and H) – qty. 2 per cartridge
    - Cartridge plugs (URG-2000-DUSTCAP and DUSTPLUG) – qty. 2 per cartridge
        - For these experiments, the clean PUF media will be installed in the glass tubes which will be stored inside the cartridge caps, plugged on the ends with orange cartridge plugs, wrapped in clean aluminum foil, and placed in a resealable bag for transport to and from the field.
- Sampling Setup
    - Protective enclosure (e.g., 18 in X 18 in X 12 in) for pumps/batteries – qty. 5
    - Sampling pumps - SKC AirCheck HV30 pump – qty. 5
    - Flathead screwdriver for adjusting pump flowrate
    - Battery - 12V DC, 17Ah sealed lead-acid battery or equivalent – qty. 5
    - Battery Power Adapter – qty. 5
    - Sampling stand (vertical rod that connects to the enclosure) – qty. 5
    - Three pronged clasps for the ring stand – qty. 5
    - Tygon flexible plastic vacuum tubing (½ inch OD, 5/16 inch ID, 3/16 inch wall)
    - Measuring tape
- Flow Measurement
    - Flowmeter – BIOS DryCal DC- Lite (Model DCL-H Rev. 1.08) or equivalent
    - PUF cartridge flow adapter (PUF cartridge with tubing for connecting to BIOS)
- Sample Handling and Storage
    - Brown cardboard shipping box for sample tubes (a box roughly 7"x5"x5" will

hold a day's worth of samples)
    - Bubble wrap or bubble envelopes for sample protection during shipment
    - Sample cooler for storing samples (10qt or larger as needed to hold sample shipping box(s) and ice packs)
    - Re-freezable ice packs
    - Zip closure bags for storage/shipment of PUF cartridges
- Sample Tracking
    - SVOC Air Samples Data Collection Sheet and Chain of Custody Form (see Appendix I)
    - Pen (Black, Permanent Ink)
    - Time enabled device - cell phone is ok
    - Shipping labels
- Spare and other potentially useful supplies
    - Plastic kitchen size garbage bag (non-scented)
    - Aluminum foil
    - Packaging tape
    - Kimwipes
    - Scotch tape
    - Extra labels

3.2 Training Requirements

All training required is provided by the US EPA. Sample collection will be conducted by qualified scientific staff trained in the use of the specific field monitoring equipment. Training will include a demonstration and hands-on training by qualified persons with air sampling expertise. At a minimum, all SOPs and operating instructions will be reviewed, understood, and followed exactly by the field staff. Training records will be maintained by the exposure study project lead (Kent Thomas).

4.0 Cautionary Notes or Special Considerations

The sampling systems are powered by sealed lead acid batteries (gel cell). The battery and pump system should be protected from excessive heat or cold (<50 °F, >104 °F). Pumps and batteries are shielded from direct exposure by means of an outer protective enclosure (see Figure 1). Precautions must be taken against shorting across the battery terminals or reversing polarity of the power leads. Shorting of the battery may cause a rapid discharge that will generate excessive heat and may result in a fire or severe burns. Reversing the polarity (attaching positive to negative) may damage the pump or battery. Red (+) wires should be attached to the red (+) battery terminal and black (-) wires should be attached to the black (-) terminal.

Sampling may take place outside and personnel should be equipped to shield the samples from possible weather events. Plastic trash bags or a tarp or other plastic bin can be used to

cover the pumps/batteries. If sampling is stopped, the entire apparatus may be covered, simply stop the pump and put the cap back onto the PUF sampler.

Sampling stands should be set-up so that they are provided protection from tampering. Sampling stands must also be placed and secured such that no harm will come to children or others playing in the vicinity of the equipment. Protective enclosures and plastic fencing are provided and should be used to insure safety.

5.0 Procedures

The following procedures are listed below for Section 5.0.

1. Pump and Sampling Set-up
2. Pre- and Post-Sampling Flow Rate Measurement
3. Sample Collection
4. Labeling the Samples
5. Pump/Equipment Take Down
6. Return Filter Samples to the EPA Laboratory

NOTE: Set up the pump system at the correct location in advance of the anticipated on-field activity so all sampling stations will be ready prior the beginning of the monitored on-field activity.

5.1 Pump and Sampling Set-up

5.1.1 Transport the sampling setup and flow measurement equipment to the field location. This will include protective enclosures, sampling pumps, batteries, power cords, sampling stands, 3-pronged clasps, vacuum tubing, small screwdriver, and measuring tape to the field location along with the data collection sheets, PUF cartridge flow adapter, flow meter, pen, and any materials needed to secure the monitoring site.

5.1.2 Three sampling sites will be identified for each field location and positioning of the SVOC samplers is defined in the project Quality Assurance Project Plan (QAPP). Two sites will be located next to or as close as possible to the activity field. One site will be located off of the field, in an upwind position if possible. One sampler will be located at each site and an additional sampler will be positioned at one of the on-field locations to collect a duplicate sample. The project QAPP provides guidance for drawing a map of the field to document the position of samplers. (See D-SED-IEMB-030-SOP-01 Collection of Field and Activity Metadata During Exposure Characterization Study Field Sampling for details.)

5.1.3 Figure 1 shows the sampler configuration and the major components are identified. Use this diagram to setup each sampling location. Note that only part of the URG-

2000 cartridge is being used to collect the SVOC sample (the larger cap containing the impactor plate has been removed).

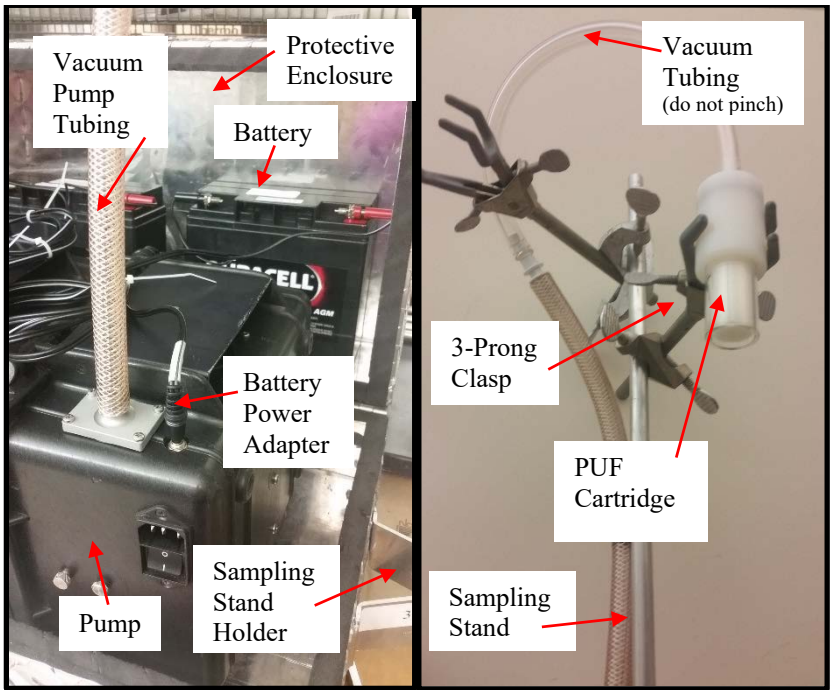


Figure 1: SVOC Sampler Configuration

5.1.3.1 Open the metal protective case and set it on the ground.

5.1.3.2 Setup the sampling stand by inserting one end of the sampling stand into the holder on the metal protective enclosure and attaching the 3-prong clasp to the other end of the sampling stand.

5.1.3.3 Adjust the placement of the clamp on the ring stand until the measured distance between the ground and the clamp is approximately 1 meter.

5.1.3.4 Open the pump, remove the tubing tucked inside the lid, and connect one end to the hose barb found on the right rear of the pump unit. The attached hose should be snug and not easily removed without use of applied force. Set the pump into the protective enclosure on its side so that the hose is positioned straight up.

5.1.3.5 The vacuum tubing will connect to the PUF cartridge once installed. For now, loop the tubing up and over into the clasp. The upper loop of tubing will ensure the tubing will not kink once the sampler is attached. (*Note: If the tubing kinks, secure the tubing with a zip tie (not tape) to the sampling stand to maintain the upper loop.*)

5.1.3.6 Place the battery into the lid of the protective enclosure. Attach the battery power adapter to the pump unit by inserting the adapter into the 12V input jack on the right rear of the pump unit near the hose barb. Attach the battery adapter leads to the appropriate terminals of the battery. Attach the red lead to the positive (+) red terminal and the black lead to the negative (-) black terminal. Make sure good contact is made.

5.2 Pre- and Post-Sampling Flow Rate Measurement

Note: Flow rate measurement will take place before and after field sample collection.

5.2.1 A "dummy" PUF cartridge and tubing (collectively referred to as the SVOC flow adapter) will be sent along in a bag marked "flow adapters for SVOC sampling."

5.2.2 Remove the SVOC flow adapter and remove the orange/red plug from the end of the "dummy" PUF cartridge. Inset this end into the vacuum tubing connected to the sample pump.

5.2.3 The other end of the "dummy" PUF cartridge will be connect to a piece of tubing. Connect this tubing to the bottom barb (outlet) on the BIOS flowmeter.

5.2.4 Turn on the pump by opening the pump lid and sliding the on/off switch located on the pump deck to the "on" position (up). Allow the pump to run for 3-5 minutes to warm-up and stabilize.

Note: The pump flow rate should be close to 20 lpm because the flow rate was adjusted to this point using the flow adjustment screw in the laboratory prior to field deployment. This will minimize the amount of time required to fine adjust the pump flow after the PUF sampler is attached.

5.2.5 After the stabilization period, press the "on" button of the BIOS unit.

5.2.6 Press and hold the "Read" button for approximately 3 seconds. This will activate the unit to make continuous measurements and automatically average 3 replicate readings.

5.2.7 Observe the flow rate values being displayed.

5.2.8 If needed, adjust the flow rate to 20.0 ± 1.0 lpm. While observing the calibrator display, use a small screwdriver to adjust the flow adjustment screw (located just to the left of the on/off switch) to adjust the flow rate. Adjustment should be made by gently turning the screw clockwise or counterclockwise to increase or decrease the pump flow rate. Make adjustments in partial turns.

5.2.9 Once adjusted to 20.0 ± 1.0 lpm allow the flow to stabilize for one minute. After 3 readings, record the average flow rate on the SVOC Air Samples Data Collection Sheet and Chain of Custody Form in the 'Flow' column. There is space for the measurement before and after sample collection (Appendix I).

5.2.10 Turn off the pump, remove the "dummy" PUF cartridge from the sample pump tubing and move-on to check the flow on the other sampling setups.

5.3 Sample Collection

5.3.1 The PUF cartridge will be sent wrapped in aluminum foil and stored in a resealable bag labeled with the sample ID (see Section 5.4 for details). Prior to the anticipated start of the monitored on-field activity, determine the appropriate sample ID and retrieve the appropriate bag. Section 5.4 gives details about how the sample media is labeled. Briefly, the ID numbers will have the form TCRS-R-VV-W-X-Y-Z where position W (F for field or D for duplicate) and Y (on-field position 1, on-field position 2, off-field position 8) will help you identify the appropriate filter media for each sampling station.

Note: The next step will begin handling of the PUF cartridge. Please clean your hands to the best of your ability using water and kimwipes. For SVOC sampling, technicians should use clean hands and minimize contact with the sample cartridge rather than don gloves as most nitrile gloves contain phthalates that can contaminate these samples. Technicians can use the aluminum foil that comes wrapped around the cartridge as a barrier to minimize contact with the sample cartridge.

5.3.2 Remove and unwrap the PUF cartridge being sure to retain the bag and place the aluminum foil inside to keep it clean. (*If the foil becomes soiled, throw it away.*)

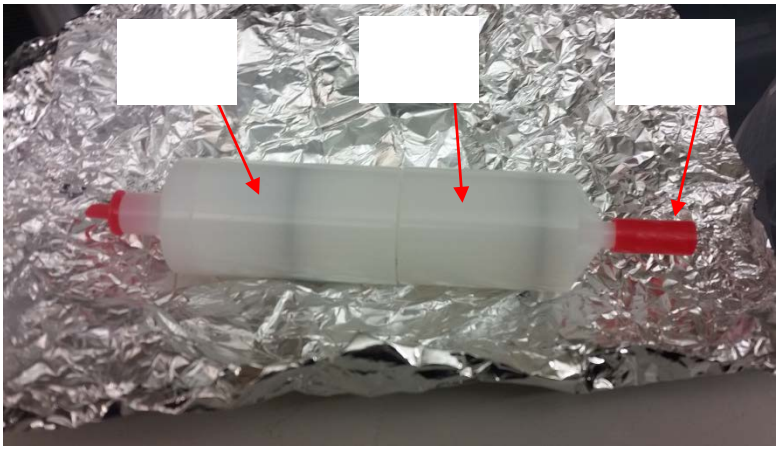


Figure 2: PUF filter cartridge

5.3.3 Looking at the PUF cartridge, you will see that the caps are two different sizes. Remove the longer orange/red plug from the smaller/shorter cap and place it into the resealable bag.

5.3.4 Insert the open end of the PUF cartridge into the vacuum tubing attached to the sample pump. Secure the PUF cartridge on the sampling stand using the 3-prong clasp being sure that the clasp is centered on the upper, shorter cartridge cap (see Fig. 1).

Note: The next step will expose the PUF filter media. DO NOT touch the PUF plug (white foam-like piece). If you MUST touch the glass tube, do so with a clean, dry kimwipe or gloves if you must. Gloves should NOT be worn unless absolutely necessary for cleanliness as compounds from the gloves may interfere with this SVOC measurement.

5.3.5 Remove the bottom, longer cartridge cap (with the smaller orange plug) and place it back into the resealable bag. Place the resealable bag in or under the pump or pump box for safe keeping until sample retrieval. DO NOT separate the resealable bag from the sampling media as this will increase the likelihood that samples will be mixed up.

5.3.6 The sampling setup should now look exactly like Figure 1. Double check the length of tubing and remove any kinks. Adjust the clamp so the inlet to the PUF cartridge is at 1.0 ± 0.1 meters

5.3.7 Sample collection should be initiated prior to the start of the monitored on-field activity so that sampling at all three locations is underway at the time the on-field activity begins. Ideally, sample collection will be initiated within 30 minutes of the start of the monitored activity (with a maximum time of 60 minutes prior). To collect field samples, turn on the pump by opening the pump lid and sliding the on/off switch located on the pump deck to the “on” position (up).

5.3.8 Record the sample start time on the SVOC Air Samples Data Collection Sheet and Chain of Custody Form (Appendix I)

5.3.9 Close the pump lid and settle it into the protective case with the vacuum tubing pointing up. Double check the tubing for kinks and correct if necessary.

5.3.10 Sampling may continue for a period after participant on-field activities are completed so that all personal participant samples can be collected as quickly as possible. Ideally, sample collection will be completed within 30 minutes of the completion of the monitored activity (with a maximum time of 60 minutes after). As soon as feasible following the monitored on-field activity, open the pump lid and turn off the pump by sliding the on/off switch located on the pump deck to the "off" position (down).

5.3.11 Record the sample stop time on the SVOC Air Samples Data Collection Sheet and Chain of Custody Form (Appendix I).

5.3.12 Retrieve the original resealable bag containing the aluminum foil, cartridge cap, and orange plug. Double check the sample ID to be sure the sample is appropriately labeled. Remove the longer cartridge cap from the bag and press it on to the open end of the PUF cartridge. Detach the PUF cartridge from the vacuum tubing and insert the long orange plug onto the shorter cartridge cap. Wrap the entire cartridge back in the aluminum foil (if clean and available, otherwise omit) and place it in the resealable bag with the sample ID.

5.3.13 Place the sample in the cooler chilled with frozen ice packs until placed into freezer storage (no higher than -4ºC) until they are shipped to the EPA Laboratory (see Section 5.6).

5.3.14 Repeat the flow rate measurement (described in Section 5.2) and record the stop flow on the SVOC Air Samples Data Collection Sheet and Chain of Custody Form (Appendix I).

5.4 Labeling the Samples

5.4.1 Labels for these samples will be generated at the EPA lab (using the convention outlined in Section 5.4.2) and placed on the resealable bags containing the clean PUF cartridges before they are transported to the field. At the end of sampling period, the sampled PUF will be returned to this bag in accordance with Section 5.3.

5.4.2 Samples are labeled according to the convention:

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

where:

TCRS designates the tire crumb rubber research study.

R designates the participant identification number
Use 0 for these samples as they are not associated with a specific participant.

VV designates the field ID
This two-digit code will be a unique identifier for each field numbered in the range of 70-79.

W designates the sample type
F = sample
D = duplicate sample
B = field blank
C = field control

X designates the analysis method
Use B to denote field air SVOC sampling.

Y designates the sample collection location
1 or 2 will be for on-field air locations
8 will be for the off-field air location

Z designates the parent/sub-sample as needed
Use 0 to designate these samples as the parent sample.
Use L to designate laboratory QC samples.

5.5 Pump/Equipment Take Down

5.5.1 Remove the sample pump from the protective enclosure.

5.5.2 Disconnect the tubing from the sample pump and store it inside the pump box.

5.5.3 Disconnect the power adapter from the sample pump and battery. Store the adapter inside of the pump box.

5.5.4 Re-pack all supplies into their original shipping containers and prepare for departure and transport of all supplies to the EPA laboratory.

5.6 Return Filter Samples to the EPA Laboratory

5.6.1 All filter media should be stored at the field location in a cooler chilled with frozen

ice packs until they can be shipped to EPA. The samples should be shipped back to the EPA laboratory within two days following sample collection. Samples may be driven back to the laboratory provided they are stored in a cooler with frozen ice packs.

5.6.2 For sample shipment, pack the cooler just prior to shipping as follows:

5.6.2.1 Pack the PUF sample cartridges in bubble wrap inside a brown cardboard box. Tape the box shut and add it to the bottom of the cooler.

5.6.2.2 Add a layer of frozen ice packs to completely cover the storage boxes.

5.6.2.3 Repeat the previous 2 steps as needed until all filter media has been packed for transport.

5.6.2.4 As available and as necessary to keep the exposed filter media from shifting and breaking during transport, add any available ice packs to the cooler followed by unexposed filter media and any padded packing material.

5.6.2.5 Snap a photo of the sample data collection and COC forms (retain in case of damage during shipping, discard after they are recorded by EPA) and then add the data collection and COC forms to a resealable bag. Place the bag on top of the cooler contents and then seal the cooler.

5.6.3 Mail the packed cooler to the EPA laboratory using next day air FedEx or similar overnight delivery service. Address the shipment to:

US EPA Chemical Services
Kent Thomas or Scott Clifton
109 T.W. Alexander Drive
Building E Loading Dock, Rm E178
Research Triangle Park NC 27709-0002
Telephone:919-541-7939

5.6.4 Immediately notify Kent Thomas (thomas.kent@epa.gov) or Scott Clifton (clifton.matthew@epa.gov) of the incoming shipment via email. Include the shipment tracking number.

6.0 Quality Control

The quality control requirements will allow assessment of the quality of the samples collected. Determination of possible contamination and reproducibility of the method will

be targeted as data quality indicators.

6.1 Field Blanks and Field Controls

A minimum of 1 field blank and 1 spiked field control will be collected each sampling day. The project's quality assurance project plan will detail the quality control sample types, numbers, and deployment plan (see Section B5 of Quality Assurance Project Plan Addendum for the Tire Crumb Research Study - Exposure Characterization Pilot Study (D-SED-IEMB-006-QAPP-02)).

6.1.1 As stated in Section 5.2.1, the PUF cartridge will be sent plugged with orange fittings, wrapped in aluminum foil, and stored in a resealable bag. Resealable bags containing PUF cartridges meant for field sampling and for field blanks and controls will be marked with the sample ID (see Section 5.4)

6.1.2 Field blank(s) and field control(s) will be taken to the synthetic turf field along with the sample filters. The field blank(s) and field control(s) will NOT be opened or deployed but should be handled just like field samples in that they should be transported to the field and put into cold storage at the same time as the field samples. These blanks will NEVER be removed from the resealable storage bags.

6.1.3 The field blank(s) and field control(s) will be stored and shipped along with the sample filters.

6.2 Duplicate Samples

Duplicate samples shall be collected at a single on-field location during each day of field measurement. Two sampling systems (pump/inlets) shall be positioned within 2 meters of each other and operated as specified in Section 5. The purpose of duplicate samples is to determination of precision of the sampling method in its entirety.

6.3 BIOS Flow Calibrator Calibration

The BIOS flow meter will be used to determine the flow rate through the sampling media. Because the unit is equipped with a mass flow controller and because of sampling conditions (pump not operated at max flow and pressure drop sufficiently low), the flow rate can be considered stable during sampling.

This BIOS flow meter was calibrated by the manufacturer and no adjustment or additional calibration of this device is needed. The flow meter should be sent back to the manufacturer or to another source for re-calibration if it has been greater than 1 year since the calibration

certification date or if flow meter is not operating properly.

7.0 General Sampling Precautions

- Pumps should never be operated without a filter in-line.
- Pumps should be calibrated against the reference BIOS. Calibration should take place just prior to sampling initiation and after sampling as noted in section 5.2.
- The flow rate will be used in conjunction with the total elapsed sampling time to calculate total air volumes sampled and integrated analyte concentrations observed during the sample capture period.

8.0 Possible Corrective Actions for Observed Problems During Sampling

8.1 Pump Failure

- If a pump fails, correct any obvious errors such as kinked lines, battery not fully charged, etc. If possible, replace the pump or battery.
- Document any pump failures in the data collection sheet.

8.2 Possible Contamination of Filters or Supplies

Clean resealable bags and aluminum foil will be available. Simple replace the contaminated items being sure to transfer the sample ID sticker to a new resealable bag.

When possible, a spare PUF sampling cartridge will also be sent along. This PUF cartridge may be used in place of a contaminated cartridge if a field sample cartridge is contaminated prior to sample collection.

Contact the NERL-EMMD staff scientists for possible replacement items or directions for decontamination. Be sure to document in the data collection sheets any suspicion of possible contamination of filters or supplies.

9.0 Recordkeeping

9.1 Data Sheets

All information concerning sample collection will be recorded by the appropriate operator on the SVOC Air Samples Data Collection and COC form attached in Appendix I.

9.2 Calculations

The sample flow rate is directly measured using the average of the pre and post BIOS flow measurements. The elapsed time in minutes is the sum total of minutes the pump operated during the sampling episode. A normal 3 hour run period should have approximately 3 hours X 60 min/hour = 180 minutes. Sample volume calculations will be made during data processing, not as part of the field sampling activities.

9.3 Chain-of-Custody

The original of the SVOC Air Samples Data Collection Sheet and Chain of Custody Form will accompany the filter samples back to the NERL-RTP laboratory. A copy of the SVOC Air Samples Data Collection Sheet and Chain of Custody Form is attached in the Appendix I.

Sample collection, shipping, receipt, and analysis will be indicated on the sample COC form by responsible parties. Original copies of all data forms will be returned to the EPA project coordinator Kent Thomas (thomas.kent@epa.gov) and maintained in the NERL TCRS project files.

Appendix I. SVOC Air Samples Data Collection Sheet and Chain of Custody Forms

Each form included in this appendix has been given a unique form number (COC-XX) at the request of JTI so that all sample collection forms for this study can be more easily tracked.

Four forms are attached and are differentiated by the Field Location ID and the sub-header referring to field or blank samples. For air samples, locations 1 and 2 designate on-field air sampling locations and 8 designates the off-field air location. The lasts form has a sub-header designating its use for blanks/controls.

TIRE CRUMB EXPOSURE STUDY-- AIR SAMPLING DATA AND COC SHEET

COC-08

Field Locations -- Active PM & SVOC Air Samples

		Deployment	Recovery
Study Name: TCRS	**Date**		
Field ID No:	**Operator**		

Sample Chain of Custody

Field Location ID	1										
					Shipped to EPA,RTP		**Received & Stored at EPA, RTP**		**Analyzed**	
PM Filter ID	**Start Time**	**Start Flow**	**Stop Flow**	**Stop Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

SVOC	**Start Time**	**Start Flow**	**Stop Flow**	**Stop Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
TCRS-0-98-F-B-1-0 Field air SVOCs - Loc 1		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- AIR SAMPLING DATA AND COC SHEET

COC-09

Field Locations -- Active PM & SVOC Air Samples

		Deployment	Recovery
Study Name: TCRS	Date		
Field ID No:	Operator		
Field Location ID 2			

Sample Chain of Custody

PM Filter ID	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date

Sample ID	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

Sample ID	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

SVOC	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date

TCRS-0-98-F-B-2-0 Field air SVOCs - Loc 2	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

Sample ID	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

TIRE CRUMB EXPOSURE STUDY-- AIR SAMPLING DATA AND COC SHEET

COC-10

Field Locations -- Active PM & SVOC Air Samples

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID	**8**		

Sample Chain of Custody

PM Filter ID	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

SVOC	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- AIR SAMPLING DATA AND COC SHEET

COC-12

Active SVOC Air Field/Lab Blanks and Controls

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID			

Sample Chain of Custody

	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Field notes:	
Receipt notes:	
Analysis notes:	

	Initials	Date	Initials	Date	Initials	Date

Sample ID	
Field notes:	
Receipt notes:	
Analysis notes:	

SVOC	Initials	Date	Initials	Date	Initials	Date

Sample ID	
Field notes:	
Receipt notes:	
Analysis notes:	

	Initials	Date	Initials	Date	Initials	Date

Sample ID	
Field notes:	
Receipt notes:	
Analysis notes:	

Appendix II. Battery Tender – Portable Battery Chargers - Owner's Manual

(4BankBatteryChargerUserManual.pdf attached)

Appendix III. BIOS DryCal DC-Lite Flow Calibrator Manual

(DryCalDCLiteFlowCalibratorManual.pdf attached)

Portable Battery Chargers

Designed for six cell lead-acid batteries from 1.2 – 200Ah.

IMPORTANT SAFETY INSTRUCTIONS

CAREFULLY READ AND

SAVE THESE INSTRUCTIONS

DOWNLOAD MANUAL

This manual can be read or downloaded from the BATTERY TENDER® website @ www.batterytender.com

WARNING AND CAUTION LABEL DEFINITIONS:

WARNING indicates a potentially hazardous situation which, if not avoided, could result in serious injury or death.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury.

CAUTION

CAUTION used without the safety alert symbol indicates a potentially hazardous situation, which if not avoided, may result in property damage.

GENERAL PRECAUTIONS

WARNING

Always charge the battery in a well ventilated area. Explosive hydrogen gas may escape from the battery during charging. Keep open flames, electrical sparks and smoking materials away from the battery at all times. Failure to do so could result in serious injury or death.

NOTE :

Gas hot water heaters are a source of open flame to be avoided.

CAUTION

Locate the charger as far away from the battery as is allowed by the length of the output cable harness. NEVER set the charger above or below the battery. Gasses or fluids from the battery may corrode and damage the charger.

CAUTION

Do not set the charger on a combustible surface. Locate in a well ventilated area to dissipate heat generated by the charger.

CAUTION

NEVER use a battery charger unless the battery voltage matches the output voltage rating of the charger. For example, do not use a 12-volt charger with a 6-volt battery and vice versa.

⚠ WARNING

Do not expose the charger to rain or snow to avoid risk of electric shock or fire.

⚠ WARNING

Do not use attachments or accessories that are not recommended or sold by the battery charger manufacturer. Doing so may cause electric shock, fire, or other unforeseen situations resulting in serious injury or death.

⚠ CAUTION

When handling electric power cords, always pull by the plug rather than by the cord. This reduces the risk of damage to both the plug and cord, and minimizes the likelihood of electric shock.

CAUTION

Make sure all electric power cords are located so that they cannot be stepped on, tripped over, or otherwise subjected to damage or stress.

CAUTION

Study all of the battery manufacturer's precautions and specific recommendations for safe operation such as not removing cell caps while charging and the recommended rates of charge (charger output current). This is important to avoid damage to the battery.

CAUTION

When leaving a battery charger connected to a non-sealed, flooded battery for extended periods of time (weeks, months, etc.), periodically check individual cell fluid levels against manufacturer's recommendations for safe operation.

CAUTION

If the battery releases an excessive amount of gas or if the battery gets hotter than 130°F (55°C) during charging, disconnect the charger and allow the battery to cool. Overheating may result in plate distortion, internal shorting, drying out or other damage.

P/N 392-0215-RC

⚠ WARNING

NEVER disassemble the charger or attempt to do internal repairs. Take it to a qualified service technician. Assembling the charger incorrectly may result in the risk of electric shock or create a fire hazard. If the supply cord is damaged, it must be replaced by the manufacturer, its service agent or similarly qualified persons in order to avoid a hazard.

PERSONAL PRECAUTIONS

⚠ WARNING

Battery posts, terminals and related accessories contain lead and lead components, chemicals known to the State of California to cause cancer and birth defects or other reproductive harm. Wash hands after handling.

1. Someone should be within range of your voice or close enough to come to your aid when you work near a lead-acid battery;
2. Have plenty of fresh water and soap nearby in case battery acid contacts skin, clothing, or eyes;
3. Wear complete eye protection and clothing protection. Avoid touching eyes while working near battery;
4. If battery acid contacts skin or clothing, wash immediately with soap and water. If acid enters an eye, immediately flood eye with running cold water for at least 10 minutes and get medical attention immediately;
5. **NEVER** smoke or allow a spark or flame in vicinity of battery or engine.
6. Be extra cautious to reduce risk of dropping a metal tool onto battery. It might spark or short-circuit battery or other electrical part that may cause an explosion;
7. Remove personal metal items such as rings, bracelets, necklaces, and watches when working with a lead-acid battery. A lead-acid battery can produce a short-circuited current high enough to weld a ring or the like to metal, causing a severe burn;
8. Use the charger for charging a lead-acid battery only. It is not intended to supply power to an extra low-voltage electrical system or to charge dry-cell batteries. Charging dry-cell batteries may cause them to burst and cause injury to persons and damage to property;

NOTE

There are some wet, non-spillable, lead acid batteries on the market whose manufacturers' make the claim that they are dry-cell batteries. These batteries are sealed, gas-recombinant, starved electrolyte, possibly with AGM (Absorbed Glass Matte) type construction. It is perfectly safe to use the INTERNATIONAL BATTERY TENDER® to charge these types of batteries. The dry-cell battery warning is intended for non-rechargeable, alkaline and other similar types of batteries. If you have any doubt about the type of battery that you have, please contact the battery manufacturer before attempting to charge the battery.

9. **NEVER** charge a visibly damaged or frozen battery.
10. Do not recharge non-rechargeable batteries.

PREPARING TO CHARGE

1. If it is necessary to remove battery from vehicle to charge it, always remove grounded terminal from battery first. Make sure all accessories in the vehicle are off in order to prevent an arc;
2. Be sure area around battery is well ventilated while battery is being charged. Gas can be forcefully blown away by using a piece of cardboard or other nonmetallic material as a fan;
3. Clean battery terminals. Be careful to keep corrosion from coming in contact with eyes;
4. Add distilled water in each cell until battery acid reaches level specified by battery manufacturer. This helps purge excessive gas from cells. Do not overfill. For a battery without cell caps, carefully follow manufacturers' recharging instructions;
5. Study all battery manufacturers' specific precautions such as removing or not removing cell caps while charging and recommended rates of charge;
6. Determine voltage of battery by referring to owner's manual and make sure it matches output rating of the battery charger.
7. Locate charger:
    a. Locate the charger as far away from battery as the DC cables permit;
    b. Never place the charger directly above or below the battery being charged. Gases or fluids from the battery will corrode and damage the charger;
    c. Never allow battery acid to drip on the charger when reading gravity or filling battery;
    d. Do not operate the charger in a closed-in area or restrict ventilation in any way.
    e. Do not set a battery on top of the charger.
8. Connect and disconnect DC output clips only after setting any charger switches to the off position and removing AC cord from the electric outlet. Never allow clips to touch each other.
9. Follow these steps when battery is installed in a vehicle. A spark near battery may cause a battery explosion. To reduce risk of a spark near battery:
    a. Position AC and DC cords to reduce risk of damage by hood, door, or moving engine parts like fan blades, belts, and pulleys.
    b. Check polarity of battery posts. A positive (pos, p, +) battery post may have a larger diameter than a negative (neg, n, -) post;
    c. Determine which post of battery is grounded (connected) to the chassis. If negative post is grounded to the chassis (as in most vehicles), see item (d). If positive post is grounded to the chassis, see item (e);
    d. For a negative-grounded vehicle, connect the positive (red) clip from the battery charger to the positive (pos, p, +) ungrounded post of battery. Connect the negative (black) clip to the vehicle chassis or engine block away from battery. Do not connect the clip to carburetor, fuel lines, or sheet-metal parts. Connect to a heavy gauge metal part of the frame or engine block;

e. For a positive-grounded vehicle, connect the negative (black) clip from battery charger to negative (neg, n, -) ungrounded post of battery'. Connect the positive (red) clip to the vehicle chassis or engine block away from battery. Do not connect clip to carburetor, fuel lines, or sheet-metal parts. Connect to a heavy gauge metal part of the frame or engine block;

f. Connect charger AC supply cord to an electric outlet;

g. When disconnecting the charger, turn switches to off, disconnect AC cord, remove clip from vehicle chassis, and then remove clip from battery terminal.

10. Follow these steps when battery is outside the vehicle. A spark near the battery may cause a battery explosion. To reduce risk of a spark near battery:

a. Check polarity of battery posts. A positive (pos, p, +) battery post may have a larger diameter than a negative (neg, n, -) post;

b. Attach at least a 24 inch long 6-gauge (AWG) insulated battery cable to the negative (neg, n, -) battery post;

c. Connect the positive (red) charger clip to the positive (pos, p, +) post of battery;

d. Position yourself and the free end of cable as far away from battery as possible, then connect negative (black) charger clip to free end of cable;

e. Do not face battery when making final connection;

f. Connect charger AC supply cord to an electric outlet;

g. When disconnecting the charger, always do so in reverse sequence of connecting procedure and break first connection while standing as far away from the battery as is practical.

USER INSTRUCTIONS

AUTOMATIC CHARGING AND BATTERY STATUS MONITORING: All BATTERY TENDER® chargers are completely automatic and may be left connected to both AC power and to the battery that it is charging for long periods of time. The charger output power, voltage, and current depends on the condition of the battery it is charging. BATTERY TENDER® chargers have 2 status indicator lights that provide a visual means to determine the operating mode of the charger and hence the condition of the battery connected to the charger.

The two-colored status indicator lights are available to determine whether the charger is operating in one of the 3 primary charge modes: the bulk mode (full charge, constant current, battery is 0% to 85% charged), the absorption mode (high constant voltage, battery is 85% to 100% charged), or the storage/float maintenance mode (low constant voltage, battery is 100% to 103% charged).

When the battery is fully charged, the green status indicator light will turn on and the charger will switch to a storage/maintenance charge mode. The BATTERY TENDER® charger will automatically monitor and maintain the battery at full charge.

ELECTRICAL CONNECTIONS BETWEEN THE CHARGER AND THE BATTERY: Before charging, connect the alligator clips or ring terminals to the battery terminals. Then connect the charger AC power cord to the AC power outlet. When you want to disconnect the charger from the battery, first disconnect the charger AC power cord from the AC power outlet. Then disconnect the charger leads from the battery terminals.

⚠ WARNING

Always unplug or turn OFF the battery charger before connecting or disconnecting the charger clamps to the battery. Connecting or disconnecting clamps with the charger on could cause a spark resulting in a battery explosion. A battery explosion may rupture the battery case causing a discharge or spray of sulfuric acid which could result in serious injury or death.

CONNECTIONS FOR ALL LEAD-ACID BATTERY TYPE: (See item 10 under General Precautions.)

< In General: First connect the red positive (+) charger output lead to the positive terminal of the battery. Then connect the black negative (-) charger output lead to the negative terminal of the battery. However, pay particular attention to the next two items and the instructions under item 18 under General Precautions.

< As an added measure of safety, particularly when working with standard, flooded, lead acid batteries, UL recommends that the second, negative (-) charger output lead connection be made to the grounded equipment chassis rather than directly to the negative battery post.

< In similar fashion, for positive ground systems, the positive post of the battery is now at the same electrical potential as the grounded equipment chassis. Therefore UL recommends that the positive (+) charger output lead connection be made at the grounded equipment chassis rather than directly to the positive battery post.

ATTENTION: BATTERY TENDER® CHARGERS HAVE A SPARK FREE CIRCUITRY. The output alligator clips or ring terminals will not spark when they are touched together. The BATTERY TENDER® chargers will not produce an output voltage until it senses at least 3 volts from the battery. It must be connected to a battery with the correct polarity before it will start charging a battery. Therefore, if you plug the AC power cord into an AC power outlet, and if the output alligator clips or ring terminals are not connected to a battery, and if you touch the alligator clips or ring terminals together, there will be no electrical spark.

NOTE:

THE OUTPUT CLIPS OR RING TERMINALS MUST BE CONNECTED TO A BATTERY BEFORE THE CHARGER CAN PRODUCE AN OUTPUT VOLTAGE.

If the charger is hooked up backwards, the amber light will continue flashing (International Plus and EURO400), indicating that a charge has not been initiated (WP800 does not show any light at all). The alligator clips or accessory ring terminals must be connected to the battery, with the proper polarity, Red to Positive (+ output to + battery post) and Black to Negative (- output to - battery post), before the charger will generate any output voltage.

WORKING WITH A DEAD BATTERY OR A BATTERY WITH A VERY LOW VOLTAGE:

If you try to charge a dead battery having a voltage below 3 Volts, BATTERY TENDER® chargers will not start. An internal safety circuit prevents the BATTERY TENDER® chargers from generating any output voltage unless it senses at least 3 Volts at the charger output. In this situation, the amber light will continue to flash (International Plus and EURO400), indicating that a charge has not been initiated (WP800 does not show any light at all).

NOTE:

If a 12 Volt, Lead-Acid battery has an output voltage of less than 9 volts when it is at rest, when it is neither being charged nor supplying electrical current to an external load, there is a good chance that the battery is defective. As a frame of reference, a fully charged 12-Volt, Lead-Acid battery will have a rest-state, no-load voltage of approximately 12.9 volts. A fully discharged 12-Volt, Lead-Acid battery will have a rest-state, no-load voltage of approximately 11.4 volts. That means that a voltage change of only 1.5 volts represents the full range of charge 0% to 100% on a 12-Volt, Lead-Acid battery. Depending on the manufacturer, and the age of the battery, the specific voltages will vary by a few tenths of a volt, but the 1.5-volt range will still be a good indicator of the battery charge %.

STATUS INDICATING LIGHTS: If neither light is lit, then the battery is not properly connected and/or the charger is not plugged into AC power. The following describes light operation:

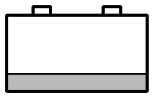
< **AMBER LIGHT FLASHING** – The amber light flashing indicates that the battery charger (International Plus and EURO400) has AC power available and that the microprocessor is functioning properly. If the amber light continues to flash, then either the battery voltage is too low (less than 3 volts) or the output alligator clips or ring terminals are not connected correctly.

< **AMBER LIGHT ON STEADY** – Whenever the amber light is on steady, a battery is connected properly and the charger is charging the battery. The amber light will remain on until the charger completes the charging stage.

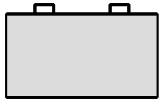
< **GREEN LIGHT FLASHING** – (International Plus and EURO400 only) When EURO400 shows a green light flashing, the battery is 80% charged and may be used if necessary. When the green light is flashing, and the amber light is on (International Plus), the battery is greater than 80% charged and may be removed from the charger and used if necessary. Whenever possible, leave the battery on charge until the green light is solid.

< **GREEN LIGHT ON STEADY** – All chargers: When the green light stops flashing and burns steady, the charge is complete and the battery can be returned to service if necessary. It can also stay connected to maintain the battery for an indefinite period of time

STATUS INDICATING SYMBOLS: The following symbols are located next to the status indicator lights.



The symbol next to the AMBER light represents a partially charged battery. The solid band across the bottom is green in color. The background is yellow. The green area indicates the charged portion of the battery and the yellow area represents the uncharged portion.



The symbol next to the GREEN light represents a fully charged battery. The entire area inside the battery outline is green.

TROUBLESHOOTING CHECK LIST:

1. CHARGER LIGHTS DO NOT TURN ON:
   a. Remove the charger from the AC outlet and recheck that the battery charger clamps are connected to the correct terminals and are making a clean tight connection.
   b. Check to make sure AC outlet is supplying power by plugging in a lamp, an appliance, or a voltage meter.
2. THE GREEN LIGHT GOES ON IMMEDIATELY WHEN CHARGING A DISCHARGED BATTERY:
   a. The battery may be defective, take battery to the dealer to be tested.
3. CHARGER IS CHARGING BUT THE GREEN LIGHT DOES NOT GO ON:
   a. The battery may be defective, take battery to the dealer to be tested.
   b. The battery has an excessive current draw, remove battery from equipment.
4. THE AMBER LIGHT COMES ON WHEN STORAGE CHARGING BATTERIES:
   a. The battery may be defective, take battery to the dealer to be tested.
   b. The battery has an excessive current draw, remove battery from equipment.

- *This appliance can be used by children aged from 8 years and above and persons with reduced physical, sensory or mental capabilities or lack of experience and knowledge if they have been given supervision or instruction concerning use of the appliance in a safe way and understand the hazard involved.*

- Children shall not play with the appliance. Cleaning and user maintenance shall not be mad by children without supervision.

- The supply cord cannot be replaced. If the cord is damaged the appliance should be scrapped.

- Examine the battery charger regularly for damage, especially the cord, plug and enclosure, if the battery charger is damaged, it must not be used until it has been repaired.

This symbol indicates separate collection for electrical and electronic equipment

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Butler, New Jersey, USA 07405
Phone (973) 492 8400
Toll Free (800) 663 4977
Fax (973) 492 8270

Email sales@biosint.com
Web www.biosint.com

DryCal® DC-Lite Manual

MK01-1 Rev. C 1.09

DryCal® DC-Lite Specifications

Size 5" x 5" x 2.75" • 127 mm x 127 mm x 70 mm
Weight 42 oz • 1200 g
Flow Ranges | Air Flow Accuracy Specifications based on averaged readings. Lower limit is based on self-tested maximum leakage.

Model	Optimum Flow Range (±1%)	Extended Flow Range
L	10–500 ml/min	1 ml/min–500 ml/min
ML	50 ml/min–2 L/min	5 ml/min–5 L/min
M	100 ml/min–7 L/min	10 ml/min–12 L/min
MH	200 ml/min–20 L/min	20 ml/min –20 L/min
H	500 ml/min–30 L/min	50 ml/min–30 L/min

Contact Bios for extended flow range specifications, or visit our website at www.biosint.com/products/dclite_models.htm

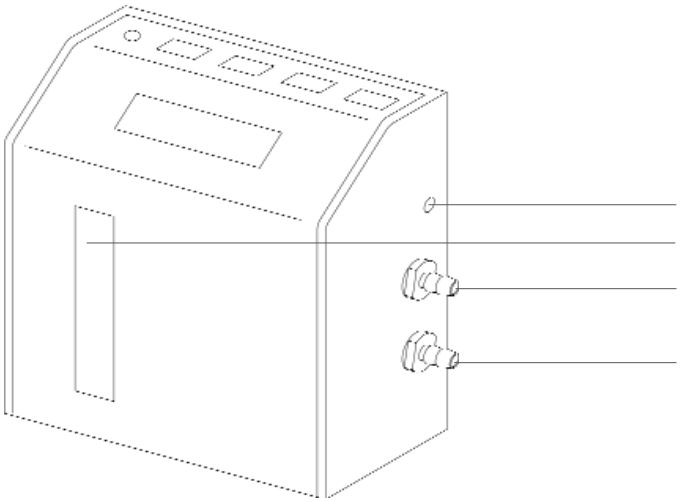
Battery System 6V rechargeable, sealed lead-acid, 6-8 hours typical operation
AC Battery Charger | Power Adapter Wall-mounted, single-station charge, input: 100 to 120 VAC, 60 Hz., output: 12 VDC. Optional input: 200 to 240 VAC, 50 Hz., output 12 VDC.
Operating Modes Single reading, 10 readings, or auto-mode.
Temperature Range 0–55 °C
Humidity Range 0–70% non-condensing
Printer Port Standard parallel (Not compatible with printers that require Microsoft® Windows™)
Warranty Product, 1 year; battery, 6 months

The annual recalibration program offered by Bios is elective and is not included as a warranty item. All specifications are subject to change.

Please contact Bios or visit our web site at www.biosint.com for the most current information.

Table of Contents

1.0 DC-Lite Features



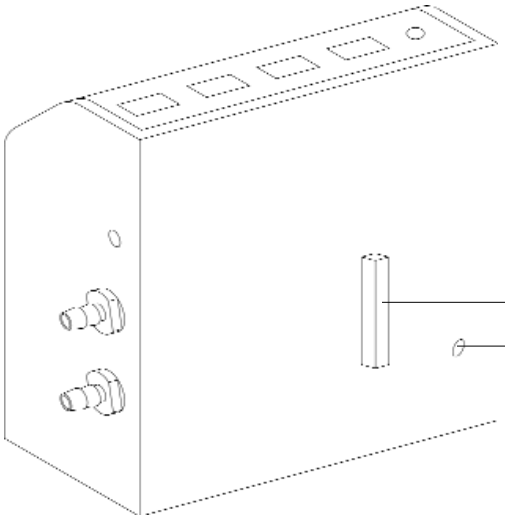
Front and Side

Charging Jack

Cell Viewing Window

Inlet Port
Connect tubing here for pressure applications

Outlet Port
Connect tubing here for suction applications



Back and Side

Parallel Printer Port

Reset Button

2.0 Unpacking Checklist

Your DryCal DC-Lite has been packaged with care and includes all components necessary for operation. Please take a moment to check that you have received the following items. If you believe you have not received a full shipment or have any other questions, please contact Bios immediately.

Your DryCal DC-Lite Includes

- Single-Station Battery Charger
- Tubing Kit
- Additional High Flow Tubing (with DCL-MH and DCL-H only)
- Certificate of Calibration
- Instruction Manual
- Registration Card

3.0 General Description

The DryCal DC-Lite is a field-portable primary flow calibrator used for industrial hygiene, environmental and laboratory flow measurement applications.
The DC-Lite uses patented dry piston technology and infrared sensors to obtain volumetric flow rates quickly and accurately.

Housed in a small, sturdy case, each unit employs a variety of popular user conveniences such as push-button read and auto-read functions, a large alphanumeric display, battery level indicator, 5-minute automatic shut-off and a parallel printer port for data-logging.

4.0 Theory of Operation

The DryCal DC-Lite can be used to measure air flow rates for either a vacuum flow source (connected to the outlet port) or a pressure flow source (connected to the inlet port). Before a reading is initiated, or between readings, a computer-directed valve performs a bypass function. This allows the air to pass through the DryCal valve, bypassing the flow-measuring cell which is then able to reset.

As a reading is initiated (by pressing the **Read** button) the internal valve closes and the flow source evacuates or pressurizes the air in the flow-measuring cell. The piston rises at the rate of evacuation or pressurization. A precision encoder system provides two finely collimated light beams with a known distance between the beams.

After a suitable acceleration interval the piston breaks the first infrared light beams as it passes. The flow reading is completed when the second infrared beam

is broken. A crystal clock measures the time interval as the piston passes the two infrared light beams. The internal computer then calculates the volumetric flow based upon these parameters.

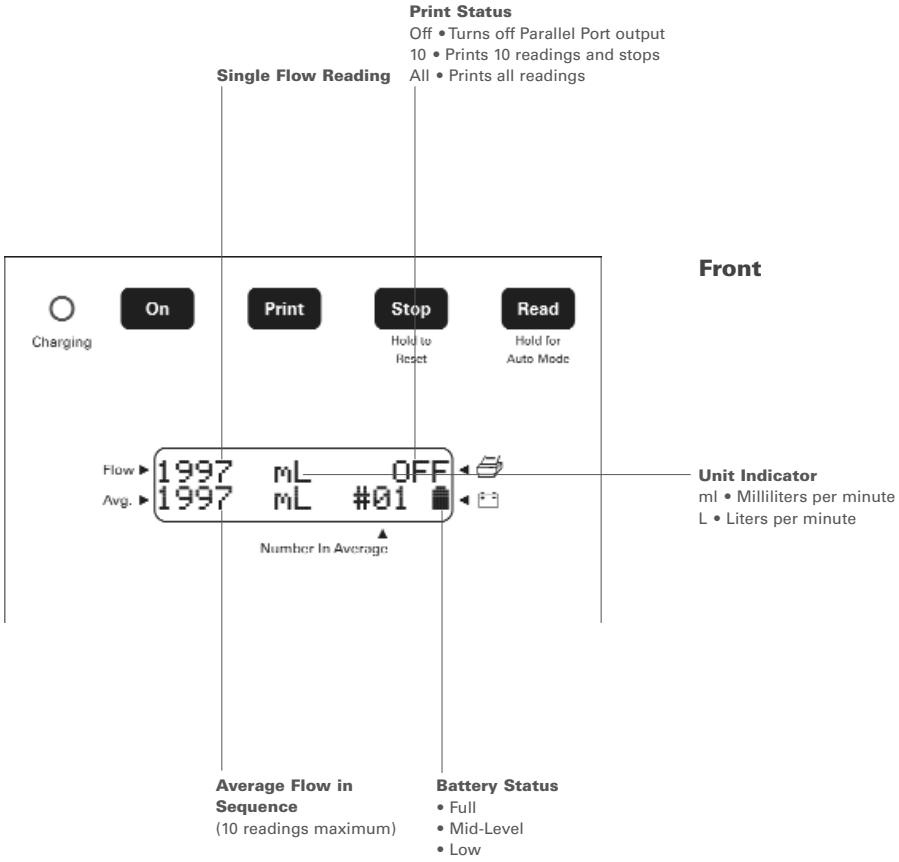
After a completed cycle, the valve is opened by the computer and the piston resets. The flow measurement is instantly displayed on the LCD in milliliters per minute (ml) or liters per minute (L).

Any time the valve is open, the air flow is allowed to pass through the DryCal valve, bypassing the flow-measuring cell.

5.0 Operating Instructions

The following pages will guide you through the operation of your DC-Lite primary flow calibrator.

5.1 DC-Lite Button Panel



5.2 Turning the Power On

The DC-Lite has an energy saving 5-minute auto shut-off feature.

1. Press the **On** button to turn the DC-Lite on.
2. An initializing screen will display the microprocessor revision number, then the standard screen will be displayed.

5.3 Disabling & Re-Enabling the 5-Minute Auto-Shutoff Feature

5-minute auto-shutoff is the default setting for the DC-Lite. This feauture can be disabled if your application requires a longer standby time. The 5-minute auto-shutoff feature must be disabled each time the unit is powered on or reset.

The DC-Lite features protective circuitry that prevents the battery from becoming over-depleted. If the battery is allowed to become too weak, the DC-Lite may automatically shut off due to low battery voltage. This is more likely to occur more if the 5-minute auto-shutoff feature is disabled.

Disabling the 5-minute auto-shutoff feature

1. Press and hold the **Read** button, then press the **On** button (or the **Reset** button if the unit is already on).
2. The display will read, “Auto-Off Disabled” until the **Read** button is released.

Re-enabling the 5-minute auto-shutoff feature

With the unit on, push the **Reset** button.

5.4 Taking Readings

Taking Single Readings

The inlet and outlet ports are located on the right side of the unit. The lower port is for suction (outlet) and the upper port is for pressure (inlet). All successive readings will automatically be used to calculate the average flow. The unit will automatically clear the average after ten readings and begin averaging a new sequence.

A reading has been initiated when the green LEDs in the flow cell viewing window turn on, the valve can be heard closing and the piston begins to move up the flow cylinder.

1. Connect tubing between the flow source and the DC-Lite with sampling medium in-line if the application requires it. Turn the DC-Lite and flow source on.

2 Press and release the **Read** button to obtain a single flow measurement. The flow measurement will appear on the LCD.
3 Continue this procedure to obtain the required number of flow readings.

Taking Auto-Repeat Readings

Readings can be taken continuously in the auto-repeat mode for hands-free operation. The unit will automatically clear the average after ten readings and begin averaging a new sequence.

1 Press and hold the **Read** button until a reading starts then release. This will begin a continuous read session.
2 To stop the continuous read session, press the **Stop** button once. The display will indicate the current flow reading (Flow), the average flow value (Average) and the number of readings in the average (Number in Average) up to 10.

5.5 Resetting the Averaging Sequence

The number of readings in an averaging sequence can be reset to (00) at any time by pressing and holding the **Stop** button for 2 full seconds.

5.6 Printing

The DC-Lite must be turned on prior to connecting a printer cable to the back of the unit. Failure to do so will result in the display reading "Nexus Control." If this occurs, remove the printer cable and reset the unit using the white recessed **Reset Button** located on the back of the unit as described in Section 5.10.

The DC-Lite does not support any printers except those supplied by Bios. The DC-Lite sends basic ASCII text in IBM/Centronics parallel format to a printer. Although it may work with older and some stand-alone, IBM-compatible office printers (printers that do not require drivers to be installed on an attached computer in order to operate), we do not recommend their use.

If you wish to experiment nonetheless, try the "Wide 1" and "Wide 2" formats to test compatibility. You may get one page per line or other incompatible results.

Bios offers the BP-1 stand-alone battery powered printer for hard copy output of DryCal data. This printer is small, portable, convenient and easy to use. It makes an excellent dedicated printer for use with Bios products.

Bios cannot guarantee compatibility with any printer other than the Bios BP-1 portable thermal printer.

Print Setup

1 The flow source should be turned on and connected to the appropriate air boss on the right side of the DC-Lite.
2 Turn the DC-Lite on before connecting the printer cable. Failure to do so will result in the display reading "Nexus Control". If this occurs, remove the printer cable and reset the unit using the white recessed **Reset Button** located on the back of the unit as described in Section 5.10.
3 Plug the printer cable into the parallel printer port located on the back of the DC-Lite. Make sure DC-Lite and the printer are on.

Selecting a Print Setting

After the printer setting selection has been made a print mode selection (All, 10 or Off) must also be made to initiate printing. The **Print** button will toggle between three print settings.

1 The default setting is "Off." When the power is turned on the printer setting will always be in the "Off" position.
2 To engage the printer, press the **Print** button once for the "Print 10" setting (this will allow the printer to print ten readings and stop). Press the **Print** button twice for the "Print All" position (to print continuously).
3 After the printer setting selection has been made, a **Read** mode selection (single or auto) must also be made to initiate the flow measurement process as described in Section 5.4.

5.7 Stop & Reset

A flow reading can be stopped at any time by pressing and releasing the **Stop** button. This process opens the valve and allows air to bypass the flow-measuring cell. The piston will fall to the bottom of the flow-measuring cell.

The DC-Lite can be reset by pressing and holding the **Stop** button for two full seconds. During a reset, the display is cleared and the number of readings in an averaging sequence is reset to zero.

5.8 Resetting a Printed Sequence

When connected to a printer, the reset process initiates a printed heading for a sequence of readings and resets the number of readings in an averaging sequence to zero. The printed heading includes a column for each flow reading (Flow), the running average (Average) and the number of samples in the average (# Samples).

5.9 Printing to a PC

Bios International offers a parallel-to-serial converter kit, part PSC-1, that allows the information from a DC-Lite to be printed to a computer via the HyperTerminal utility included with Microsoft® Windows™.

This information can be imported into many commonly used spreadsheet programs, such as Microsoft Excel or Quattro Pro. The DryCal parallel-to-serial converter kit includes everything you will need to print flow readings from your DryCal to a Windows-based PC.

Bios International only guarantees compatibility with parallel-to-serial converters purchased through Bios International. Bios International does not offer technical support on serial port configuration. For assistance with determing the correct Com Port number or port configuration, please contact your IT professional.

5.10 Hard Reset Button

If for any reason the DC-Lite does not respond to push-button commands, it may be necessary to reset the instrument. For this purpose there is a white recessed button on lower right side of the back panel near the parallel printer port. The button resets the unit back to the initializing screen and the printer setting will revert to the "Off" position. Before resetting, be sure to remove the printer cable from the back of the DC-Lite. Failure to do so will result in the display reading "Nexus Control." If this occurs, remove the printer cable and reset the unit again.

6.0 Battery System

The DryCal DC-Lite is powered by an internal lead-acid battery. The battery will power the instrument for 6–8 hours of continuous use and has a typical service life of approximately 2–5 years, depending on use. The DC-Lite provides a convenient 5-minute automatic shut-off feature to extend battery life. Use of a printer does not affect the battery life.

The DC-Lite can be charged by the Bios single-station charger when plugged into a standard 115V AC power source outlet (220V AC optional). Provided that the battery has sufficient charge to operate the DC-Lite, the DC-Lite can be charged indefinitely using the AC wall adapter provided.

Although the DC-Lite may be plugged into AC power, if the battery is exceptionally weak the DC-Lite may not function. Please read all setup and charging instructions indicated in this manual before using equipment.

6.1 Charging the Battery

Before using your DryCal DC-Lite, be sure that the battery system has been fully charged to ensure that unit will perform without interruption. Using the DC-Lite with a low battery will not affect the product's accuracy.

The DC-Lite is equipped with a battery indicator that provides battery charge indication at three levels. When the battery indicator on the display is empty the unit will continue to operate for a limited period of time before shutting itself off.

To Charge the DC-Lite

To view the actual charging status during the charging period, disconnect the battery charger and wait 3–5 minutes. When the indicator is solid black the battery is fully charged. Bios recommends leaving the DC-Lite on charge when not in use to prevent battery degradation.

1. Connect only the appropriate Bios 12VDC charger, provided with the DC-Lite calibrator, into a standard wall outlet.
2. Insert the charger barrel plug into the charging jack located on the right side of the DC-Lite housing above the inlet and outlet air bosses. A green Charge LED will illuminate while the unit is charging. Full charge takes 8 to 12 hours, and the DryCal can charge while being used.

6.2 Battery Maintenance & Storage

The DC-Lite's lead-acid battery will not exhibit the memory effect common to nickel-cadmium batteries. It may be left on charge for an indefinite time period without damage.

Long-term storage without charging can damage the battery pack, therefore if the DC-Lite cannot be left charging continuously, it should be fully charged at least once every three months and should be placed in storage only after achieving a full charge.

7.0 Isolating the DryCal from Other Instruments

The DryCal DC-Lite will mimic the flow source being used. Therefore, if the flow source exhibits air flow pulsation, Bios recommends the use of an isolation device.

Use of a 25mm, 0.8m filter cassette makes a suitable load for most flow rates used in industrial hygiene applications. This method stabilizes variations in flow due to the slight pulsation caused by the stroke of the pump's piston.

In addition, when taking flow readings with the DryCal DC-Lite, an internal valve closes, placing an insertion pressure spike into the flow stream. Generally, the

pressure spike is invisible to the flow source; however, it can cause an interaction with some instruments (example: some mass flow controllers, Magnehelic manometers and rotameters). The most common solution is to isolate the DryCal with a restriction as described in Sections 7.1–7.4.

7.1 Use with Instruments that Contain Internal Mass Flow Controllers (MFCs)

For some flow instruments with MFCs and large dead volumes (example: some PM 2.5 monitors) results may not correlate between the instrument's display and the DryCal. To eliminate these discrepancies, Bios offers an active regulation device, part DC-IR-H, to provide a constant insertion pressure.

7.2 Use with Personal Air Samplers

The DryCal DC-Lite may be used to calibrate or check the flow rate of personal air samplers. To ensure accurate flow calibrations, Bios recommends the use of an isolating flow restriction as described in Section 7.0. A standard MSHA approved respirable dust filter or equivalent 25–37 mm 0.5 micron casette should be sufficient to provide an appopriate isolation.

7.3 Calibrating Rotameters

When calibrating rotameters the DryCal DC-Lite should be used as a transfer standard only. Do not use the DC-Lite in series with a rotameter. For optimum accuracy, use a rotameter over its mid-range.

1. Attach an isolating load or sample medium, with a pressure drop of about 8 to 12 inches of water column, in series with a stable pump and a DryCal.
2. Calibrate the sampling pump at the desired flow setting (ie: 2.00 Lpm) with the DryCal. When the desired flow setting is obtained, disconnect the DryCal and attach the tubing to the outlet boss of the rotameter.
3. When the rotameter ball stabilizes, mark the rotameter for the true flow rate (2.00 Lpm for example) using tape and a permanent marker to denote the calibrated flow setting or note the point on a rotameter flow chart. Repeat this procedure for any additional flow settings.

7.4 Use with Magnehelic Manometers

High-capacitance spring-loaded gauges such as Magnehelic manometers can cause vibration of the DryCal piston. This is not a defect in the DryCal. The piston is accurately mirroring the transient internal vibrations of the gauge. This type of gauge must be isolated from the DryCal by inserting a suitable restriction between the gauge and the calibrator

8.0 Maintenance, Quality Assurance

Although the DryCal DC-Lite is a rugged instrument, certain care and maintenance requirements must still be met.

Current service and calibration information and pricing can be found at www.biosint.com/service/dclite.htm.

8.1 Maintenance

When not in use always store your DC-Lite in a clean, dry environment. When possible leave the unit on charge. Wipe only with a damp cloth and do not spray with liquid solvents or use abrasive cleaners.

8.2 Leak-Test Procedure

A quality assurance self-test feature is provided to verify proper integrity of the flow cell. It is recommended that the self-check leakage test be conducted periodically as part of an on-going quality assurance program.

Passing the leak test does not ensure proper function of the DC-Lite. It does ensure that total leakage is within the product's allowable limits. To ensure proper function of the DC-Lite annual factory calibration is recommended.

To Initiate the Leak-Test

The leak-test tubing accessory is a short piece of latex tubing with a red plug that is found in the tubing kit shipped with your DC-Lite. Place the leak-test tubing accessory over the top (inlet) air boss. The low flow range DC-Lite requires a miniature leak-test tubing accessory that is supplied in addition to the standard tubing kit. Any maintenance to the DryCal must be performed by Bios maintenance personnel.

1. Press and hold the **Stop** button while pressing the **On** button. If the DC-Lite is already on, press and hold the **Stop** button while pressing the hard reset button on the back of the unit as described in Section 5.10. After a leak-test is initiated, the display will read "Leak Test, Invert & Push Read."
2. Invert the DC-Lite so the piston moves to the top of the cell. While the piston is resting at the top of the cell press the **Read** button and the internal

valve will close. Return the unit to an upright position and it will time the descent of the piston.

3 Place the DC-Lite on a flat, vibration-free surface.

4 Observe the location of the piston to ensure that it is at the top of the cell when the test begins (the test may take as long as 15–20 minutes). If the test is completed successfully, the display will read: "Test OK Push Read."

5 Push the **Read** button as directed and the internal valve will open and the piston will fall.

6 Repeat the test with the leak-test tubing accessory connected to the lower (outlet) air boss. If the unit fails the Leak-test, the display will read: "Maintenance Reqd Push Read."

8.3 Air Containing Particulates

As of January 1, 2001, the DryCal DC-Lite comes standard with either a 5-micron or 30-micron inlet filter inside the inlet fitting (depending on model ordered). Additionally, all older DC-Lites sent in for calibration will be retrofitted with new style inlet filters, free of charge. However, air containing cigarette smoke or other excessive dust and particulates should be additionally pre-filtered. An additional particulate filter, part AF-516, is available for this purpose. The filter should be placed ahead of the DryCal in the flow stream, on the inlet side.

8.4 Return Authorization

Prior to returning your DryCal for repair or recalibration, please contact Bios International for technical support, troubleshooting assistance and an RMA number if necessary.

You can telephone Bios at (800) 663 4977 or (973) 492 8400, or send an email to service@biosint.com.

8.5 Shipment

When shipping the DryCal DC-Lite please ensure that the packaging is adequate to protect the instrument. When possible the DC-Lite should be shipped in the original packaging. Bios International Corp. is not responsible for damage that occurs during shipment.

8.6 Long-Term Storage

DryCal calibrators can remain on charge until needed without causing damage to the battery. If the DryCal is stored for long periods of time the battery should be charged at least once every three months.

Always store DryCal calibrators in a clean, dry environment and recharge the unit prior to use after long-term storage.

8.7 Calibration

As a quality assurance measure, Bios recommends annual calibration of all measurement instruments, although how often you have your DryCal calibrated is an internal quality control decision. The determining factors are whether the unit passes the internal leak-test, quality system requirements if applicable, and the conditions in which the unit is used. Units used in a laboratory setting may require calibration less frequently than a unit that is used in a dusty environment. The annual calibration program is an elective and is therefore not included as a warranty item. "As received" flow test data and expedited "48 hour" turnaround service are also available at an additional cost. Please contact the factory for more information on available calibration services and pricing.

Calibration Includes

- Cleaning (if required)
- Valve adjustment (if required)
- Battery capacity test
- Internal computer program upgrade as necessary
- Mechanical upgrades as necessary
- Dynamic Performance Test
- NIST-Traceable Calibration Certificate

9.0 Calibration Statement

The DC-Lite is dynamically tested by comparing it to a Laboratory Standard primary piston prover of much higher accuracy (±0.25%), but of similar operating principles. Flow generators of ±0.01% stability (included in prover accuracy) are used for the comparison. Use of provers of similar construction to the devices under test assures the validity of the flow generator as a transfer standard.

The primary Laboratory Standards are qualified by direct measurement of their dimensions (diameter, length of measured path, time base) against NIST-traceable gauges and instruments. A rigorous analysis of their accuracy in accordance with the International Guide to Uncertainty in Measurements has been performed, assuring their traceable accuracy. Test procedures assure temperature matching of the Laboratory Standards to the devices under test.

The calibration dates of the laboratory standards for each parameter (diameter,

encoder spacing, time base) are included in our calibration reports, along with identification of the devices used for calibration, their calibration dates and NIST calibration numbers.

10.0 Limited Warranty

The Bios DryCal DC-Lite is warranted to the original end user to be free from defects in materials and workmanship under normal use and service for a period of one year from the date of purchase as shown on the purchaser's receipt. The DC-Lite's battery is warranted for 6 months from the original purchase date. If the unit was purchased from an authorized reseller a copy of an invoice or packing slip showing the date of purchase may be required to obtain warranty service.

The obligation of Bios International Corporation under this warranty shall be limited to repair or replacement (at our option), during the warranty period, of any part which proves defective in material or workmanship under normal use and service provided the product is returned to Bios International Corporation, transportation charges prepaid.

Notwithstanding the foregoing, Bios International Corporation shall have no liability to repair or replace any Bios International Corporation product:

1. Which has been damaged following sale, including but not limited to damage resulting from improper electrical voltages or currents, defacement, misuse, abuse, neglect, accident, fire, flood, act of God or use in violation of the instructions furnished by Bios International Corporation,
2. When the serial number has been altered or removed or
3. Which has been repaired, altered or maintained by any person or party other than Bios International Corporation's own service facility or a Bios authorized service center.

This warranty is in lieu of all other warranties, and all other obligations or liabilities arising as a result of any defect or deficiency of the product, whether in contract or in tort or otherwise. All other warranties, expressed or implied, including any implied warranties of Merchantability and fitness for a particular purpose, are specifically excluded.

In no event shall we be liable for any special, incidental or consequential damages for breach of this or any other warranty, express or implied, whatsoever.

Notes

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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 the Collection of Tire Crumb Active Field Ambient Air Samples for VOCs using Thermal Desorption Tubes and Low-Flow Pumps

SOP ID: D-EMMD-AQB-024-SOP-01	Effective Date: August 23, 2017

SOP was Developed: ☒ In-house ☐ Extramural: enter organization

SOP Discipline*: Field Collection

Alternative Identification:

SOP Contact Signature

Name: Karen Oliver

Signature/Date: Karen D Oliver

Digitally signed by KAREN OLIVER
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=KAREN OLIVER, dnQualifier=0000034610
Date: 2017.08.23 16:04:48 -04'00'

Management Signature

Name: Surender Kaushik

Title: AQB Branch Chief

Signature/Date: TADEUSZ KLEINDIENST

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Date: 2017.08.23 16:27:15 -04'00'

QA Signature

Name: Sania W. Tong Argao

Title: EMMD QA Manager

Signature/Date: Sania W. Tong Argao

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Date: 2017.08.23 15:58:26 -04'00'

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

Standard Operating Procedure for the Collection of Tire Crumb Active Field Ambient Air Samples for VOCs using Thermal Desorption Tubes and Low-Flow Pumps

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Contents

NOTICE
This Analytical Procedure has been prepared for use by the Air Quality Branch of the U.S. Environmental Protection Agency, Research Triangle Park, North Carolina and may not be specifically applicable to the activities or objectives of other organizations. This procedure has not been fully validated and should be used for research purposes only. Adequate QA/QC measures must be implemented with this procedure to allow assessment of data quality.

1.0 Scope and Application

This method applies to the collection of air samples from stationary locations on or near synthetic turf fields with crumb rubber infill. Samples will be analyzed for a suite of volatile organic compounds (VOCs). This SOP incorporates many of the procedures previously detailed in standard operating procedure (SOP) D-EMMD-ABQ-004-SOP-01 (Formerly ECAB-152.1) "SOP for Carbopack X Sorbent Tube Handling: Field Deployment and Shipping." The streamlined field deployment checklist in this SOP will be useful to field operators.

2.0 Summary of Method

Portable, battery operated, air sampling pumps attached to 3.5-inch x ¼ inch o.d. stainless steel thermal desorption (TD) tubes packed with Carbopack X adsorbent are used to capture gas-phase VOCs from ambient air. Air samples are collected from two stationary locations (duplicate samples at one of these locations) at each synthetic field as close as possible to where activities occur without posing an obstruction or safety hazard. A third location will be sampled upwind and at a sufficient distance from the field to represent background.

3.0 Definitions

COC	chain of custody	NERL	National Exposure Research Laboratory
EMMD	Exposure Methods and Measurement Division	PTFE	polytetrafluoroethylene
FEP	fluorinated ethylene propylene	qty	quantity
ID	identification	TD	thermal desorption

4.0 Precautions

The sampling systems are powered by internal battery packs. The battery pack must be completely charged before operating the pump. The battery and pump system should be protected from excessive heat or cold (<-4 °F, >104 °F) as specified in the operator's manual.

Sampling will take place at outside and inside activity fields. Equipment should be shielded as much as possible from moisture and should not be used during periods of heavy rain (protective enclosures are not provided). Sampling stands should be set up so that they are provided protection from tampering. Sampling stands must also be placed and secured such that no harm will come to children or others playing in the vicinity of the equipment.

5.0 Personnel Qualifications

Required training is provided by the US EPA. Sample collection will be conducted by qualified scientific staff trained in the use of the specific field monitoring equipment. Training will include a demonstration and hands-on training by qualified persons with air sampling expertise. At a minimum, all SOPs and operating instructions will be reviewed, understood, and followed exactly by the field staff. Training records will be maintained by the EPA exposure study project lead (currently Kent Thomas).

6.0 Sampling Media and Supplies

6.1 Sampling Media

Supelco FLM Carbopack X deactivated 89-mm (3.5 in. x 0.25 in. o.d.) stainless steel thermal desorption tube (part no. 28686-U, Millipore Sigma, St. Louis, MO) fitted with 0.25-in. brass Swagelok fittings with combined (one-piece) polytetrafluoroethylene (PTFE) ferrules (**Note**: Conditioned TD tubes will be capped and stored inside the glass culture tubes, which will be stored inside sealed metal cans for transport to and from the sampling site.)

6.2 Sampling Setup

- Sampling pumps - SKC Pocket Pump (Cat. No. 210-1000) – qty. 4
- Charging System – SKC Pocket Pump Multi-charger (Cat. No. 223-247)
- Tripod sampling stand - custom modified – qty. 3
- Tygon flexible vacuum tubing (5/16 in. o.d., 3/16 in. i.d.)
- ¼ in. Swagelok stainless steel straight union with PTFE ferrules and a knurled nut for holding sampling tube—qty. 4 each
- ¼ in. o.d. "U" shaped acrylic adapter for attaching Swagelok fitting to Tygon tubing – qty. 4 each
- Measuring tape (at least 6 ft length)

6.3 Flow Measurement

- Flowmeter – MesaLabs flow calibrator, low-flow (or equivalent) (Defender 520 model, Lakewood, CO)

6.4 Sample Handling and Storage

- Powder-free nitrile gloves
- Kimwipes
- Metal tray
- Clean forceps (e.g., Teflon coated, 5 in. stainless steel)
- CapLok tool (part no. C-CPLOK, Markes International, Gold River, CA)
- Glass culture tubes (part no. 45066A-25150, Kimble/Kontes, Vineland, NJ) – qty. 12 per sampling day (10 for TD tube storage and 2 for spares)
- Unlined caps for glass culture tubes (custom order, Scientific Specialties Service, Inc., Randallstown, MD) – qty. 1 per tube
- Septrseal Septa and Teflon liners for culture tube caps (part no. B69800-24 and B68800-24, Scientific Specialties Service, Inc.) – qty. 1 each type per tube fluorinated ethylene propylene (FEP) Teflon tubing, 0.25 in. o.d
- Clean unlined gallon metal cans with lids (part no. 5501-07B, SKS Bottle and Packaging, Inc., Mechanicville, NY)

  Note: Conditioned TD tubes will be capped and stored inside the glass culture tubes, which will be stored inside sealed metal cans for transport to and from the sampling site.

- Cooler (example part no. 5248-5286-5296, Coleman Products, Inc., Wichita, KS)
- Foam inserts for coolers (Instapack quick foam packaging, Sealed Air Corporation, Danbury, CT)
- Rex protective sleeves, 1.021 in. i.d. by 5.750 in. length with wall thickness of 0.125

in. (custom order, Yazoo Mills, Inc., New Oxford, PA)
- Closed cell foam, assorted thicknesses

6.5 Sample Tracking

- Data Collection and Chain of Custody (COC) Record forms (see Appendix A) and meta data form shown in Appendix C
- Pen (Black, archival quality Ink)
- GPS and time enabled device - Garmin or cell phone with GPS capabilities
- Shipping labels

6.6 Spare and other potentially useful supplies

- Paint can opener
- Resealable closure bags
- Packaging tape
- Extra labels

7.0 Quality Control and Quality Assurance

The quality control requirements will allow assessment of the quality of the samples collected. Determination of possible contamination and reproducibility of the method will be targeted as data quality indicators.

7.1 Field Blanks and Field Controls

A minimum of one field blank and one field control will be collected on each sampling day.

As stated in Section 8.4.2, the thermal desorption tubes will be sent sealed with brass fittings, secured in glass culture tubes, and stored in a metal can. Metal cans containing thermal desorption tubes meant for field sampling and for field blanks will be pre-labeled with sample ID codes.

Field blanks and controls will not be removed from their packing in the metal can; they will remain sealed and returned to the laboratory.

7.2 Duplicate Samples

Duplicate samples will be collected at a single on-field location during each day of field measurement. Two sampling systems (pump/inlets) shall be positioned on the same sampling tripod and operated as specified in Section 8.3. The purpose of duplicate samples is to determine the precision of the sampling method in its entirety.

7.3 Mesa Labs Defender Flow Calibrator

The Mesa Labs Defender flow calibrator will be used at the beginning and end of each sampling period to measure the pump flow rate. The flow calibrator should have been certified within the past year by the manufacturer or the flow rates verified against a unit that is under current certification and deemed appropriate (within 2%). The sampling pump is equipped with a flow controller and the flow rate is stable during sampling.

8.0 Procedures

8.1 General Sampling Considerations

- Samplers must be placed in an area representative of the average ambient conditions (i.e., away from roadways, parking lots or high traffic areas).
- Pump flow rates are measured using the Mesa Labs Defender. Flow measurements should take place immediately prior to and immediately after sampling as noted in sections 8.5.5 through 8.5.14.
- The flow rate will be used in conjunction with the total elapsed sampling time to calculate total air volumes sampled and integrated analyte concentrations observed during the sample capture period.

8.2 Pre-Deployment Preparation

- Charge batteries and flow meter
- Test sampling pumps
- Clean field supplies (forceps, glass culture storage tubes, trays, etc.)
- Condition TD tubes for use
- Prepare spiked tubes for use as field controls and lab controls
- Pre-label glass culture tubes and data collection forms with sample codes.
- Pack all field supplies

8.3 Pump and Sampling Equipment Set-up

8.3.1 Transport the sampling setup and flow measurement equipment to the field location (listed in sections 6.1-6.6). This will include sampling pumps, sampling stands, vacuum tubing, and measuring tape along with the data collection sheets, flow meter, pen, and any materials needed to secure the monitoring site.

8.3.2 Three sampling sites will be identified for each location. Two sites will be located on the activity field. One site will be located off of the field, in an upwind position if possible. One sampler will be located at each site and an additional sampler will be positioned at one of the on-field locations (using same tripod stand) to collect duplicate samples.

Note: Ensure that the information regarding specific site locations and conditions have been recorded as meta data (on the sheet shown in Appendix C) according to SOP D-SED-IEMB-005-SOP-01.

8.3.3 Figure 1 shows the sampler configuration with major components identified. Use this diagram to setup each sampling location.

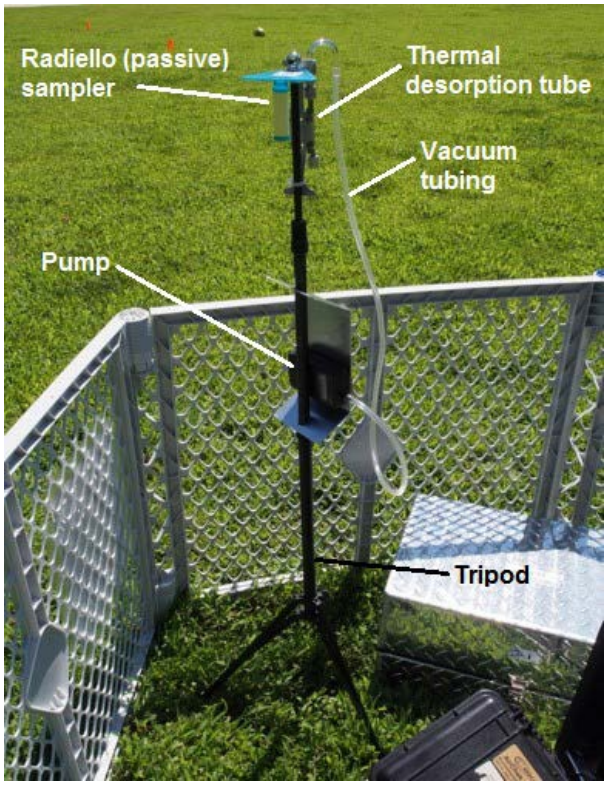


Figure 1: VOC Sampler Configuration

1. Set up the sampling stand by spreading the tripod base and extending the vertical rod. The tripods are each outfitted with a TD tube holder, a Radiello passive tube holder (see SOP D-EMMD-AQB-019-SOP-01 for Radiello deployment procedures) and a metal plate to mount the pump.
2. Connect one end of the tubing to the hose barb found on the pump. The attached hose should be snug and not easily removed without the use of applied force. Attach the pump to the metal plate on the tripod such that the pump is between the plate and the tripod post. This will provide some shielding to the pump.
3. Turn the pump on by sliding down the protective cover, pressing any keypad button to activate the pump, and using the up (Δ) or down (∇) arrow buttons simultaneously to toggle from "Hold" to "Run." Allow the pump to run while the rest of the equipment is being set up.
4. Adjust the tripod so the TD tube inlet is at a height of 1.0 ± 0.1 meters (35.4 in. - 43.3 in.) above ground level.

8.4 Sample Setup

8.4.1 Refer to SOP D-EMMD-ABQ-004-SOP-01 for instructions on handling the TD tubes.

8.4.2 The TD tubes will be sent with the inlets capped with brass fittings, sealed in glass culture tubes, and stored in a metal can. Thermal desorption tubes meant for field blanks and controls will be labeled as such. Tubes meant for sampling will be pre-labeled with the appropriate sample ID codes. Spare sampling tubes will be shipped to the sampling locations and can be used in the event of damage to the pre-labeled sampling tubes. Be sure

to retain the fittings, culture tubes, cans, and packaging for return to the laboratory for future use.

8.4.3 To set up sampling, determine the appropriate sample code and sampling location. Enter the TD tube ID number of the pre-labeled tube on the corresponding Data Collection and COC Record Form.

8.4.4 Immediately before sampling is set to begin, don a pair of nitrile gloves and remove the glass-enclosed TD tube from the metal can labeled with the field sampling date. Remove the vial cap and then the Teflon strips from the culture tube one at a time. Remove the Teflon strips using forceps and place them on a clean surface such as a laboratory tissue or clean aluminum foil. Next remove the TD tube from the glass culture tube and return the Teflon strips to the glass culture tube.

8.4.5 Remove the brass fitting from the outlet side (non-grooved end) of the TD tube and insert this end into the vacuum tubing. Gently place the brass fitting back into the glass culture tube. Secure the TD tube to the sampling stand using the clamp. Ensure that the other end of the vacuum tubing is attached to the pump inlet.

8.4.6 Remove brass fitting from the inlet side (grooved end) of the TD tube and place it back into the glass culture tube, and then return the glass culture tubes to the metal can. The sampling setup should now look exactly like Figure 1. Double check the length of tubing and remove any kinks.

8.5 Conducting Active Sample Collection

8.5.1 To collect field samples, turn on the pump by sliding down the protective cover, press any keypad button to activate the pump, and then press both the up (Δ) and down (∇) keys together to turn the pump on. Allow the pump to run for at least five minutes to warm-up and stabilize.

Note: The up (Δ) and down (∇) keys pressed at the same time toggle from "Hold" to "Run" and from "Run" to "Hold".

8.5.2 The pump flow rate should be set to 100 mL/min in the laboratory prior to field deployment. This will minimize the amount of time required to fine adjust the pump flow after the TD tube is attached.

Note: Refer to the pump manual for complete instructions on how to adjust flows. Basic flow adjustment instructions are shown on the PocketPump Quick Guide in Appendix B.

8.5.3 Insert the non-grooved end of the TD tube into the Swagelok fitting of the Tygon tubing sample train and tighten the knurled nut finger tight. Insert the TD tube into the mounting bracket on the sampling stand by inserting the grooved end of the TD tube into the "ears" of the mounting bracket. This is the sample start time.

8.5.4 Record the sample 'Start Time' on the Data Collection and COC Record Form (Appendix A).

8.5.5 Measure the air sampling flow rate through the TD tube by attaching the grooved end of the TD tube to a length of Tygon tubing that is then attached to the upper port (suction) of the Mesa Labs Defender flow calibrator. Press the "ON" button (lower right corner) of the flow calibrator and hold for approximately one second until the display lights up and the optical light in the cell illuminates.

Note: If the on button is depressed too long it will turn the unit off and you must then press the "ON" button again to turn on.

8.5.6 The display should show the Defender information and highlight the word "Measure." Press the Enter button twice to begin flow measurements. This will activate the unit to make continuous measurements and automatically average 3 replicate readings.

Note: Be sure that the Defender unit is set up to measure the volumetric flow rate in units of mL/min. Do not measure as STP. Refer to the manual on how to change the setup features.

Note: If the Defender unit is used in bright sunlight, the flow cell may need to be shielded in order for the sensors to work properly. Simply hold the unit close to your body or hold the unit in a shaded area while taking the readings.

8.5.7 Observe the flow rate values being displayed. Flow rates should be 100 ± 10 mL/min.

8.5.8 If needed, adjust the flow rate to 100 ± 10 mL/min. While observing the calibrator display, use the up and down arrow buttons on the pump keypad to adjust the flow. The security code "✸ Δ ∇ ✸" must be pressed in sequence within 10 seconds to change operating parameters (refer to Appendix B for full details).

8.5.9 Once adjusted to 100 ± 10 mL/min allow the flow to stabilize for one minute, reset the calibrator by pressing the "Stop" button followed by holding the "Read" button for three seconds. The unit will automatically collect three flow readings and average them. Record the average flow rate of the three readings on the datasheet in the 'Start Flow' column of the Data Collection and COC Record Form (Appendix A).

8.5.10 Remove the flow calibrator tubing from the TD tube and attach the TD screen inlet to the grooved end of the TD tube.

8.5.11 Close the protective sliding cover on the pump and attach it into the bracket near the bottom of the sampling stand with the vacuum tubing pointing out. Double check the tubing to verify there are no kinks.

8.5.12 After sampling for the desired time period (presumed to be an approximate three-hour duration), re-check the flow and record the average flowrate in the 'Stop Flow' column of the Data Collection and COC Record Form. To do so, simply remove the inlet screen from the TD tube, attach the flow calibrator with the Tygon tubing, turn on the flow calibrator, measure three readings, and record the average reading on the form. No stabilization wait time is required as the pump has been running continuously.

8.5.13 Open the pump protective sliding cover and turn off the pump by pressing the up (Δ) and down (∇) keys simultaneously to toggle pump mode to "Hold." The pump enters "sleep" mode after five minutes in "Hold" without activity (there is no manual off option).

8.5.14 Record the sample 'Stop Time' on the Data Collection and COC Record Form (Appendix A).

8.5.15 Retrieve the glass culture tubes containing the brass fittings from the metal can. Make sure the label on the glass vial and the TD tube ID (etched on the TD) match those shown on

the data sheet. Don a clean pair of nitrile gloves. Remove the TD tube from the mounting bracket and remove the inlet screen. Place the brass fitting caps on the TD tube. Hand tighten the brass fittings and then tighten an additional one-eighth turn using the CapLok tool. Perform the tug test described in SOP D-EMMD-ABQ-004-SOP-01 to ensure that the caps are properly tightened.

Note: Do not overtighten the brass nuts as it may distort the metal body of the TD tube or be hard to remove. Only a small fraction past finger tight is required.

8.5.16 Open the glass culture tube and place the lid onto the metal tray or clean surface.

8.5.17 Retrieve the TD tube. Gently transfer the TD tube to the labeled glass culture tube (Figure 2) being sure that the glass culture tube has a Teflon chip in the bottom to provide cushioning. Insert the two Teflon strips into the glass culture tube one at a time in order to minimize stress on the glass.

Figure 2: Thermal desorption tube in a glass culture tube

8.5.18 Replace the cap to the glass culture tube.

8.5.19 Place the glass culture tube (containing TD tube) into the cardboard sleeves in the metal can and place the two foam discs at the top of the can between the metal lid and the culture tube tops. Securely place the metal lid back on the can.

8.5.20 Samples should be stored at room temperature and shipped back to EPA the day of or the day after collection (see Section 8.8).

8.6 Labeling the Sample

8.6.1 Labels for these samples will be generated at the EPA lab (using the convention outlined in Table 1.). The labels will be placed on the glass culture tubes and on the data collection forms before they are transported to the field

Table 1. Sample Identification Scheme

ID Code	Example Code	Description
Sample Identification: TCRS-R-VV-W-X-Y-Z		
TCRS		tire crumb rubber research study
R	0	participate identification number (Use 0 for these samples as they are not associated with a specific participant.)
VV	70-79	two-digit code will be a unique identifier for each field numbered in the range of 70-79.
W (sample type)	F	sample
	D	duplicate sample
	B	field blank
	C	field control (spiked blank)
X (analysis method)	D	field air VOC sampling
Y		sample collection location
	1 or 2	on-field air locations
	8	off-field air location
Z	0	parent/ sample

8.7 Pump/Equipment Take Down

1. Remove the sample pump from the tripod stand.
2. Disconnect the tubing from the sample pump and return pump to its protective case.
3. Re-pack all supplies into their original shipping containers and prepare for transport of all supplies to the EPA laboratory.

8.8 Return Samples to the EPA Laboratory

1. All samples should be shipped back to the EPA laboratory as soon after sampling is complete as possible.

2. Pack the sample cooler immediately prior to shipping by placing as many as three metal cans into the cooler and replacing the associated packing material on top of the cans to prevent the contents of the cooler from shifting. Photocopy or photograph the Data Collection and COC Record Forms (retain in case of damage during shipping, discard after they are recorded by EPA) and then add the completed forms to a zipper storage bag. Place the bag on top of the cooler contents and then seal the cooler.
3. Ship the packed cooler to the EPA laboratory using next day air UPS or similar overnight delivery service. EPA generally will prepare and provide a return shipping label. If applicable, address the shipment to:

US EPA Chemical Services
Attn: Karen Oliver/Lillian Alston (919-541-2337)
Bldg E Loading Dock, Room E-178

109 T.W. Alexander Drive
Durham, NC 27711

4. Immediately notify Karen Oliver (oliver.karen@epa.gov) of the incoming shipment via email. Include the shipment tracking number.

9.0 Possible Corrective Actions for Observed Problems During Sampling

9.1 Pump Failure

- If a pump fails, correct any obvious errors such as kinked lines, battery not fully charged, etc. If possible, replace the pump.
- If a replacement pump is unavailable, stop data collection immediately and contact the NERL/EMMD supervising scientist. Examples of pump failure include: failure to reach desired flow rate; or failure to maintain the desired flow rate.
- Document any pump failures in the Data Collection and COC Record Form.

9.2 Possible Contamination of Filters or Supplies

Contact the NERL/EMMD staff scientists for possible replacement items or directions for decontamination. Be sure to document any suspicion of possible contamination of filters or supplies in the Data Collection and COC Record Form.

10.0 Recordkeeping

10.1 Data Sheets

All information concerning sample collection will be recorded by the appropriate operator on the VOC Data Collection and COC Record Form and on the Field Sampling Location Record Form (see SOP D-SED-IEMB-005-SOP-01). Examples are attached in Appendices A and C, respectively.

10.2 Calculations

The sample flow rate is directly measured using the average of the pre- and post-sampling Mesa Labs flow calibrator measurements. The elapsed time in minutes is the sum total of minutes the pump operated during the sampling episode. A normal 3-hour run period should have approximately 180 minutes.

Collected air volume is calculated as follows:

$$VV = \frac{(TT \; xx \; FF)}{1000}$$

Where, V = Sample volume in liters

T = Elapsed time in minutes

F = Average flow in mL/min

10.3 Chain-of-Custody

The original Data Collection and COC Record Form will accompany the filter samples

back to the NERL-RTP laboratory. A copy of this form is attached in Appendix A.

Subsequent analysis (i.e. thermal desorption/GC-MS analysis) will be indicated on the sample Data Collection and COC Record Form by responsible parties. Original copies of all data forms will be maintained in the NERL project files.

11.0 References

D-EMMD-ABQ-004-SOP-01. (Formerly ECAB-152.1.) Standard Operating Procedure for Carbopack X Sorbent Tube Handling: Field Deployment and Shipping. 2015.

D-EMMD-AQB-019-SOP-01. Standard Operating Procedure for Radiello Carbopack X Diffusive Sampler Handling: Field Deployment and Shipping for Tire Crumb Exposure Studies. 2017.

D-SED-IEMB-005-SOP-01. Standard Operating Procedure for the Collection of Field and Activity Metadata During Exposure Characterization Pilot Study Field Sampling. 2017.

Appendix A: Data Collection and COC Record Forms for Fields 1, 2, and 8

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Field Locations -- Passive (Radiello) and Active VOC Samples

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID 1			

Sample Chain of Custody

Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	

Radiello Tube ID	Holder ID		Start Time	Stop Time	Initials	Date	Initials	Date	Initials	Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

PE Tube ID	Start Time	Start Flow	Stop Flow	Stop Time	Initials	Date	Initials	Date	Initials	Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Field Locations -- Passive (Radiello) and Active VOC Samples

			Deployment	Recovery
Study Name:				
TCRS		**Date**		
Field ID No:				
		Operator		
Field Location ID	2			

Sample Chain of Custody

Radiello Tube ID	Holder ID		Start Time	Stop Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

PE Tube ID	Start Time	Start Flow	Stop Flow	Stop Time	Initials	Date	Initials	Date	Initials	Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

Form Rev 06/21/2017 daw

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET
Field Locations -- Passive (Radiello) and Active VOC Samples

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID 8			

Sample Chain of Custody

Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Initials	Date	Initials	Date	Initials	Date

Radiello Tube ID	Holder ID		Start Time	Stop Time	Initials	Date	Initials	Date	Initials	Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

PE Tube ID	Start Time	Start Flow	Stop Flow	Stop Time	Initials	Date	Initials	Date	Initials	Date
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Active (PE) VOC Field/Lab Blanks and Controls

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID			

Sample Chain of Custody

	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
PE Tube ID	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Place Field/Lab Blank ID Label Here	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

Place Field/Lab Blank ID Label Here	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

PE Tube ID	Initials	Date	Initials	Date	Initials	Date

Place Field/Lab Control ID Label Here	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

Place Field/Lab Control ID Label Here	**Field notes:**	
	Receipt notes:	
	Analysis notes:	

Form Rev 06/21/2017 daw

Appendix B: SKC PocketPump Quick Guide

PocketPump Programming Sequences »

- **To change the pressure in Constant Pressure mode,**
  With pump in hold, press [▲▼] then ●▲▼● (SET and P flashes) Press ▲ or ▼ to increase or decrease When done, press ●
- **To change data display from standard to enhanced,**
  With pump running, press [▲▼] then ●▲▼● then ●▲▲●
- **To change data display from enhanced to standard,**
  With pump running, press [▲▼] then ●▲▼● then ●▼▼●
- **To change temperature scale from F to C or C to F,**
  With pump running, press [▲▼] then ●▲▼● then [●▼]
- **To change back pressure units from mm to ins or ins to mm,**
  With pump running, press [▲▼] then ●▲▼● then [▲●]

Form #37715 Rev 1104

SKC

PocketPump®
Personal Low Flow Sampler

Quick Guide »

SKC Inc., 863 Valley View Road, Eighty Four, PA 15330

PocketPump Terms »

Keypad is located beneath the sliding cover

Star button ●
- To scroll through displays or, with other buttons, to set pump operations

Up and down arrow buttons ▲ ▼
- To increase or decrease flow or pressure during setup and to set up pump operations

Button sequence
- To enter commands correctly during pump setup, they must be in sequence

Underlined sequence ●▼▲●
- To be pressed within 10 seconds of previous command

Bracketed sequence [▲▼]
- To be pressed simultaneously

Security code ●▲▼●
- To prevent unauthorized changes to the pump's sampling program

PocketPump Programming Sequences »

- **To activate pump (e.g. to change pump from SLEEP to HOLD),**
  Press any button.
- **To change pump from HOLD to RUN or RUN to HOLD,**
  Press [▲▼]
- **To clear old data,**
  With pump running, press [▲▼] then ●▲▼● then ● ●
- **To select operating mode (switch from constant flow to constant pressure),**
  With pump running, press [▲▼] then ●▲▼● then ●▼▲●
- **To change the flow rate in Constant Flow mode**
  With pump in hold, press [▲▼] then ●▲▼● (SET flashes) Press ▲ or ▼ to change flow rate. When done, press ● ●
- **To calibrate the flow in Constant Flow mode,**
  With pump in hold, press [▲▼] then ●▲▼● then ● (ADJ flashes) Press ▲ or ▼ to adjust flow until pump and calibrator are in agreement. When done, press ●

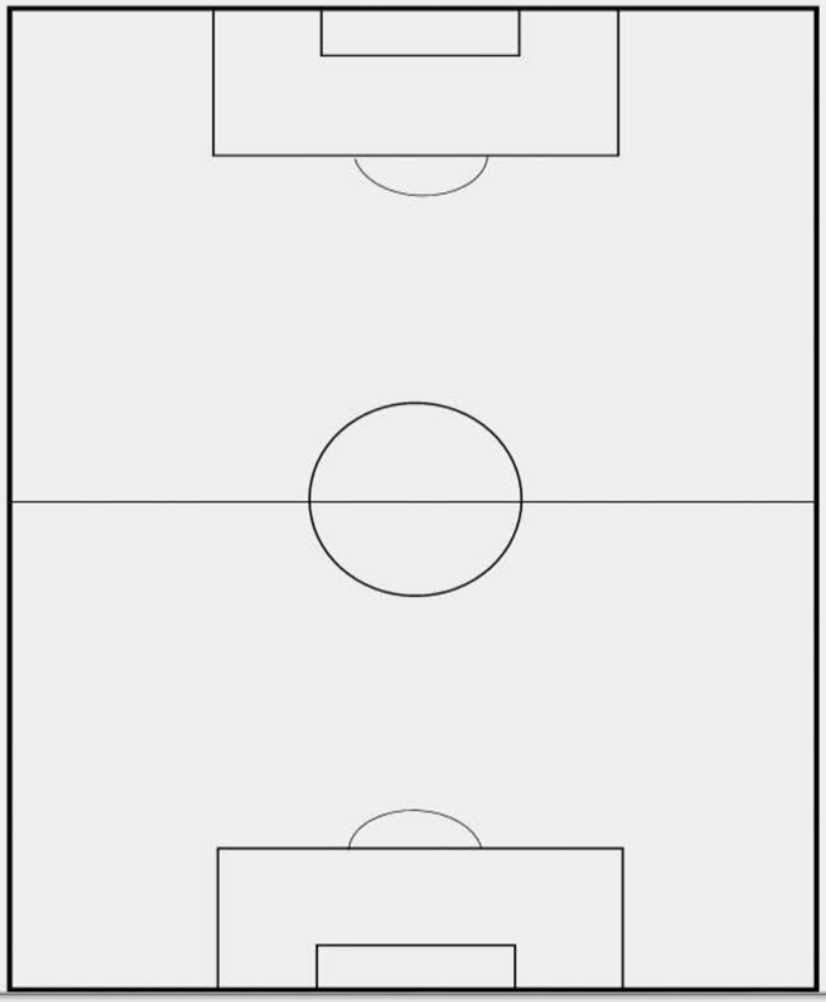
Appendix C: Field Sampling Location Record Form

Field Sampling Location Record Form

Field ID Number

Field SVOC wipe locations S
Field metals wipe locations M
Field SVOC drag sled locations D
Air sample station locations A
(For off-field upwind air sample station estimate and record distance from field center; record type of ground surface)

Draw Magnetic North Direction Arrow Here



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 Radiello Carbopack X Diffusive Sampler Handling: Field Deployment and Shipping for Tire Crumb Exposure Studies

SOP ID: D-EMMD-AQB-019-SOP-01	Effective Date: August 23, 2017

SOP was Developed: ☒ In-house ☐ Extramural: enter organization

SOP Discipline*: Field Collection

Alternative Identification:

SOP Contact Signature

Name: Karen Oliver

Signature/Date: KAREN D. OLIVER

Digitally signed by KAREN OLIVER
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=KAREN OLIVER, dnQualifier=0000034610
Date: 2017.08.23 16:06:18 -04'00'

Management Signature

Name: Surender Kaushik

Title: AQB Branch Chief

Signature/Date: TADEUSZ KLEINDIENST

Digitally signed by TADEUSZ KLEINDIENST
Date: 2017.08.23 16:24:08 -04'00'

QA Signature

Name: Sania W. Tong Argao

Title: EMMD QA Manager

Signature/Date: Sania W. Tong Argao

Digitally signed by Sania W. Tong Argao
DN: cn=Sania W. Tong Argao, o=US EPA, ou=ORD/NERL/EMMD, email=Tong-Argao.Sania@epa.gov, c=US
Date: 2017.08.23 16:00:55 -04'00'

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

Standard Operating Procedure for Radiello Carbopack X Diffusive Sampler Handling: Field Deployment and Shipping for Tire Crumb Exposure Studies

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Revision History

Version No.	Name	Date of Revision	Description of Change(s)
1	EPA and JTI, listed on cover page	August 18, 2017	Original SOP.

Contents

1.0 Scope and Application

This standard operating procedure (SOP) describes sample handling techniques for Carbopack X Radiello Samplers that are used for diffusive sample collection of volatile organic compounds (VOCs) in ambient air for Tire Crumb Exposure Studies. Direction is provided for field deployment and shipping.

2.0 Summary of Method

Ambient and personal air samples are collected using Radiello radial diffusive samplers containing Carbopack X sorbent. Prior to sampling, the Radiello stainless steel mesh cartridges containing Carbopack X are conditioned according to D-EMMD-AQB-008-SOP-01, "Standard Operating Procedure for Carbopack X Sorbent Tube Conditioning Using the Markes International Model TC-20 Sample Tube Conditioner" by placing each Radiello cartridge into an empty industry standard PerkinElmer-style stainless steel thermal desorption (TD) tube. Conditioned tubes are sealed with 0.25-in. Swagelok caps with combined polytetrafluoroethylene (PTFE) ferrules and are stored in glass culture tubes with Teflon-lined caps in a refrigerator until needed. The culture tubes are then placed in protective sleeves in a metal can and stored in a refrigerator until the time of shipment.

(Note: TD tubes containing Radiello cartridges are visually denoted by one of the two end caps having a stainless steel nut substituted for a brass nut to differentiate the tubes containing Radiello cartridges from standard TD tubes that have all brass caps and nuts.)

In the field, the Radiello cartridges are removed from the TD tubes, placed in polypropylene diffusive bodies and deployed for sampling for a designated time period. The samplers are then retrieved and prepared for return shipment to the analytical laboratory where they are analyzed according to either D-EMMD-AQB-006-SOP-01, "Standard Operating Procedure for Desorbing Volatile Organic Compounds from Carbopack X Sorbent Tubes Using the PerkinElmer TurboMatrix ATD," or D-EMMD-AQB-014-SOP-01, "Standard Operating Procedure for Desorbing Volatile Organic Compounds from Carbopack X Sorbent Tubes Using the PerkinElmer TurboMatrix ATD 650" and D-EMMD-AQB-003-SOP-01, "Standard Operating Procedure for Determination of Volatile Organic Compounds Desorbed from Carbopack X Diffusive Sampling Tubes Using the Agilent 6890N/5975 GC-MSD."

3.0 Definitions

COC	chain of custody	PVC	polyvinyl chloride
DI	deionized	QAPP	quality assurance project plan
DQO	data quality objective	SOP	standard operating procedure
FEP	fluorinated ethylene propylene	TD	thermal desorption
PTFE	polytetrafluoroethylene	VOC	volatile organic compound

4.0 Health and Safety Warnings

Glass vials might occasionally break during shipment. The field and laboratory staff should exercise caution when packing and unpacking the glass vials from the shipping containers.

5.0 Cautions/Interferences

5.1 The operator should handle the TD tubes and Radiello yellow diffusive bodies only while wearing clean nitrile or cloth gloves so that the tubes do not become contaminated with body oils, hand lotions, perfumes, etc.

5.2 To maintain sample integrity, labels should not be attached to the TD tubes or Radiello bodies and ink markings should not be made on the TD tubes or Radiello bodies (see section 9.4).

5.3 Only combined (one-piece) PTFE ferrules should be used with the 0.25-in. storage end caps as other types of ferrules might not seal adequately or might score the tube.

5.4 A CapLok tool should be used to tighten the fittings one-eighth to one-quarter turn past finger tight so that they are neither too tight nor too loose.

5.5 Care must be taken when transferring the Radiello Carbopack X cartridge from the TD tube to the Radiello diffusion body. The cartridge must be gently pushed out of the TD tube using the supplied "push rod" so as not to damage the wire screen containing the sorbent. Care must also be taken when transferring the cartridge back into the TD tube.

5.6 Sorbent material can sometimes leak from the Radiello Carbopack X cartridge, most likely due to a damaged cartridge or improper handling. Laboratory and field personnel should watch for any significant loss of sorbent material when end caps are changed. Any suspect tube should be removed from the sampling/analysis queue.

5.7 Since the Radiello Carbopack X cartridge itself does not have a unique identifier, it is important that each tube be returned to its assigned uniquely identified TD tube when transferred from the diffusion body back to a TD tube.

5.8 The tubes should be shipped in an airtight, non-VOC-emitting container to minimize their exposure to possible contaminants in the ambient air.

5.9 The refrigerator in which the tubes are stored should be free of solvents and chemicals to prevent possible contamination of the tube samples.

5.10 The laboratory in which the tubes are handled should be free of VOCs to prevent possibility of contamination.

5.11 Depending on the data quality objectives (DQOs) for a particular study, Radiello bodies and TD tubes may be cleaned after each use to minimize any chance of contamination.

6.0 Personnel Qualifications

Field study personnel should have experience handling TD tubes and collecting trace-level VOC samples using TD tubes.

7.0 Equipment and Supplies

In general, all sampling equipment and supplies, excluding the VOC-free refrigerator and vacuum oven, are shipped to sampling sites in packaged field kits prepared by the VOC laboratory. The following equipment and supplies are needed:

- Refrigerator, VOC free

- Precision vacuum oven (model 19, Precision Scientific Inc., Chicago, IL)
- Radiello Carbopack X cartridges (part no. RAD 141, Millipore Sigma, St. Louis, MO) loaded in empty stainless steel 89-mm stainless steel TD tubes (part no. 21822-U, Millipore Sigma) and fitted with precleaned and assembled 0.25-in. brass Swagelok fittings with combined (one-piece) PTFE ferrules (part no. 23094-U, Millipore Sigma) **Note: TD tubes containing Radiello cartridges are visually denoted by one of the two end caps having a stainless steel nut substituted for a brass nut to differentiate the tubes containing Radiello cartridges from standard TD tubes that have all brass caps and nuts.**
- Radiello yellow diffusive body (part no. RAD 1201, Millipore Sigma), triangular support plate (part no. RAD 121, Millipore Sigma), and vertical adapter for personal sampling (part no. RAD 122 Millipore Sigma)
- Nitrile gloves (part no. 55091, 55092, or 55093, Kimberly-Clark, Neenah, WI or equivalent)
- CapLok tool (part no. C-CPLOK, Markes International, Gold River, CA)
- Glass vials (part no. 45066A-25150, Kimble/Kontes, Vineland, NJ)
- Unlined caps for glass vials (custom order, Scientific Specialties Service, Inc., Randallstown, MD).
- Septrseal Septa and Teflon liners for glass vial caps (part no. B69800-24 and B68800-24, Scientific Specialties Service, Inc.)
- Clean, unlined gallon metal cans with lids (part no. MET-03098, 1195 Qorpak Inc., Washington Pike Bridgeville, PA 15017)
- Cooler (example part no. 5248-5286-5296, Coleman Outdoor Products, Inc., Wichita, KS)
- Foam inserts for coolers (Instapack quick foam packaging, Sealed Air Corporation, Danbury, CT)
- Rex protective sleeves, 1.021-in. i.d. by 5.750-in. length with wall thickness of 0.125 in. (custom order, Yazoo Mills, Inc., New Oxford, PA)
- Closed cell foam, assorted thicknesses
- Assorted tools, including but not limited to metal forceps or tweezers, wrenches of various sizes, and paint can openers
- Fluorinated ethylene propylene (FEP) Teflon tubing, 0.25-in. o.d.
- 600-mL Pyrex beaker (part no. 1000)
- Tech Wipes, three-ply tissue (part no. 350/50353, Horizon Industries, Tyler, TX)
- Zipper storage bags, 5 × 7-in. 3 mil (model #S-14444, 100/carton, Uline, Pleasant Prairie, WI) – used to store and ship the diffusion caps
- Zipper storage bags, 16 × 16-in. 3 mil (model #S-10835, 100/carton, Uline, Pleasant Prairie, WI) – to contain COC forms, data sheets, and labels when shipped to the field)
- Aluminum foil, food service grade (Western Plastics, Calhoun, GA) – for baking out diffusion caps
- Cable ties
- Bubble wrap

- Paper tape and sample ID labels
- Shipment chain of custody (COC) forms (Appendix A), field monitoring data and COC sheets (Appendix B), and laminated field deployment procedure (Appendix C)

8.0 Quality Control/Quality Assurance

8.1 Before storing, before and after shipping, and before and after sample collection, the end caps on the TD tubes should be checked for tightness by tugging simultaneously on each of the end caps in opposite directions to verify that the tubes are properly sealed.

8.2 TD tubes containing Radiello cartridges are visually denoted by one of the two end caps having a stainless steel nut substituted in for a brass nut to differentiate the tubes containing Radiello cartridges from standard TD tubes that have all brass caps and nuts.

8.3 Staff must handle all sampling media with gloves to ensure there is no contamination.

8.4 Staff must inspect Radiello diffusive bodies to ensure they are free of debris prior to deployment.

9.0 Procedures

9.1 Preparation of TD tubes for outgoing field shipments

1. Retrieve the conditioned TD tubes and "tug test" tubes as described in section 8.1 to ensure end caps are secure.
2. Use paper tape or generated labels on the outside of the appropriate glass vials to clearly label "Field Spikes," "Field Blanks," and "Spares" (Figure 1). (Refer to the project-specific Quality Assurance Project Plan (QAPP) to determine how field spikes and field blanks are prepared and used for the current project.)

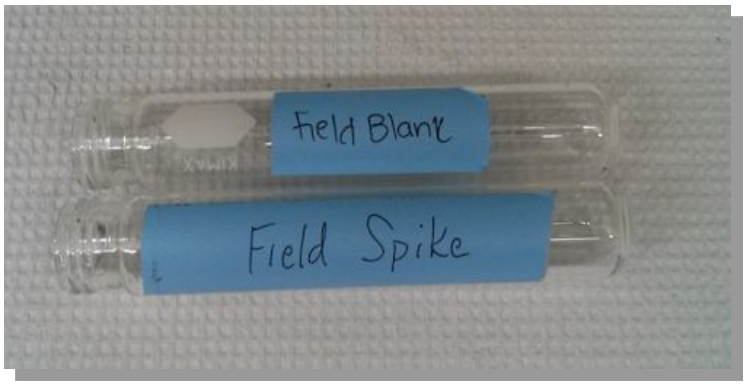


Figure 1. Labeled field blank and field spike.

3. Record the TD tube number of all outgoing tubes in the designated laboratory notebook.
4. Record the conditioning date next to each outgoing tube and the exposure dates next to the field spikes.
5. Make a copy of the laboratory notebook page to be sent to the field (Figure 2).

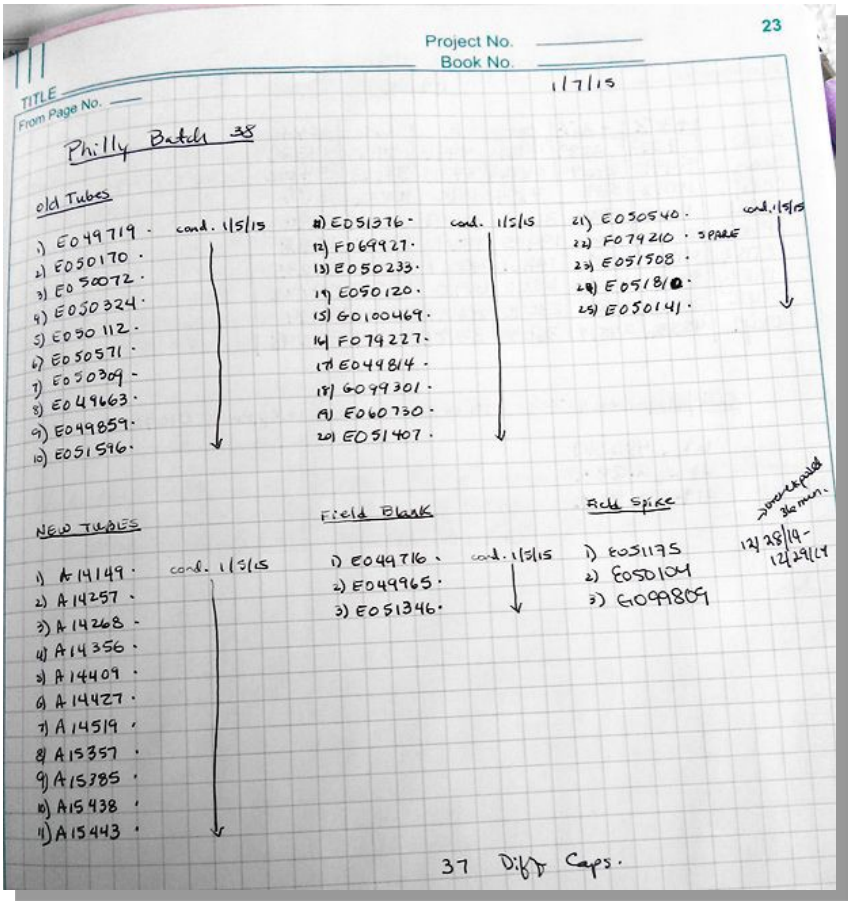


Figure 2. Laboratory notebook entries.

9.2 Packaging TD Tubes for Shipment to or from the Field

Note: Prior to the first use wash all metal shipping cans with soap and deionized (DI) water. Wipe them down with Tech Wipes and let them air dry.

1. Insert a short piece of 0.25-in. FEP Teflon tubing as a protective chip in the bottom of a glass vial to prevent the TD tube from bouncing around and possibly breaking the glass. Perform the tug test (see section 8.1), and then place the sealed TD tube into the glass vial.
2. Use forceps or tweezers to insert two 5.5-in. pieces of 0.25-in. o.d. FEP Teflon tubing strips into the glass vial beside the TD tube one at a time to prevent the TD tube from bumping the sides of the glass vial and cracking or breaking the glass (Figure 3).

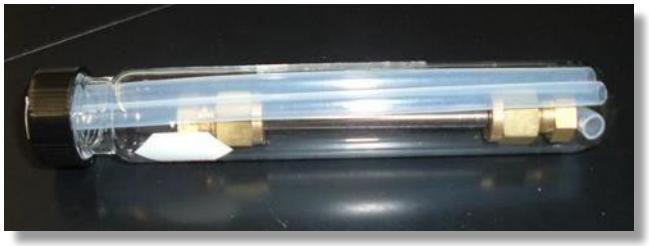


Figure 3. Protective Teflon tubing inserted into glass vial.

3. Screw the cap, which is fitted with a septum and Teflon liner, onto the glass vial.
4. Attach a sample ID label to the glass vial and to the field data sheet; this step streamlines the sample deployment process for the field operators.
5. Line the bottom of a metal can with a 6-in.-diameter foam disk, and then insert 19 cardboard protective sleeves (1.021-in. i.d. by 5.75-in. length) to cushion the glass vials.
6. Place the glass vial(s) into the metal can inside the protective sleeves, and insert a piece of foam cut roughly the size of a cardboard sleeve into the metal can to ensure that the protection sleeves fit snugly inside the can, as shown in Figure 4. (***Note*:** The seven protection sleeves in the center are preassembled with bubble wrap and bound together with cable ties, which allows for easy insertion or removal of the outer band of tubes, as shown in Figure 5.)

Figure 4. Tubes packed in metal can.

Figure 5. Tubes packed in inner protective sleeve.

7. Place two pieces of round foam (6.5-in. and 6-in. diameter) in the top of the metal can and seal the metal can with the lid (Figure 6).

Figure 6. Foam inserts in shipping canister.

8. Store the metal can in a refrigerator at 4 ºC until the tubes are to be sent to the field for

sample collection or back to the laboratory for analysis.

9. Ensure that any tubes that were removed from the metal canisters are repackaged as described in Section 9.2 steps 1-2 and that all procedures in section 9.2 for packing and sealing the metal cans are followed.
10. Pack the diffusive bodies in a zipper storage bag and place it inside a metal shipping canister.
11. Place the metal cans in coolers (three cans per cooler) fitted with foam packaging, as shown in Figure 7.

Figure 7. Metal cans packed in cooler for shipment.

12. Pack field data sheets and COC forms, spare preprinted sample ID labels, and return shipping labels inside a large (16 in. × 16 in.) zipper storage bag and place inside the cooler before shipping. Be sure to sign and date the COC form prior to shipping the cooler to the field.

9.3 Cleaning and Preparing Diffusive Bodies for Shipment to the Field

Laboratory personnel should inspect diffusive bodies for overall integrity prior to each return shipment to the field and should clean them as necessary using the following procedure. Compromised diffusive bodies should be discarded by VOC laboratory staff. When handling diffusive bodies, both field and laboratory personnel must wear cotton or nitrile gloves at all times to ensure the caps are not contaminated with body oils. Use the following procedure to clean the diffusive bodies:

1. Immerse the diffusive bodies in a beaker with mild laboratory detergent and DI water and sonicate for 20 minutes. (***Note***: Be sure the diffusive bodies remain submerged by weighting down with a smaller beaker.)
2. Rinse the diffusive bodies with plenty of tap water followed with DI water.
3. Lay diffusive bodies on several layers of laboratory tissues and air dry. Store the clean diffusion bodies in clean zipper storage bags.

9.4 Receiving Sample Media

(***Note***: Steps 1-10 are "check-in" steps that may be completed in an office or laboratory upon receipt of sampling media to ensure the integrity of the media and provide time for organization prior to deployment to the field. Alternatively, these steps may be completed in the field immediately prior to deployment.)

1. Remove the shipping canisters from the cooler.
2. Unpack the glass vials containing the tubes from the shipping canister by opening the lid of the can with a flathead screwdriver or paint can opener.
3. Remove the foam pieces from the top of the can, and remove the bound inner unit of tubes from the can.
4. Check in the TD tubes one by one by verifying that the glass vials are not cracked and that the caps are on the glass vials. Verify that the storage end caps are still in place on the TD tube and then check the TD tube numbers against the COC form. If any of the TD tubes have end caps that have slipped off or if the glass vials are broken, the operator should select a different tube for field deployment.
5. While wearing clean nitrile or cloth gloves, uncap the glass vial and remove the two Teflon tubing strips using tweezers, forceps, or small pliers one strip at a time so as not to crack or break the glass vial (Figure 8). Remove the cap from the glass vial and inspect the inside of the cap to ensure the cap septa and liner are in place. Replace if necessary.

Figure 8. Removing TD tube from the glass vial.

6. Remove the TD tube from the vial and perform the tug test to ensure the storage end caps are not loose (Section 8.0). If a problem is noted, choose a different TD tube for field deployment. Return the tube to the glass vial being sure that the protective Teflon chip is still on the bottom of the vial. (***Note***: Since the glass vials provide secondary containment, if a situation arises where all of the spare diffusive sampling tubes are used, then a tube can be selected for which the glass vial was broken or cracked. All relevant information on tube selection must be recorded on the field monitoring data sheet should this situation arise.)

7. Slide the Teflon strips into the glass vial, cap the vial, and place the glass vial holders in the shipping can. Repeat these steps for each TD tube that has been received and then place the foam pieces and lid on the can and seal. (***Note***: If these check in steps are being performed in the field at the time of deployment, the operator will not be placing the tubes or associated Teflon pieces back in the glass vials and metal shipping containers.)

8. Inspect all Radiello yellow diffusive bodies prior to use. Do not deploy any Radiello yellow diffusive bodies that have holes in them or have debris on the surface. Spare Radiello yellow diffusive bodies are provided in every shipment and should be used in these instances. Compromised Radiello yellow diffusive bodies should be returned by field staff to the VOC laboratory along with notes detailing concerns or problems regarding suspect Radiello yellow diffusive bodies. (***Note***: Cloth or nitrile gloves are to be worn for all activities involving handling of the diffusive body and/or TD tube and Radiello cartridge.)

9. Store the container(s) of TD tubes, tools, spare supplies, pens, diffusion caps, and a notebook containing the field data sheets in a storage box that can be transported easily to the field site.

10. List any observations regarding condition of sample media upon receipt on the field monitoring data and COC sheet (Appendix B) that accompanied the tube shipment.

11. If the tubes have been stored in a refrigerator, allow them to come to room temperature (~ 30 min to 1 h) before using them for sample collection.

9.5 Deployment of Radiello Diffusive Samplers

1. Assembly of the sampler for fixed site monitoring requires a tripod sampling stand (Figure 9A), a triangular support plate that has been modified to fit the tripod, a yellow diffusive body, a 1/8-inch diameter push rod, and a Radiello Carbopack X cartridge contained within the TD tube (Figure 9B).

A **B**

Figure 9. Tripod Sampling Stand (A); Radiello yellow diffusive body, support plate, push rod and TD tube containing a Radiello Carbopack X cartridge (B).

2. For fixed site sampling, the triangular support plate should be mounted on the top of the tripod with the threaded portion of the support plate that holds the diffusive body facing the base of the tripod (Figure 10). Extend and lock the sections of the tripod and place at the designated sampling area.

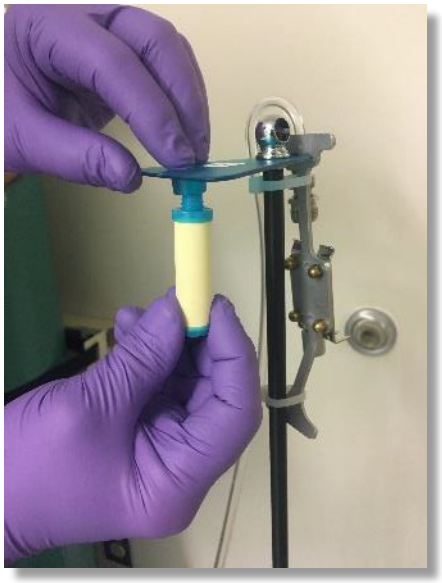


Figure 10. Assembly of support plate on tripod.

3. For each tube, ensure that the preprinted sample ID label is on the field data sheet and the other on the glass vial (Figure 11A). Record the field sample TD tube number, the ID

code of the corresponding triangular support plate and the sampling location information on the field data collection form (Figure 11B) The field data collection form is located in Appendix B.

Figure 11A. Retrieve TD tube packed inside of glass vial from shipping container.

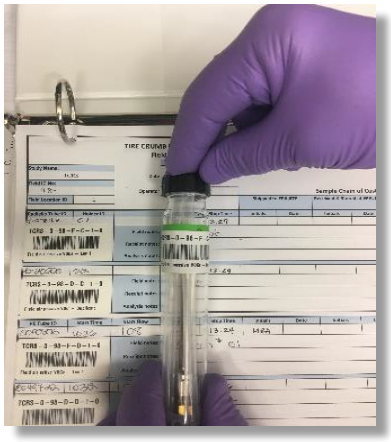


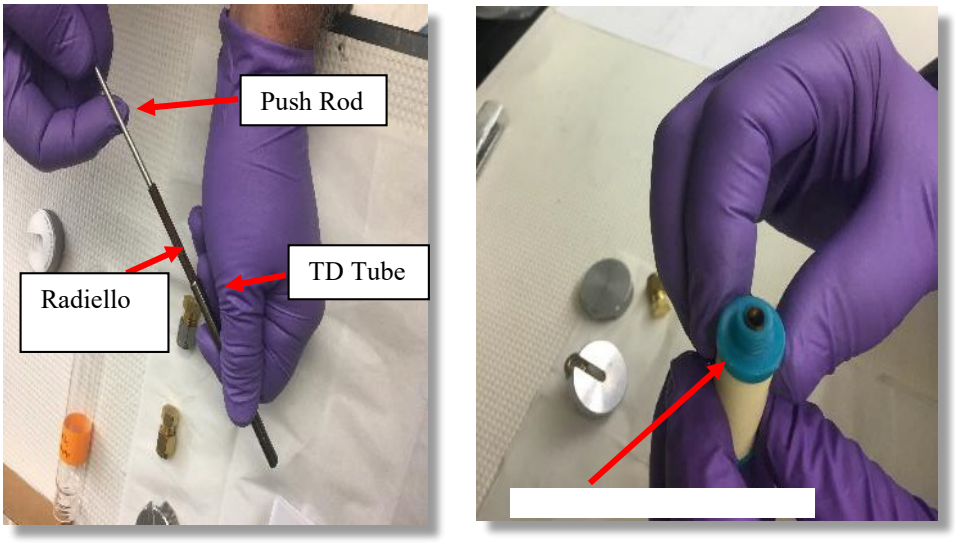
Figure 11B. Verifying labels on the vial and field data collection form

4. Record the TD tube number(s) of the field blank(s) and field spike(s) on the field data sheet. For QA samples, place one of the preprinted sample ID labels on the field data sheet and the other on the glass vial. Field blanks and field spikes must remain sealed with the storage end caps and are deployed alongside field samples.
5. To deploy field samples, using the CapLok tool, remove the end caps from both ends of the TD tube (Figure 12).

Figure 12. Remove end caps with Caplok tools.

6. Insert the 1/8-inch push rod in the grooved end of the TD tube and carefully push the Radiello Carbopack X cartridge out of the TD tube (A) and into the opening of yellow diffusive body (B) (See Figure 13). (***Note:*** A correctly centered cartridge should not

stick out even by half a millimeter. If it does, the cartridge is not correctly positioned and is out of axis).



A **B**

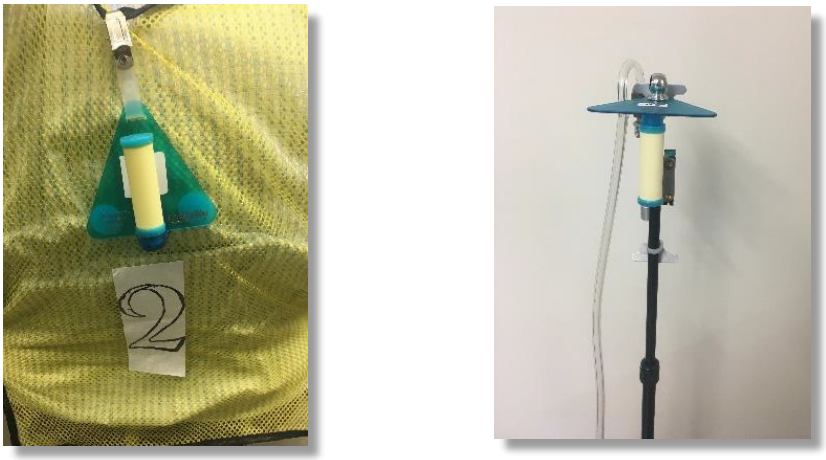
Figure 13. Push rod (A) used to push Radiello cartridge into the Radiello diffusive body (B).

7. Place the empty TD tube, end caps and Teflon strips and chip back into the glass vial and replace the vial cap.

8. Screw the yellow diffusive body containing the Radiello cartridge into the triangular support plate mounted on the top of the tripod. The diffusive body should be positioned underneath the support plate facing the ground (Figure 14). (***Note***: Be sure to minimize contact with the diffusive body and gloved hands to prevent contamination.)

Figure 14. Screw the Radiello diffusive body onto the triangular support plate.

9. Attach the sampler to the person (Figure 15A) or sampling tripod (Figure 15B). During personal sampling, ensure the sample body is not covered by clothing or hair.



A **B**

Figure 15. Radiello samplers deployed for personal monitoring (A) or stationary monitoring (B).

10. Record the start time and additional sampling details on the field monitoring data sheet. An example data sheet entry is shown in Figure 16 (***Note***: The sample ID code will vary according to the design of a particular field study.

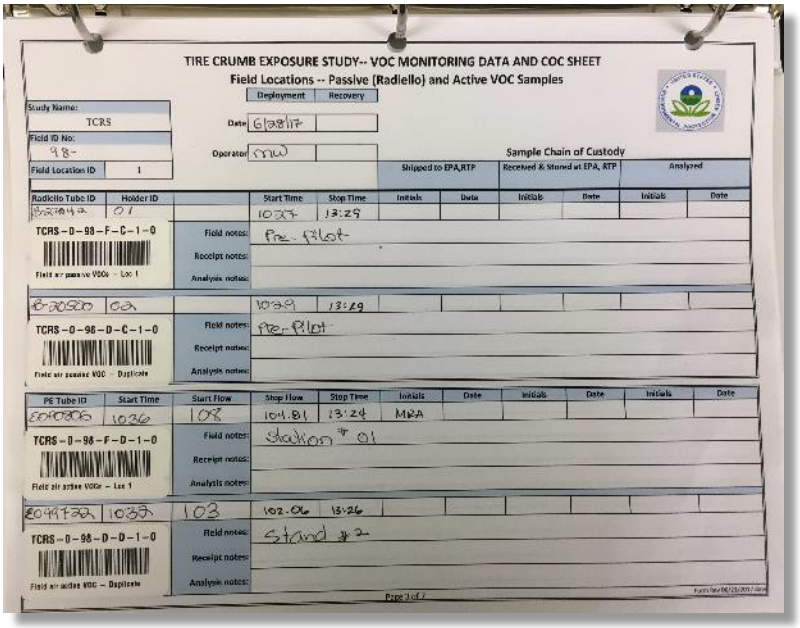
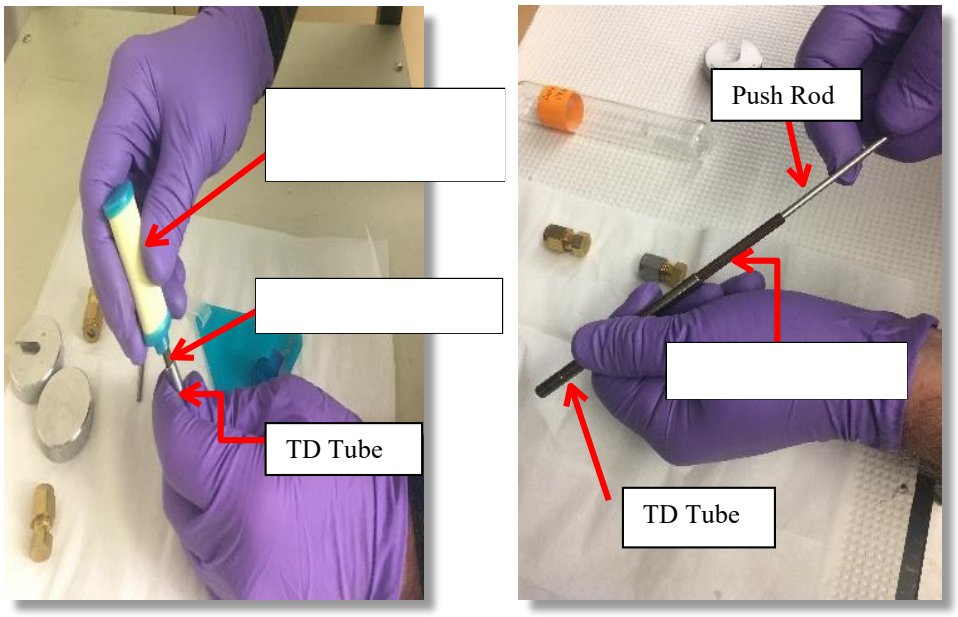


Figure 16. Enter sample ID code and tube number on data sheet.

11. Deploy for 3 hours (nominal).
12. At the end of the 3-hour deployment period, record the stop date and time on the field monitoring data sheet and any additional sampling/field comments (Figure 16).
13. Unscrew the yellow diffusive body from the triangular support plate.
14. Retrieve the exposed Radiello cartridge from the diffusive body using a pair of stainless steel forceps (Figure 17A). Carefully place the end of the Radiello cartridge into the non-grooved end of the TD tube and use the 1/8-inch push rod to gently push the cartridge completely into the TD tube (Figure 17B). (***Note***: Be sure the Radiello cartridge is returned to its ***original*** TD tube. TD tube numbers must be verified against the information recorded on the field data monitoring form).



A **B**

Figure 17. Radiello Cartridge (A) inserted back into the original PE tube using push rod (B).

15. Use CapLok tools as show in Figure 12 to tighten end caps on each end of the TD tube and perform the "tug test" to ensure end caps are tight. Place the TD tube back into the appropriate glass vial as shown in Figure 18. Ensure the Teflon chip and two Teflon strips are also packed inside of the glass vial to provide stability (Figure 18A). The tube should not move in the glass vial when packed correctly (Figure 18B).

A **B**

Figure 18. Tube packed into glass vial with Teflon chip and strips (A) and capped (B).

16. Return glass vials containing TD tubes back to the shipping canister. Place used Radiello diffusive bodies in a bag marked "Used" and return with samples for assessment for reuse.
17. Record tube numbers for any spare or unused tubes. In the comments section of the field data sheet indicate if the tube was a spare and/or was unused (Figure 16). Ensure that all entries on the field monitoring sheet are filled on correctly with start times, dates and sampling notes as well as stop dates, times and sampling notes. (***Note:*** All tubes shipped from the field must be documented on the field data sheet by field staff.).
18. Prepare the tubes for return shipment to the laboratory according to the procedure outlined in section 9.2. Field staff can also refer to the "Field Procedure for Diffusive Sample Collection Using Radiello Samplers – Deployment and Shipping" in Appendix C. A laminated version of this procedure will be shipped to field sites at the start of a study.

10.0 Data and Records Management

10.1 Details of the tube deployment (start/stop times, date, sample ID code, TD tube serial number, and operator's name) are listed on the field deployment data sheet (Appendix B).

10.2 Data sheets are returned to the laboratory with samples for analysis where they are placed in designated three-ring binders.

10.3 For large studies, field staff might be asked to populate electronic versions of field data sheets and send them to the laboratory manager.

10.4 Information from the field data sheets is combined with the corresponding analytical data in either Excel format or by a data manager as appropriate to a particular study and outlined in that study's QAPP.

10.5 Laboratory staff should refer to the Field Packing Checklist (Appendix D) and the Incoming Field Sample Checklist (Appendix E) when preparing outgoing field shipments and checking in incoming field samples.

11.0 References and Supporting Documentation

D-EMMD-AQB-008-SOP-01 (formerly 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-006-SOP-01 (formerly 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-014-SOP-01. 2016. Standard Operating Procedure for Desorbing Volatile

Organic Compounds from Carbopack X Sorbent Tubes Using the PerkinElmer TurboMatrix ATD 650. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

D-EMMD-AQB-003-SOP-01 (formerly 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.

Appendix A: Shipment Chain of Custody Form

CHAIN-OF-CUSTODY DECLARATION FORM

GENERAL INFORMATION

Monitoring Project: _____

Date of Initiation: _____

Sample Collection Date: _____

Total No. of Samples for Custody Transfer: _____

THE FOLLOWING TRANSFER OF CUSTODY DECLARATIONS APPLY TO ALL SAMPLES THAT ARE LISTED DIRECTLY IN EACH DESCRIPTION AND ONLY TO THOSE SAMPLES.

TO THE FIELD

DESCRIPTION OF SHIPMENT (Number and type of samples covered by this declaration):

Relinquished by :	Received by:
(Print Name and Organization)	(Print Name and Organization)
(Signature, Date and Time)	(Signature, Date and Time)
	Condition Acceptable? ____ **Yes** ____ **No**

FROM THE FIELD

DESCRIPTION OF SHIPMENT (Number and type of samples covered by this declaration):

Relinquished by :	Received by:
(Print Name and Organization)	(Print Name and Organization)
(Signature, Date and Time)	(Signature, Date and Time)
	Condition Acceptable? ____ **Yes** ____ **No**

Appendix B: Tube Field Monitoring Data and Chain of Custody Sheet

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Field Locations -- Passive (Radiello) and Active VOC Samples

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID 1			

Sample Chain of Custody

Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Initials	Date	Initials	Date	Initials	Date

Radiello Tube ID	Holder ID		Start Time	Stop Time
Place Sample ID Label Here		**Field notes:**		
		Receipt notes:		
		Analysis notes:		
Place Sample ID Label Here		**Field notes:**		
		Receipt notes:		
		Analysis notes:		

PE Tube ID	Start Time	Start Flow	Stop Flow	Stop Time
Place Sample ID Label Here		**Field notes:**		
		Receipt notes:		
		Analysis notes:		
Place Sample ID Label Here		**Field notes:**		
		Receipt notes:		
		Analysis notes:		

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Field Locations -- Passive (Radiello) and Active VOC Samples

		Deployment	Recovery
Study Name: TCRS	Date		
Field ID No:	Operator		

Sample Chain of Custody

Field Location ID	2				Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Radiello Tube ID	**Holder ID**		**Start Time**	**Stop Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

PE Tube ID	**Start Time**	**Start Flow**	**Stop Flow**	**Stop Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Place Sample ID Label Here		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

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TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Field Locations -- Passive (Radiello) and Active VOC Samples

		Deployment	Recovery
Study Name:			
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID 8			

Sample Chain of Custody

Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Initials	Date	Initials	Date	Initials	Date

Radiello Tube ID	Holder ID		Start Time	Stop Time
Place Sample ID Label Here		Field notes:		
		Receipt notes:		
		Analysis notes:		
Place Sample ID Label Here		Field notes:		
		Receipt notes:		
		Analysis notes:		

PE Tube ID	Start Time	Start Flow	Stop Flow	Stop Time
Place Sample ID Label Here		Field notes:		
		Receipt notes:		
		Analysis notes:		
Place Sample ID Label Here		Field notes:		
		Receipt notes:		
		Analysis notes:		

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

Active (PE) VOC Field/Lab Blanks and Controls

Study Name:		Deployment	Recovery
TCRS	Date		
Field ID No:			
	Operator		
Field Location ID			

Sample Chain of Custody

PE Tube ID		Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
		Initials	Date	Initials	Date	Initials	Date

Place Field/Lab Blank ID Label Here	Field notes:	
	Receipt notes:	
	Analysis notes:	

Place Field/Lab Blank ID Label Here	Field notes:	
	Receipt notes:	
	Analysis notes:	

PE Tube ID		Initials	Date	Initials	Date	Initials	Date

Place Field/Lab Control ID Label Here	Field notes:	
	Receipt notes:	
	Analysis notes:	

Place Field/Lab Control ID Label Here	Field notes:	
	Receipt notes:	
	Analysis notes:	

Form Rev 06/21/2017 daw

Appendix C: Field Procedure for Diffusive Sample Collection Using Radiello Samplers – Deployment and Return Shipping

Health and Safety Warnings

Glass vials containing TD tubes might occasionally break during shipment. Exercise caution when packing and unpacking the glass vials from the shipping containers.

Cautions/Interferences

- The operator should handle the TD and diffusive bodies only while wearing clean cloth or nitrile gloves.
- Labels should not be attached to the TD tubes or Radiello diffusive bodies, and ink markings should not be made on the TD tubes or Radiello diffusive bodies. Markers should not be used around the TD tubes or Radiello diffusive bodies.
- Radiello cartridges must be seated properly in the diffusive body. They should not stick out of the diffusive body by even a millimeter. Any protrusion from the diffusive body indicates the Radiello sampling cartridge is not properly seated.
- When sampling is complete, a CapLok tool should be used to tighten the storage end caps one-eighth to one-quarter turn past finger tight so that they are neither too tight nor too loose. A tug test should be performed to ensure adequate end cap tightness.
- The laboratory/area in which the samples are stored and handled should be free of VOCs to prevent any possibility of contamination.

Procedure

1. Diffusive Sample Collection

***Note*: Steps 1 through 5 are usually performed in the lab/office upon receipt of the sampling media to facilitate organization of the field deployment of the TD tubes. Steps 6 through 15 are performed in the field.**

1. Unpack the TD tubes from the shipping can by opening the lid of the can with a screwdriver as necessary, remove the foam pieces from the top of the can, remove the inner tube holder of glass vials from the can, and remove the remaining glass vials from the shipping can if applicable.
2. Check in the TD tubes one by one by verifying that the glass vials are not cracked and that the caps are on the glass vials. Verify that the storage end caps are still in place on the TD tube. If any of the TD tubes have storage end caps that have slipped off or if the glass vials are broken, the operator should select a different tube for field deployment.
3. Check the TD labels against the labels on the field data sheet.
4. While wearing clean nitrile gloves, uncap the glass vial and remove the two Teflon tubing strips using tweezers, forceps, or small pliers one strip at a time so as not to crack or break

the glass vial.

5. Remove the TD tube from the vial and perform the tug test to ensure the storage end caps are not loose. If a problem is noted, choose a different TD tube for field deployment. Return the tube to the glass vial being sure that the protective Teflon chip is still on the bottom of the vial.
6. Slide the Teflon strips into the glass vial, cap the vial, and place the glass vial holders in the shipping can. Repeat these steps for each TD tube that has been received and then place the foam pieces and lid on the can and seal.
7. Inspect all Radiello yellow diffusive bodies prior to use. Do not deploy any Radiello yellow diffusive bodies that have holes in them or have debris on the surface. Spare Radiello yellow diffusive bodies are provided in every shipment and should be used in these instances. Compromised Radiello yellow diffusive bodies should be returned by field staff to the VOC laboratory along with notes detailing concerns or problems regarding suspect Radiello yellow diffusive bodies.
8. Store the container(s) of TD tubes, tools, spare supplies, pens, diffusion caps, and a notebook containing the field data sheets in a storage box that can be transported easily to the field site.

Note: Perform the following steps** a**t the field site beginning immediately prior to deployment of the Radiello Sampler tubes while wearing clean gloves and working steadily:

9. Following the procedures above, allow tubes to warm up for 30 minutes prior to deployment.
10. Remove a TD tube from a glass vial and use a CapLok tool to loosen and remove the storage end cap from the sampling end of the tube. Place the Teflon chip and strips, and storage end cap (as applicable) back into the glass storage vial and cap the glass vial.
11. Record the appropriate TD tube number on the field data sheet next to its corresponding sample ID label. Store the glass vial as appropriate.
12. Using the 1/8-inch push rod, insert in the grooved end of the TD tube and carefully push the Radiello Carbopack X cartridge out of the TD tube and into the opening of yellow diffusive body. (***Note***: A correctly centered cartridge should not stick out even by half a millimeter. If it does, the cartridge is not correctly positioned and out of axis).
13. Attach the Radiello sampler to the person or sampling tripod. During personal sampling, ensure the sample body is not covered by clothing or hair
14. Follow steps 8 through 12 for each TD tube to be deployed for sample collection.
15. Do not uncap the field blanks or field spikes, but instead hang them in place with both of the storage end caps still sealing the TD tube. Record the TD tube serial number for the field blank(s) and field control(s) on the field data sheet. (***Note***: This deployment procedure will depend on the design of a particular study; some studies might specify that the field blank(s) and field spike(s) travel to the field and then are returned to the field office for

storage during the sampling period.)

16. Once all Radiello samplers are positioned, mount the tripod as appropriate at the site. Record the start time and additional sampling details and/or comments on the field monitoring data sheet.

Note: Perform the following steps at the field site at the end of the sampling cycle.

17. At the end of the 3-hour deployment period, record the stop date and time on the field monitoring data sheet and any additional sampling/field comments.
18. Retrieve the exposed Radiello cartridge from the diffusive body using a pair of stainless steel forceps. Carefully place the end of the Radiello cartridge into the non-grooved end of the TD tube and use the 1/8-inch push rod to gently push the cartridge completely into the TD tube. (***Note***: Be sure the Radiello cartridge is returned to its original TD tube. TD tube numbers and labels must be verified against the information recorded on the field data monitoring form.)
19. Use CapLok tools to tighten storage end caps on each end of the TD tube and preform the “tug test” to ensure end caps are tight.
20. Place the TD back into the appropriate glass vial. Ensure the Teflon chip and two Teflon strips are also packed inside of the glass vial to provide stability.
21. Return glass vials containing TD tubes back to the shipping canister. Place used Radiello diffusive bodies in a bag marked “Used” and store appropriately for cleaning.
22. Verify that the field sample ID label and TD tube number on the vial match those recorded on the field monitoring data sheet. Record the stop time and any additional sample collection data on the field monitoring data sheet beside the appropriate field ID label/TD tube serial number.
23. Return all TD tubes and supplies to the storage box/cooler for return to the field office.

2. Shipment to the Laboratory

1. To prepare the TD tube shipment to the laboratory, check to see that the Teflon tubing chip is in the bottom of each glass vial to prevent the TD tube from bouncing around and possibly breaking the glass.
2. Use tweezers/forceps to insert two Teflon tubing strips into each glass vial beside the TD tube to prevent the TD tube from bumping the sides of the glass vial and cracking or breaking the glass.
3. Screw the vial cap onto the glass vial after checking the vial cap to be sure that the liner and septum are in place. Replace with a spare liner or septum if necessary.
4. Place the glass vial(s) into the metal can(s) loaded with cardboard protection sleeves to cushion the glass vials. Insert the piece of foam that is cut roughly the size of a cardboard sleeve into the metal can to ensure that the protection sleeves fit snugly inside the metal can. Place the center tube holder insert into the middle of the can.

5. Place the two pieces of round foam in the top of the metal can and seal the metal can with the lid.
6. Place the metal cans in the same shipping box or container that they were originally shipped in and fill the container with bubble wrap or other packaging material to cushion the contents.
7. Fill out the COC form and return it along with the field data sheets in this shipment. ***Note****:* Make a photocopy of these documents for retention at the field office prior to shipping.
8. Seal the box securely for shipment, attach the return shipping label for delivery, and be sure that the package will not be in transit over a holiday or weekend.

Appendix D: Field Packing Checklist for Laboratory Staff

It is helpful to perform these activities a week or two before the next shipment.

Activity	Performed
Count how many clean TD tubes are in the fridge and ready for deployment. This will give you an idea of how many batches you might need to analyze this week to free up tubes for the next shipment.	☐
Count how many glass vials and vial caps are currently available for field use.	☐
Be sure you have enough coolers.	☐
Be sure you have enough tubes for the field spike exposures. It is always a good idea to ask if there is a need for any additional field spikes outside the norm before starting the field spike exposures.	☐
Check to see if you have enough hang tag packets for shipping the coolers. The sooner you know the better in case more need to be ordered.	☐
Be sure you have enough protective Teflon strips and chips to pack into each vial. You might need to check incoming shipments stored in the fridge for extra Teflon strips/chips if you do not have enough for outgoing shipments.	☐
Before you start packing, check to see if any additional sites have been added or if sites have been removed for each field location. It is also helpful to pass this information along to the staff member who is preparing the sample ID labels.	☐
Be sure you have enough clean Radiello diffusive bodies. Write down how many you pack per shipment and ensure they are clean.	☐
Always write down the TD tubes numbers packed in each batch in the designated lab notebook. Include the exposure date for field spikes and lab controls and the conditioning dates for all other tubes packed.	☐
QA packed shipments and any inventory forms that are shipped to ensure tube numbers are typed correctly. Templates for inventory sheets are on the desktop of the spare computer in D260 in the folder labeled “Packing Lists”.	☐
Always pack field spikes and field blanks in vials labeled with paper tape: “Field Spike,” “Field Blank,” or “Spare.”	☐
Store outgoing shipments on the top shelf on the right-hand side of the refrigerator.	☐
Copy outgoing COC signature pages and tube inventory lists and store in designated field study COC binders.	☐
Store all emails/correspondence relevant to a particular batch with copies of the COC.	☐

Appendix E: Incoming Field Sample Checklist for Laboratory Staff

Activity	Performed
Be sure all check-in notes, signatures, and date are recorded on the original COC in permanent waterproof black pen.	☐
Sign and date the signature page of the COC as well as the boxes next to each received sample.	☐
If any additional tubes are received that are not listed on the COC by field operators, record those tube numbers on the COC and initial/date receipt of those tubes as well.	☐
In general, one **unused/unexposed** tube is sent back with each shipment. This tube is considered the shipping blank.* Write **"shipping blank"** in the "Receipt Comments" section and assign the appropriate Sample ID to this tube on the COC in the box where the sample ID label is placed according to the format designated in the associated QAPP. Examples of recently used Sample ID number formats for shipping blanks are shown below: <u>For Regional Shipping Blank/Spare Tube</u> Region-Site-Week-Type ##-99-##-SB Example: 06-99-12-SB Region 6, Site 99, Week 12, Shipping Blank ***Note***: The designated site for the shipping blank sample is 99. The newly assigned sample ID should also be written on paper tape and placed on the vial that the tube is stored in. <u>For Philly Shipping Blank/Spare Tube</u> Site-Week-Type-PHL 99-##-SB-PHL Example: 99-25-SB-PHL Site 99, Week 25, Shipping Blank, Philly ***Note***: The designated site for the shipping blank sample is 99. The newly assigned sample ID should also be written on paper tape and placed on the vial that the tube is stored in. Label the glass vial with the shipping blank sample code before storing. *If multiple spare/unused TD tubes are shipped back, select only one as the shipping blank and assign an appropriate Sample ID. The remaining unused tubes should be conditioned prior to reuse.	☐
Cross-check TD tube numbers and Sample IDs on the tubes against those on the COC.	☐
Note any observations in the "Receipt Comments" section. The following are some suggestions of the info to enter so that everyone checking in samples is making the same types of check-in comments, which helps when flagging data in the database. • "SE loose" – the sampling end cap on the grooved end of the tube is loose; tightened • "RE loose" – the rear end cap is loose; tightened • "Vial cracked" • "Cap off vial" • "SE end cap off"/ "SE end cap nut off"	☐

• "RE end cap off"/ "RE end cap nut off" • "Tube dirty" • "SE ferrule missing/RE ferrule missing" • "Tube number incorrect"; "Correct tube number is …." • "Sample ID is incorrect"; "Correct sample ID is…." • "Tubes switched in vial; switched tubes to match COC" • "Tube invalid no analysis"	
Remove all protective Teflon tubing strips and chips to ensure they are available for the next outgoing shipments. If any strips or chips are dirty, they can be wiped off with lint-free Kimwipes, and in extreme cases can be rinsed with DI water and dried with house air. Never use solvents.	☐
Remove white lined vial caps/black lined vial caps and replace them with white vial caps that are not shipment-ready before they are stored.	☐
Set all tubes to the side that are considered invalid and will not be analyzed. These are usually tubes that are found on the ground. Inform the VOC lab manager of these tubes and verify that they will not be analyzed.	☐
Store all checked-in tubes inside their glass vials, place them in a cardboard box, and place them in the fridge. Labels from the incoming canisters can be placed on the cardboard box to identify the samples in the box.	☐
Clean any used Radiello diffusive bodies. Discard those that are torn or unfit for additional use.	☐
Immerse the diffusive bodies in a beaker with mild laboratory detergent and DI water and sonicate for 20 minutes. ***Note***: Be sure the diffusive bodies remain submerged by weighting down with a smaller beaker.	☐
Rinse the diffusive bodies with plenty of tap water followed with DI water. Lay diffusive bodies on several layers of laboratory tissues and air dry. Store the clean diffusion bodies in clean zipper storage bags.	☐
Copy the COC after check-in is complete and place the original COC in the designated binder with any correspondence regarding that particular batch. Place the copy aside in the designated space so that the Excel tube tracking sheet can be updated. In addition, store a copy of the COC with the tubes that are refrigerated for future analysis.	☐
Acknowledge receipt of all incoming shipments for the day and any important notes/findings in the designated laboratory notebook.	☐

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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 (SOP) for Collection of Surface Wipe Samples from Synthetic Turf Fields

Number: D-SED-IEMB-026-SOP-01	Effective Date: August 21, 2017

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification:

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For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

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

STANDARD OPERATING PROCEDURE (SOP) FOR COLLECTION OF SURFACE WIPE SAMPLES FROM SYNTHETIC TURF FIELDS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the method for collecting wipe samples from synthetic turf field surfaces to measure semivolatile organic compounds (SVOCs) and metals for the United States Environmental Protection Agency (EPA) exposure characterization pilot tire crumb rubber research study (TCRS).

2.0 SUMMARY OF THE METHOD

Dermal, inhalation, and ingestion of dust at synthetic turf fields may represent important pathways of exposure to chemicals associated with tire crumb rubber, other synthetic field materials, and environmental dust deposited on the field. The concentrations of metals and semivolatile organic compounds (SVOCs) on field surfaces available for dermal transfer must be measured to determine human exposures and to compare these to the various exposure pathways and to biologic markers in blood and urine.

Surface wipe samples for metals analysis will be collected at synthetic turf field sites using a wet (water) wipe (Environmental Express, Ghost Wipe No. 4210) conforming to American Society for Testing and Materials (ASTM) E1792 (ASTM-03, 2016a) requirements. Surface wipe samples for SVOC analysis will be collected using cleanroom twill wipes (M.G. Chemicals, cotton, pre-cleaned), using a dry 30.5 cm × 30.5 cm wipe attached to a drag sled and a wipe wetted with 1:1 isopropyl alcohol:water with dimensions of 4 inch × 4 inch (10 cm × 10 cm).

3.0 DEFINITIONS

ASTM – American Society for Testing and Materials

CDC – Centers for Disease Control and Prevention

COC – Chain-of-custody

EPA – Environmental Protection Agency

FB – Field Blank

FC – Field Control spiked with target analytes

Metals – Includes both metals and the metalloid, arsenic

QAPP – Quality Assurance Project Plan

QC – Quality Control

RTP – Research Triangle Park

SOP – Standard Operating Procedure

SVOC – Semivolatile Organic Compound (generally, a compound with vapor pressure = 10^{-5} – 10^{-2} kilopascals)

TCRS – Tire Crumb Rubber Research Study

4.0 CAUTIONS

4.1 Nitrile gloves and eye protection should be worn during sample collection for metals wipe samples. Silver Shield gloves and eye protection should be worn during sample collection for SVOCs.

4.2 Field staff should keep the sampling materials and the samples out of the reach of children.

4.3 Do not wipe the template demarcating a sampling area.

4.4 Collect samples at times when it was safe to do so with regard to any activities occurring on the field. Sample collection time is not critical for these samples, but the samples should be collected at a convenient time during the overall exposure measurement activities at each field.

4.5 No photography will be performed of any activities at the fields.

5.0 RESPONSIBILITIES

5.1 The EPA project staff will prepare the surface wipe sample collection equipment and materials and deliver them to the field coordinator. EPA will provide the spiked field controls.

5.2 The field coordinator will receive the surface wipe sample collection equipment and materials. The field coordinator will create a strategy and schedule to deploy or collect the appropriate percentage of each type of field quality control (QC) samples as defined in the QAPP addendum. The field coordinator will communicate the schedule for QC samples to the field staff and distribute any additional QC sample materials. The field coordinator will distribute surface wipe sample collection equipment and materials to the field staff. Upon collection of the surface wipe samples, the field coordinator will be responsible for returning the samples with their collection records and Chain-of-custody (COC) sheets to the EPA in Research Triangle Park (RTP), NC for analysis.

5.3 The field staff will be responsible for obtaining the collection equipment and materials from the field coordinator, collection of the surface wipe sample, entering relevant information on the sample collection record sheets, and returning collected surface wipe samples and records to the field coordinator.

6.0 MATERIALS AND REAGENTS

6.1 Wipe media for SVOCs (M.G. Chemicals, Cleanroom Twill wipes, 10 cm × 10 cm and 30.5 cm × 30.5 cm, cotton, pre-cleaned)

6.2 Wipe media for metals (Environmental Express SC 4210 (or similar) Ghost Wipes, 15 cm × 15 cm, packaged pre-moistened with deionized water)

6.3 Pre-cleaned and certified amber glass jars with Teflon-lined lids, 2 oz. straight-sided (Thermo I-Chem Part No. 340-0060 or equivalent)

6.4 Plastic digestion cups, (50 mL, Environmental Express P/N SC475 or equivalent)

6.5 Masking tape

6.6 Disposable nitrile gloves

6.7 Disposable Silver Shield gloves

6.8 Protective glasses

6.9 Frozen ice packs

6.10 Cooler

6.11 Aluminum or stainless steel template for SVOC wipes (with 12” × 12” interior dimension wiping area)

6.12 Paper sampling template for metal wipes (with 12” x 12” interior dimension wiping area)

6.13 25’ measuring tape

6.14 Ink pen with black ink

6.15 Isopropyl alcohol (ACS reagent grade or better)

6.16 Disposable pre-wetted isopropanol wipes for cleaning sampling equipment

6.17 Stainless steel or aluminum tray (10” x 13” or similar)

6.18 Stainless steel and plastic forceps

6.19 Sample collection and COC record sheets

6.20 Sample ID labels

6.21 Field waste bag

6.22 DAWser 2016 drag sled (custom built by EPA) consisting of a 10 kg aluminum block (25.4 x 25.4 x 5.1 cm), clamps for securing wipe material, and an attached handle

7.0 PROCEDURES

7.1 SAMPLE COLLECTION

Wipe samples will be collected from synthetic turf fields to support characterization of chemical constituents.

7.1.1 Identification of Field Sampling Location

Individual wipe and drag sled samples will be collected from three locations at each field (see Figure 1). A separate set of samples will be collected at each location including: one wipe sample for SVOC analysis, one wipe sample for metals analysis, and one drag sled sample for SVOC analysis. It is important that the surface SVOC, surface metals, and drag sled SVOC not be collected from the exact same spots on the field. They should be collected in proximity to each other at the three locations but their sampling areas must not overlap (Figure 1).

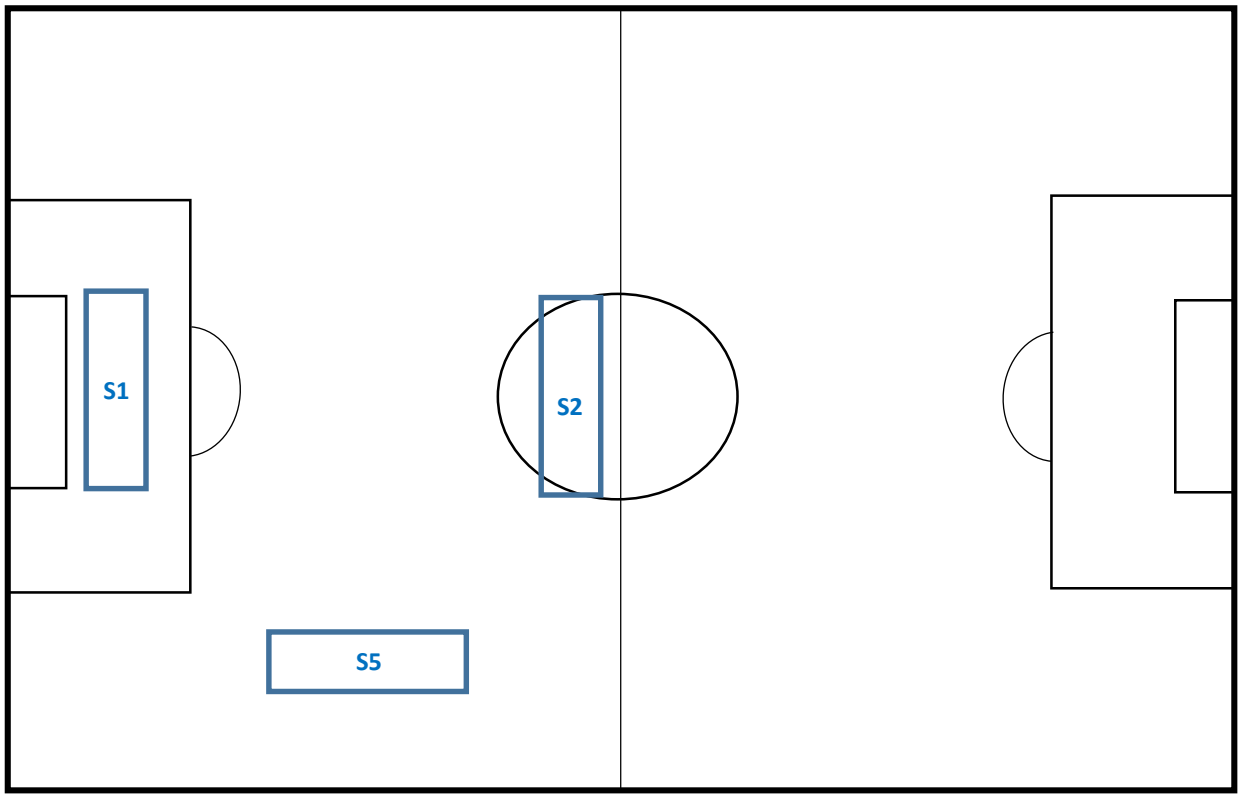

Figure 1. Surface wipe and drag sled sample collection locations on athletic fields.

7.1.2 Gloves

Prior to collecting metals surface wipe samples, put on clean, powderless nitrile gloves and keep them on during the entire sampling period. Prior to collecting surface wipe SVOC and drag sled SVOC samples, put on Silver Shield gloves.

7.2 Field Surface Wipes for Metals

Samples will be collected at positions #1, #2, and #5 as shown in Figure 1, for a total of three separate samples. No background sampling location wipe sample will be collected.

Use surface wipes to collect samples for **metals** analysis at synthetic turf field sites. Samples are collected with a wet (water) wipe material conforming to ASTM E1792 (ASTM-03, 2016a) requirements (Ghost Wipe No. 4210, Environmental Express).

Collect samples following the ASTM E1728 sample collection method (ASTM 1728-16, 2016b), a standard wet-wipe method for collecting dust from indoor floor surfaces that use water as the wetting agent. Specifically, a 929 cm^2 (1-ft^2) template is placed on the surface of the field.

Remove the wet wipe from the foil packet. Using one side of the wipe, wipe the turf surface in a S or Z-shaped pattern within the template area. After folding the wipe in half to get a fresh wipe surface, wipe the area again in a S or Z-shaped pattern perpendicular to the first wipe pattern (see Figure 2). Next, fold the wipe in half again and wipe the edges of the sampling area near the interior portion of the template. Prior to placing the wipe in a storage tube, use plastic forceps to remove full size tire crumb rubber infill granules, synthetic grass blades, and other large debris or litter. Finally, fold the wipe and place it a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage. Tightly cap the tube and transport at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Figure 2. Wiping the turf surface in a S or Z-shaped pattern within the 929 cm^2 template area.

7.3 Field Surface Wipe Samples for SVOCs

Ensure that you have a sufficient number of previously pre-cleaned SVOC wipes for the field team staff for transport to the field location.

Two types of field surface wipe samples will be collected for SVOCs analysis. One method uses a dry wipe material attached to a drag sled. The second uses an isopropanol:water-wetted wipe.

7.3.1 Drag Sled **Dry Wipe Method**: Collect surface wipe drag sled samples for SVOC analysis at synthetic turf field sites using a dry wipe material (Texwipe TX312 Cleanroom Twill, 30.5 cm × 30.5 cm, cotton) that has been cleaned by pre-extraction using a series of solvents including acetone and hexane prior to use.

Collect samples only at times when it was safe to do so with regard to any activities occurring on the field. Sample collection time is not critical for these samples, but the samples should be collected at a

convenient time during the overall exposure measurement activities at each field.

Collect samples at positions #1, #2, and #5 as shown in Figure 1, for a total of three separate samples. Samples will be collected from different areas than the areas used for metals wipe sample collection. Ensuring that there is no area overlap can be accomplished by placing templates and marking the drag sled area prior to sampling. No background sampling location wipe samples will be collected.

Using clean, Silver Shield gloves, remove the wipe material from its storage container and clamp it to a wipe sampling push sledge device (see Figure 3). The device has a 10 kg aluminum block of the dimensions 25.4 x 25.4 x 5.1 cm with clamps on one side for securing the wipe material and an attached handle for pushing the device. Secure the wipe material so that the 645 cm^2 bottom face of the block is completely covered by the wipe material.

Using a tape measure, mark a 5 m x 1 m area (5 m^2) on the synthetic turf field. The area may be marked with masking tape or surveyor flags; existing field lines may also be used to denote one or more area boundaries. With the wipe sampler starting in one corner of the marked area, drag the sled down the 5-m length and then back again. Move the drag sled one width over in the collection area. Drag the sled down the 5-m length and then back again. Repeat this until the entire 5 m^2 has been wiped with one up and one back pass. When pushing or pulling the drag sled, keep the handle at or below waist level to minimize the vertical force of the handle on the drag sled body – the goal is to achieve consistency by having the majority of the force resulting from the weight of the drag sled body.

Prior to placing the wipe back into the storage container, remove as much as possible of any synthetic grass blades, large tire crumb rubber pellets, and other large debris or litter on the sides of the wipe material that did not contact the field. With forceps, remove any large (≥ 1 mm) tire crumb rubber pellets from the portion of the wipe material that contacted the synthetic turf field. Do not attempt to remove any grass blades from the field-facing side of the filter.

Finally, fold the wipe and place it in the clean 500 mL amber glass wide mouth storage bottle with Teflon cap liner. Tightly cap the bottle and transport at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Figure 3. Wipe sampling push sledge device with cotton dry wipe material attached.

7.3.2 **Wetted Wipe Method**: Collect wetted samples at synthetic turf field sites using a wipe material (Texwipe TX312 Cleanroom Twill, 10 cm × 10 cm, cotton) that has been cleaned by pre-extraction

using a series of solvents including acetone and hexane prior to use. In the laboratory, prior to shipment to the field site, each wipe will be placed in a clean 60-mL wide-mouth amber jar, and 3 mL of 1:1 isopropanol:water will be added directly to the wipe material in the jar, dispersed across the folded wipe material as evenly as possible. The jar will then be tightly capped with Teflon-lined lids. Sample labels will be affixed to the jars, and the jars will be transported to the field site.

Collect samples at times when it was safe to do so with regard to any activities occurring on the field. Sample collection time is not critical for these samples, but the samples should be collected at a convenient time during the overall exposure measurement activities at each field.

Collect samples at positions #1, #2, and#5 as shown in Figure 1, for a total of three separate samples. Collect samples from different areas than the areas used for previous wipe sample collection. Ensuring that there is no area overlap can be accomplished by placing templates and marking the drag sled area prior to sampling. No background sampling location wipe samples will be collected.

Place a 929 cm^2 (1 ft^2) aluminum template on the surface of the field.

Using clean, Silver Shield gloves and, if needed, clean tweezers, remove the pre-wetted wipe material from its storage container. Using one side of the wipe, wipe the turf surface in a S or Z-shaped pattern within the template area (see Figure 4). After folding the wipe in half to get a fresh wipe surface, wipe the area again in a S or Z-shaped pattern perpendicular to the first wipe pattern. Next, fold the wipe in half again and wipe the edges near the interior portion of the template. Prior to placing the wipe back into its sample jar, use forceps to remove full size tire crumb rubber infill granules (≥1 mm), synthetic grass blades, and other large debris or litter. Finally, fold the wipe and place it back into its 60-mL amber wide-mouth glass jar. Tightly cap the jar and transport at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Figure 4. Wiping the turf surface in a S or Z-shaped pattern within the 929 cm^2 template area. (Note: Silver Shield gloves will be used instead of the nitrile gloves shown in this photo).

7.4 HANDLING AND PRESERVATION

7.4.1 Complete the sample collection records and COC records for the samples (Appendix A).

7.4.2 After collection and during transport from the collection site, store the surface wipe samples in a cooler with frozen ice packs.

7.4.3 Store the samples on frozen ice packs or in a refrigerator or freezer until shipment. Ship samples by overnight delivery service in a shipping cooler with frozen ice packs. SOP EMAB-185.0 *Standard Operating Procedure (SOP) for Storage and Shipping of Multimedia Samples* for post-collection handling, storage, and shipment of surface wipe samples may be consulted for additional information.

7.4.4 Ship samples and their sample collection data/COC sheets to:

US EPA Chemical Services
Kent Thomas or Scott Clifton
109 T.W. Alexander Drive
Building E Loading Dock, Rm E178
Research Triangle Park NC 27709-0002
Telephone:919-541-7939

8.0 RECORDS

A data collection system will be used to capture information associated with the collection of all samples. For the surface wipe samples, the sample collection information to be recorded will include the following, as a minimum: the sample ID, the date and time of the sample collection, the sampling location, initials or ID number of the field staff member responsible for the sample collection, and any comments regarding collection (Appendix A). Other information shall be collected as needed to ensure successful collection and interpretation of data. Please see D-SED-IEMB-030-SOP-01 for recording sample collection locations on the proper field diagram.

Section B3 in the QAPP addendum details the sample code information. Sample codes used for the EPA Tire Crumb Rubber Research Study will follow the general naming scheme used by the EPA for the tire crumb rubber characterization study.

The specific coding information for field wipe samples is extracted from the QAPP addendum:

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

Where:

TCRS
: Designates the tire crumb rubber research study

R – Participant identification number

1 – 8; each number assigned to a unique participant where participant-specific ID is required (personal air, dermal)

0 for all samples not associated with a specific participant

VV – Field ID number

Two-digit code unique to each field (We will use a different unique code for each field/participant group combination. We will not try to match to any previous field numbers so we can pre-print all labels. Numbers will go from 70 to 79).

W - Sample type designator

F = sample

D = duplicate sample

B = field blank

C = field control (spike)

X – Method type designator

E = field wet wipe metals

F = field wet wipe SVOCs

J = field drag sled SVOCs

Y - Sample collection location character

1, 2, or 5 for on-field wipe, sled, or dust sample location (corresponds to field sketch S1, S2, S5 locations in Figure 1)

Z – Parent/subsample designation character

We will use a value of zero (0) for all parent samples.

We will use the character L to designate laboratory QC samples.

Additional digits may be assigned if any sub-samples are generated.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Field blank (FB) and field control spikes (FC) samples will be prepared and used according to the schedule outlined in the QAPP addendum Table B-3. For storage, shipping, analysis and quantitation procedures, FB and FC samples will be prepared and treated in the same manner as the wipe samples.

9.2 FB will be deployed to monitor background contamination during storage and analysis. FB samples will be prepared by opening the container with wipe while wearing Silver Shield (drag sled and wet SVOC wipes) or nitrile (metals wipes) gloves, unfolding, folding, and placing into a labeled sample container. FB samples shall otherwise be treated in the same manner as the surface wipe samples.

9.3 FC will be deployed to assess recovery of target analytes from the wipe medium under the same storage and transportation conditions as the field samples. FC will be prepared by adding known

amounts of target compounds to matrix blanks which is stored in a sealed container. The container is shipped to the field and returned without opening/handling. It is stored under the same conditions as field collected samples.

9.4 At least one FB and FC should be included with each batch of wipe samples shipped to the EPA laboratory.

9.5 A duplicate sample will be collected for each of the three sample types at each field. Duplicates will be collected from a previously unsampled field area directly adjacent to its matching sample, using all of the same procedures that are used for the samples.

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Appendix A.

Sample Collection and COC Records for Field Surface Wipe and Drag Sled Samples

TIRE CRUMB EXPOSURE STUDY-- WIPE SAMPLING DATA AND COC SHEET

COC-13 Field Locations -- Metals & SVOC Field Wipe Samples

Study Name:		Collection Date	
TCRS			
Field ID No:		Operator	
Field Location ID	1		

Sample Chain of Custody

	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Metals	**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC	**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

TIRE CRUMB EXPOSURE STUDY-- WIPE SAMPLING DATA AND COC SHEET

COC-14 Field Locations -- Metals & SVOC Field Wipe Samples

Study Name:		Collection	
TCRS	Date		
Field ID No:			
	Operator		
Field Location ID	2		

Sample Chain of Custody

Metals				Collect Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date

Sample ID	Field notes:	
	Receipt notes:	
	Analysis notes:	

Sample ID	Field notes:	
	Receipt notes:	
	Analysis notes:	

SVOC				Collect Time	Shipped to EPA,RTP Initials	Shipped to EPA,RTP Date	Received & Stored at EPA, RTP Initials	Received & Stored at EPA, RTP Date	Analyzed Initials	Analyzed Date

Sample ID	Field notes:	
	Receipt notes:	
	Analysis notes:	

Sample ID	Field notes:	
	Receipt notes:	
	Analysis notes:	

TIRE CRUMB EXPOSURE STUDY-- WIPE SAMPLING DATA AND COC SHEET

COC-15

Field Locations -- Metals & SVOC Field Wipe Samples

Study Name:		**Collection**	
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID	**5**		

Sample Chain of Custody

	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
Metals	**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Sample ID

Field notes:
Receipt notes:
Analysis notes:

Sample ID

Field notes:
Receipt notes:
Analysis notes:

SVOC	**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**

Sample ID

Field notes:
Receipt notes:
Analysis notes:

Sample ID

Field notes:
Receipt notes:
Analysis notes:

TIRE CRUMB EXPOSURE STUDY-- WIPE SAMPLING DATA AND COC SHEET

COC-16

Metals Field Wipe Field/Lab Blanks and Controls

Study Name:		Collection								
TCRS	Date									
Field ID No:										
	Operator		Sample Chain of Custody							
Field Location ID			Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed			
Metals			Collect Time	Initials	Date	Initials	Date	Initials	Date	
Sample ID	Field notes:									
	Receipt notes:									
	Analysis notes:									
Sample ID	Field notes:									
	Receipt notes:									
	Analysis notes:									
Metals			Collect Time	Initials	Date	Initials	Date	Initials	Date	
Sample ID	Field notes:									
	Receipt notes:									
	Analysis notes:									
Sample ID	Field notes:									
	Receipt notes:									
	Analysis notes:									

TIRE CRUMB EXPOSURE STUDY-- WIPE SAMPLING DATA AND COC SHEET

COC-17

SVOC Field Wipe Field/Lab Blanks and Controls

Study Name:		Collection	
TCRS		Date	
Field ID No:			
		Operator	
Field Location ID			

					Sample Chain of Custody					
					Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOCs				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
SVOCs				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY - DRAG SLED SAMPLING DATA AND COC SHEET

COC-18

Field Locations -- SVOC Drag Sled Samples

Study Name:		Collection	
TCRS		Date	
Field ID No:			
		Operator	
Field Location ID	1		

Sample Chain of Custody

					Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC Drag Sled				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY - DRAG SLED SAMPLING DATA AND COC SHEET

COC-19

Field Locations -- SVOC Drag Sled Samples

Study Name:		Collection
TCRS	Date	
Field ID No:		
	Operator	
Field Location ID: 2		

Sample Chain of Custody

				Collect Time	Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC Drag Sled					Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY - DRAG SLED SAMPLING DATA AND COC SHEET

COC-20

Field Locations -- SVOC Drag Sled Samples

Study Name:		Collection	
TCRS	Date		
Field ID No:			
	Operator		
Field Location ID	5		

Sample Chain of Custody

					Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC Drag Sled				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY - DRAG SLED SAMPLING DATA AND COC SHEET

COC-21

SVOC Drag Sled Field/Lab Blanks and Controls

Study Name:	Collection	
TCRS	Date	
Field ID No:		
	Operator	
Field Location ID		

Sample Chain of Custody

					Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed	
SVOC Drag Sled				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
SVOC Drag Sled				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

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 (SOP) for Collection of Dermal Wipe Samples

Number: D-SED-IEMB-028-SOP-01	Effective Date:

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Kent Thomas

Signature: Date:

Approval

Name: Caroline Stevens
Title: Branch Chief, IEMB

Signature: Date:

Concurrence*

Name: Christine Alvarez
Title: NERL QA Manager

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

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

STANDARD OPERATING PROCEDURE (SOP) FOR COLLECTION OF HAND WIPE SAMPLES

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the method for collecting dermal wipe samples from a participant's skin in order to measure semi-volatile organic compounds (SVOCs) and metals on the skin for the EPA pilot tire crumb rubber research study (TCRS).

2.0 SUMMARY OF THE METHODS

Dermal, inhalation, and ingestion of dust at synthetic turf fields may represent important pathways of exposure to chemicals associated with tire crumb rubber, other synthetic field materials, and environmental dust deposited on the field. The concentrations of metals and SVOCs on the skin of TCRS participants must be measured to determine dermal exposures and to compare these to the other pathways and to biologic markers in blood and urine.

Each participant's skin will be wiped in the following manner:

Metals: Three dermal wipe samples will be collected for metal analysis from each participant following an on-field sports activity in the exposure characterization pilot study. One sample will be a hand wipe sample, the second sample will be from a defined area of the forearm, and the third sample will be collected from a defined area of the leg (either calf or thigh depending on which area had more exposed skin area during the sports activity). Wipe samples for metals will be collected from one hand, one arm, and one leg on the left side of the participant's body.

Semivolatile Organics: Three dermal wipe samples will be collected for SVOC analysis from each participant following an on-field sports activity in the exposure characterization study. One sample will be a hand wipe sample, the second sample will be from a defined area of the forearm, and the third sample will be collected from a defined area of the leg (either calf or thigh depending on which are had more exposed skin area during the sports activity). Wipe samples for SVOCs will be collected from one hand, one arm, and one leg on the right side of the participant's body.

3.0 DEFINITIONS

CDC – Centers for Disease Control and Prevention

COC – Chain-of-custody SOP – Standard operating procedure

FB – Field Blank TCRS – Tire Crumb Rubber Research Study

FC – Field Control (spiked control)

Metals – Includes both metals and the metalloid, arsenic QC – Quality Control

QAPP – Quality Assurance Project Plan

RTP – Research Triangle Park

SVOC – semivolatile organic compound (generally, compound with vapor pressure ~ 10^{-5} – 10^{-2} kilopascals)

4.0 CAUTIONS

4.1 Field staff will keep all sampling materials out of reach of children.

4.2 Standard laboratory protective gloves are required for this procedure to eliminate transfer of chemicals from the technician's hands onto the wipe media and to provide hygiene for participant contact. Nitrile gloves should be worn during sample collection for metals wipe samples. Silver Shield gloves should be worn during sample collection for SVOCs.

5.0 RESPONSIBILITIES

5.1 The EPA project staff will provide wipe media, digestion cups, and glass jars (collection materials) and deliver them to the field coordinator. EPA will provide the spiked field controls.

5.2 The field coordinator will receive the dermal wipe sample collection equipment and materials. The field coordinator will create a strategy and schedule to deploy or collect the appropriate percentage of each type of quality control (QC) samples. The field coordinator will communicate the schedule for QC samples to the field staff and distribute any additional QC sample materials. The field coordinator will distribute the collection materials to the field staff. Upon collection of the dermal wipe samples, the field coordinator will be responsible for returning the samples with their collection records and COC sheets to the EPA in Research Triangle Park (RTP), NC for analysis.

5.3 The field staff will be responsible for obtaining the collection equipment and materials from the field coordinator, collection of the dermal wipe samples, entering relevant information on the sample collection record and COC sheets (Appendix A) and returning collected hand wipe samples to the field coordinator.

6.0 MATERIALS AND REAGENTS

6.1 Wipe media for SVOCs (M.G. Chemicals, Cleanroom Twill wipes, 10 cm × 10 cm, cotton, pre-cleaned)

6.2 Wipe media for metals (Environmental Express SC 4210 (or similar) Ghost Wipes, 15 cm × 15 cm, packaged pre-moistened with deionized water)

6.3 Isopropanol, ACS Reagent Grade

6.4 Deionized water

6.5 Plastic digestion cups, (50 mL, Environmental Express P/N SC475 or equivalent)

6.6 Pre-cleaned and certified amber glass jars with Teflon-lined lids (2 oz. Straight-sided amber glass jars, I-Chem Part # 340-0060 or equivalent)

6.7 Disposable gloves (nitrile)

6.8 Disposable Silver Shield gloves

6.9 Stainless steel forceps

6.10 Stainless steel or aluminum tray

6.11 Easy to remove bandage tape (3M Nexcare 1" Gentle Paper Tape or equivalent)

6.11 Cooler

6.12 Frozen ice packs

6.13 Ink pens

6.1 Sample collection and COC record sheets

6.15 Sample ID labels

6.16 Rectangular flexible Teflon sheet template with outer dimensions of 11.5 x 19.0 cm and inner dimensions of 7.5 x 15 cm dimensions to provide a wipe surface area of 112 cm^2. Two templates are needed for each participant, one for metals wipe collections and one for SVOC wipe collections. (Note: if insufficient templates are available, a single template may be used for both metals and SVOCs by first cleaning the template with a wetted wipe).

7.0 PROCEDURES

7.1 SAMPLE COLLECTION

Sample collection will follow the procedures for 7.1.1 "metals" collection, followed by the procedures for 7.1.2 "SVOC" collection.

7.1.1 Collection of dermal wipe samples from participants for **metals**:

7.1.1.1 Timeline: Dermal wipes will be collected from each person, as soon as possible following his/her activity on a field.

7.1.1.2 Before starting sample collection, briefly describe the dermal wipe sampling procedures that will be used to the participant. Ask the participant if it is OK to collect the dermal wipe samples on their hands, arms, and legs. Make sure there is at least one other adult field team member present during the sample collection (parents of child participants may also be present if they wish).

7.1.1.3 Prior to collecting metals wipe samples, put on clean, powderless nitrile gloves and keep them on during the entire sampling period. New gloves should be worn for each participant.

7.1.1.4 For metal analysis, use wet (water) wipe material (Environmental Express, Ghost Wipe No. 4210) conforming to American Society for Testing and Materials (ASTM) E1792 requirements (ASTM-03, 2016a).

7.1.1.5 Hand wipe sample: Remove a wet wipe from the foil packet and unfold it to its full dimensions. With moderately firm pressure, wipe the left hand, including the back, front, and sides of the hand, fingers, and thumb. Next, fold the wipe with the exposed (contacted) surface on the inside and place into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage.

7.1.1.6 Forearm wipe sample: Select a clean Teflon template. Place it over the underside of the left forearm. Use a short piece of bandage tape to tape one short end of the template to the left arm, then a second short piece of tape to secure the other short end to the arm. The template should lay as flat against the arm as possible once taped down. Remove a wet wipe ("Ghost Wipe") from the foil packet and unfold it to its full dimensions. Then, fold it into quarters before beginning sample collection. With moderately firm pressure, thoroughly wipe the bottom (underside) of the left forearm over the entire 112 cm^2 area using a rectangular template. Next, fold the wipe one more time, with the exposed (contacted) surface now on the inside. With moderately firm pressure, thoroughly wipe the bottom (underside) of the left forearm over the entire 112 cm^2 area for a second time. Again fold the wipe with the exposed (contacted) surface on the inside and place into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage.

7.1.1.7: Leg wipe samples: Select the area to sample based on skin that was most exposed during the participant activity. The lower leg is preferred, but the lower part of the upper leg may be used if it was the most exposed area. Use the same Teflon template that was used on the forearm. Place the template over the outer side of the left leg. Use a short piece of bandage tape to tape one short end of the template to the leg, then a second short piece of tape to secure the other short end to the leg. The template should lay as flat against the leg as possible once taped down. Remove a wet wipe ("Ghost Wipe") from the foil packet and unfold it to its full dimensions. Then, fold it into quarters before beginning sample collection. With moderately firm pressure, thoroughly wipe the left leg over the entire 112 cm^2 area using a rectangular template. Next, fold the wipe one more time, with the exposed (contacted) surface now on the inside. With moderately firm pressure, thoroughly wipe the left leg over the entire 112 cm^2 area for a second time. Again fold the wipe with the exposed (contacted) surface on the inside and place into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage.

7.1.1.8 Record the sample collection information on the sample collection sheet (Appendix A).

7.1.1.9 Ensure all caps are tightly fitted and transport tubes at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C

7.1.2 Collection of dermal wipe samples from participants for **SVOCs**:

7.1.2.1 In the laboratory, prior to shipment of materials to the field site, each wipe will be placed in a clean 60-mL wide-mouth amber jar, and 3 mL of 1:1 isopropanol:water will be added directly to the wipe material in the jar, dispersed across the folded wipe material as evenly as possible. The jar will then be tightly capped with Teflon-lined lids. Sample labels will be affixed to the jars, and the jars will be transported to the field site.

7.1.2.2 Timeline: Dermal wipes will be collected from each person, as soon as possible following his/her activity on a field.

7.1.2.3 Prior to collecting wipe samples, put on clean, Silver Shield gloves and keep them on during the entire sampling period. New gloves should be worn for each participant.

7.1.2.4 Collect wipe samples for SVOC analysis using wetted (1:1 water:isopropanol) cotton wipe material (M.G. Chemicals, Cleanroom Twill, 10 x 10 cm).

7.1.2.5 Hand wipe sample: Remove the wipe from its glass storage jar to its full dimensions. With moderately firm pressure, wipe the right hand, including the back, front, and sides of the hand, fingers, and thumb. Next, fold the wipe with the exposed (contacted) surface on the inside and replace into its glass storage jar.

7.1.2.6 Forearm wipe sample: Select a clean Teflon template. Place it over the underside of the right forearm. Use a short piece of bandage tape to tape one short end of the template to the right arm, then a second short piece of tape to secure the other short end to the arm. The template should lay as flat against the arm as possible once taped down. Remove a wipe from its glass storage jar and unfold it to its full dimensions. Then, fold it into quarters before beginning sample collection. With moderately firm pressure, thoroughly wipe the bottom (underside) of the right forearm over the entire 112 cm^2 area using a rectangular template. Next, fold the wipe one more time, with the exposed (contacted) surface now on the inside. With moderately firm pressure, thoroughly wipe the bottom (underside) of the right forearm over the entire 112 cm^2 area for a second time. Again fold the wipe with the exposed (contacted) surface on the inside and place it back into its labeled storage jar and seal the cap tightly.

7.1.2.7: Leg wipe samples: Select the area to sample based on skin that was most exposed during the participant activity. The lower leg is preferred, but the lower part of the upper leg may be used if it was the most exposed area. Use the same Teflon template that was used on the forearm. Place the template over the outer side of the right leg. Use a short piece of bandage tape to tape one short end of the template to the leg, then a second short piece of tape to secure the other short end to the leg. The template should lay as flat against the leg as possible once taped down. Remove a wet wipe from its glass jar and unfold it to its full dimensions. Then, fold it into quarters before beginning sample collection. With moderately firm pressure, thoroughly wipe the right leg over the entire 112 cm^2 area using a rectangular template. Next, fold the wipe one more time, with the exposed (contacted) surface now on the inside. With moderately firm pressure, thoroughly wipe the right leg over the entire 112 cm^2 area for a second time. Again fold the wipe with the exposed (contacted) surface on the inside and place it back into its labeled storage jar and seal the cap tightly.

7.1.2.8 Record the sample collection information on the sample collection sheet (Appendix A).

7.1.2.9 Ensure all caps are tightly fitted onto the glass storage jars and place the jars into a cooler with frozen ice packs. Samples must be stored on ice packs, or in a refrigerator or freezer following collection. Samples must be shipped to the laboratory in a cooler with frozen ice packs. Upon receipt at the laboratory, samples are placed in a freezer at -20 °C

7.2 HANDLING AND PRESERVATION

7.2.1 After collection and during transport from the collection site, store the SVOC wipe samples in a cooler with ice packs. Wipe samples for metals analysis may remain at ambient temperatures.

7.2.2 Store pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) with metals wipe samples at ambient temperature at the field site and during shipment. (Note: metals wipe samples may be stored and shipped in a cooler at lower than ambient temperatures if that is most convenient). Wipe samples for SVOC analysis must be stored on ice packs, or in a refrigerator or freezer following collection, and shipped in a cooler on frozen ice packs to the laboratory. All tubes and jars must be tightly capped. Samples do not need to be shipped each day, and may be accumulated across sampling days at a field location prior to shipment. Upon receipt at the laboratory, wipe samples for SVOC analysis must be placed in a freezer at approximately -20 °C.

7.2.3 Ship samples and their sample collection data/COC sheets to:

US EPA Chemical Services
Kent Thomas or Scott Clifton
109 T.W. Alexander Drive
Building E Loading Dock, Rm E178
Research Triangle Park NC 27709-0002
Telephone:919-541-7939

8.0 RECORDS

A data collection system will be used to capture information associated with the collection of all samples. For the technician collected dermal wipe samples, the sample collection information to be recorded will include the following, as a minimum: the sample ID, the participant ID, the date and time of the sample collection, initials or ID number of the field staff member responsible for the sample collection, and any comments regarding collection (Appendix A). Other information shall be collected as needed to ensure successful collection and interpretation of data. *Section B3 in the QAPP addendum* details the sample code information.

The specific coding information for field wipe samples is extracted from the QAPP addendum:

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

Where:

TCRS
Designates the tire crumb rubber research study

R – Participant identification number
1 – 8; each number assigned to a unique participant where participant-specific ID is required (personal air, dermal)
0 for all samples not associated with a specific participant

VV – Field ID number

Two-digit code unique to each field (We will use a different unique code for each field/participant group combination. We will not try to match to any previous field numbers so we can pre-print all labels. Numbers will go from 70 to 79).

W - Sample type designator

F = sample
D = duplicate sample
B = field blank
C = field control (spike)

X – Method type designator

N = personal dermal sample metals
L = personal dermal sample SVOCs

Y - Sample collection location character

H for dermal samples collected from hands
A for dermal samples collected from arms
L for dermal samples collection from legs

Z – Parent/subsample designation character

We will use a value of zero (0) for all parent samples.
We will use the character L to designate laboratory QC samples.
Additional digits may be assigned if any sub-samples are generated.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Field blank (FB) and field control spikes (FC) samples will be prepared and used according the QAPP addendum Table B-3. For storage, shipping, analysis and quantitation procedures, FB and FC samples will be prepared and treated in the same manner as the hand wipe samples.

9.2 FB will be deployed to monitor background contamination during storage and analysis. The FB will consist of clean wipes that are removed from the container while wearing nitrile (metals) or Silver Shield (SVOCs) and handled and folded in the same manner as the actual dermal wipe samples, with the exception that no skin or other surface is wiped. They are then returned to their containers and shipped or driven to the EPA laboratory.

9.3 FC will be deployed to assess recovery of target analytes from the wipe media under the same storage and transportation conditions as the field samples. FC will be prepared by adding known amounts of target compounds to wipe media, sealed in a sample container which will remain unopened in the field and returned to the EPA laboratory.

9.4 At least one FB and FCS should be included with each batch of hand wipe samples shipped to the EPA laboratory.

9.5 No duplicate dermal wipe samples will be collected due to the increased participant burden and time they would add.

10.0 REFERENCES

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Quality Assurance Project Plan, Addendum for the Tire Crumb Research Study Exposure Characterization Pilot Study.

Appendix A.

Sample Collection and COC Records for Dermal Wipe Samples

TIRE CRUMB EXPOSURE STUDY-- DERMAL WIPE SAMPLING DATA AND COC SHEET

COC-24 **Participant -- Metals Dermal Wipe Samples**

Study Name:			Collection								
TCRS		Date									
Field ID No:											
		Operator			Sample Chain of Custody						
Field Location ID	P				Shipped to EPA,RTP		Received & Stored at EPA, RTP		Analyzed		
Participant ID	Location			Collect Time	Initials	Date	Initials	Date	Initials	Date	
1	Hand										
Sample ID		Field notes:									
		Receipt notes:									
		Analysis notes:									
Participant ID	Location			Collect Time	Initials	Date	Initials	Date	Initials	Date	
1	Arm										
Sample ID		Field notes:									
		Receipt notes:									
		Analysis notes:									
Participant ID	Location			Collect Time	Initials	Date	Initials	Date	Initials	Date	
1	Leg										
Sample ID		Field notes:									
		Receipt notes:									
		Analysis notes:									

NOTE: There will be eight of these sheets, one for each participant, with a different participant ID number from 1 – 8, with COC sheet ID numbers from COC-24 to COC-31.

TIRE CRUMB EXPOSURE STUDY-- DERMAL WIPE SAMPLING DATA AND COC SHEET

COC-32

Metals Dermal Wipe Field/Lab Blanks and Controls

Study Name:		**Collection**	
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID	**P**		

Sample Chain of Custody

				Shipped to EPA,RTP		**Received & Stored at EPA, RTP**		**Analyzed**	
Metals			**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID	**Field notes:**								
	Receipt notes:								
	Analysis notes:								
Sample ID	**Field notes:**								
	Receipt notes:								
	Analysis notes:								
Metals			**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID	**Field notes:**								
	Receipt notes:								
	Analysis notes:								
Sample ID	**Field notes:**								
	Receipt notes:								
	Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- DERMAL WIPE SAMPLING DATA AND COC SHEET

COC-33 — **Participant -- SVOC Dermal Wipe Samples**

Study Name:			**Collection**								
TCRS		**Date**									
Field ID No:											
		Operator			**Sample Chain of Custody**						
Field Location ID	**P**				**Shipped to EPA,RTP**		**Received & Stored at EPA, RTP**		**Analyzed**	
Participant ID	**Location**			**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
1	Hand									
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Participant ID	**Location**			**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
1	Arm									
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Participant ID	**Location**			**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
1	Leg									
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

NOTE: There will be eight of these sheets, one for each participant, with a different participant ID number from 1 – 8, with COC sheet ID numbers from COC-33 to COC-40.

TIRE CRUMB EXPOSURE STUDY-- DERMAL WIPE SAMPLING DATA AND COC SHEET

COC-41

SVOC Dermal Wipe Field/Lab Blanks and Controls

Study Name:		**Collection**	
TCRS		**Date**	
Field ID No:			
		Operator	
Field Location ID	**P**		

Sample Chain of Custody

					Shipped to EPA,RTP		**Received & Stored at EPA, RTP**		**Analyzed**	
SVOC				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
SVOC				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

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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 (SOP) for Collection of Dust Samples from Synthetic Turf Fields

Number: D-SED-IEMB-029-SOP-02	Effective Date: September 7, 2017

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification:

SOP Steward

Name: Kent W. Thomas

Signature:

Approval

Name: Caroline Stevens
Title: Branch Chief, NERL/SED/IEMB

Signature: Date:

Concurrence*

Name: Christine Alvarez
Title: NERL QA Manager

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

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

STANDARD OPERATING PROCEDURE (SOP) FOR COLLECTION OF FIELD DUST SAMPLES FROM SYNTHETIC TURF FIELDS

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the method for collecting dust from synthetic turf field surfaces to measure semivolatile organic compounds (SVOCs) and metals for the United States Environmental Protection Agency (EPA) exposure characterization pilot tire crumb rubber research study (TCRS).

2.0 SUMMARY OF THE METHOD

Dermal, inhalation, and ingestion of dust at synthetic turf fields may represent important pathways of exposure to chemicals associated with tire crumb rubber, other synthetic field materials, and environmental dust deposited on the field. The concentrations of metals and SVOCs on field surfaces available for dermal transfer must be measured to determine human exposures and to compare these to the various exposure pathways and to biologic markers in blood and urine.

Dust samples for SVOCs and metals analysis will be collected at synthetic turf field sites by on-field sieving of bulk dust collected as a composite from three locations on the field using a 120 mesh (150 μM) stainless steel sieves.

3.0 DEFINITIONS

COC – Chain-of-custody

EPA – Environmental Protection Agency

FB – Field Blank

FC – Field Control spiked with target analytes

Metals – Includes both metals and the metalloid, arsenic

QAPP – Quality Assurance Project Plan

QC – Quality Control

RTP – Research Triangle Park

SOP – Standard operating procedure

SVOC – semivolatile organic compound (generally, a compound with vapor pressure = 10^{-5} – 10^{-2} kilopascals)

TCRS – Tire Crumb Rubber Research Study

4.0 CAUTIONS

4.1 Nitrile gloves and eye protection should be worn during sample collection for dust samples for metals and SVOCs.

4.2 Collect samples at times when it is safe to do so with regard to any activities occurring on the field. Sample collection time is not critical for these samples, but the samples should be collected at a

convenient time during the overall exposure measurement activities at each field.

4.3 No photography will be performed of any activities at the fields.

5.0 RESPONSIBILITIES

5.1 The EPA project staff will prepare the dust sample collection equipment and materials and deliver them to the field coordinator. EPA will provide the spiked field controls.

5.2 The field coordinator will receive the dust sample collection equipment and materials. The field coordinator will create a strategy and schedule to deploy or collect the appropriate percentage of each type of field dust quality control (QC) samples as defined in the QAPP addendum. The field coordinator will communicate the schedule for QC samples to the field staff and distribute any additional QC sample materials. The field coordinator will distribute dust sample collection equipment and materials to the field staff. Upon collection of the field dust samples, the field coordinator will be responsible for returning the samples with their sample collection records and Chain-of-custody (COC) sheets to the EPA in Research Triangle Park (RTP), NC for analysis.

5.3 The field staff will be responsible for obtaining the collection equipment and materials from the field coordinator, collection of the dust samples, entering relevant information on the sample collection record sheets and COC, and returning collected dust samples and records to the field coordinator.

6.0 MATERIALS AND REAGENTS

6.1 Stainless steel sieve assembly, including 120 mesh (150 μM) screen, stainless steel lid, and stainless steel pan for SVOCs and metals dust collection (U.S. Standard stainless steel 12” x 3.25” or equivalent, pre-cleaned with deionized/carbon-filtered water and acetone and hexane rinse)

6.2 40-mL glass jars with Teflon-lined lids, pre-cleaned with acetone and hexane rinse

6.3 Plastic digestion cups, (50 mL, Environmental Express P/N SC475 or equivalent)

6.4 Disposable polypropylene spatula (6 per field in case of breakage)

6.5 Glass and/or metal funnel

6.6 Plastic funnel

6.7 Disposable nitrile gloves

6.8 Protective glasses (safety glasses or sunglasses)

6.9 Stainless steel tweezers or forceps

6.10 40-mL glass jars holding 200, 300, and 400 mg of dust for visual comparison standards

6.11 Frozen ice packs

6.12 Cooler

6.13 Ink pen with black ink

6.14 Sample collection and COC record sheets

6.15 Sample ID labels

6.16 Synthetic bristle brushes

7.0 PROCEDURES

7.1 SAMPLE COLLECTION

Field dust samples will be collected from synthetic turf fields to support characterization of chemical constituents.

7.1.1 Identification of Field Sampling Location

Samples will be collected across three locations at each field (see Figure 1). Samples will be collected as a composite by successive collections at the three locations. Separate samples will be collected for metals and SVOCs. It is important that the surface SVOC, surface metals, drag sled SVOC, and dust samples should not be collected from the exact same spots on the field. They should be collected in proximity to each other at the three locations but their sampling areas should not overlap. Ensuring that there is no area overlap can be accomplished by placing surface wipe templates and marking the drag sled area prior to sampling.

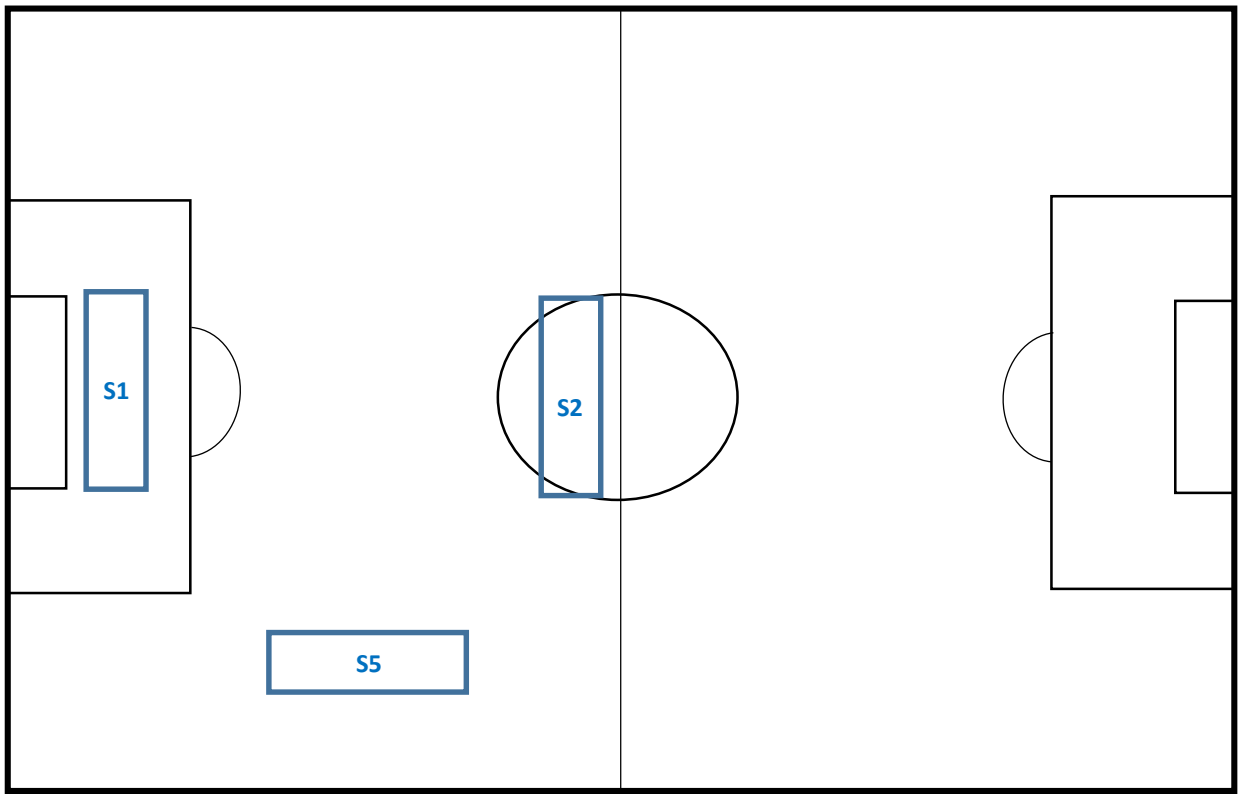

Figure 1. Field dust sample collection locations on athletic fields.

7.1.2 Gloves and Glasses

Prior to collecting metals field dust samples, put on clean, powderless nitrile gloves and keep them on during the entire sampling period. Put on protective glasses to protect against rubber particles that might be launched during collection.

7.2 Field Dust Samples for Metals

7.2.1 Composite samples will be collected across positions #1, #2, and #5 as shown in Figure 1, by successive collection and sieving of tire crumb rubber at each location. No background sampling location dust sample will be collected.

7.2.2 Remove a new plastic spatula from its wrapper.

7.2.3 Bring the 120 mesh (150 µm) stainless steel sieve and the spatula to the first sampling location.

7.2.4 Attach the receiving pan part of the sieve assembly onto the sieve screen.

7.2.5 Use the spatula to scoop crumb rubber from the field onto the sieve screen, filling the sieve to approximately 50% capacity.

7.2.6 Place the lid on the sieve screen and shake laterally in a vigorous manner for approximately two minutes (shaking can be started and stopped as needed for rest, and more than one person may contribute to the shaking).

7.2.7 Avoid getting large rubber pieces or other materials into the pan; if large pieces enter the pan, remove them with tweezers.

7.2.8 Remove the lid and sieve screen and dump the bulk rubber from the screen back onto the field from where it was collected. Do not dump the dust collected in the pan.

7.2.9 Re-distribute the rubber back into the field (this may be done after collection at each location or after all sampling has been completed).

7.2.10 Move to field sample location #2 on the field and repeat steps 7.2.4 through 7.2.8.

7.2.11 Move to field sample location #5 on the field and repeat steps 7.2.4 through 7.2.8.

7.2.12 Following sieving at the final location, remove the pan from the screen and while tilting the pan slightly use one hand to bang the side of the pan to bring as much dust as possible to one edge of the pan (note that due to static electricity it will not be possible to dislodge all dust adhering to the pan).

7.2.13 Place the plastic funnel above the open plastic digestion cup and carefully tap as much dust as possible from the pan, through the funnel, and into the digestion cup (note that due to static electricity it will not be possible to dislodge all dust adhering to the pan). A stand or tube holder may be used to hold the tube during this process, or a second person may assist in the operation. Tightly cap the plastic digestion cup after as much dust is transferred as possible.

7.2.14 If visible dust remains adhered to the pan, a synthetic bristle brush may be used to brush collected dust into one edge of the pan, and then through the funnel into the plastic digestion cup.

7.2.15 Visually evaluate the amount of dust collected. The goal is to collect approximately 300 mg or more of dust, with a minimum of approximately 200 mg. Sample amounts will be estimated by visual comparison to containers holding approximately 200, 300, and 400 mg of dust.

7.2.16 If the amount of collected dust is not 200 mg or greater based on visual comparison to the standards, it will be necessary to repeat sample collection across the three locations until at least 200 mg is collected.

7.3 Field Dust Samples for SVOCs

7.3.1 Composite samples will be collected across positions #1, #2, and #5 as shown in Figure 1, by successive collection and sieving of tire crumb rubber at each location. No background sampling location dust sample will be collected.

7.3.2 Remove a new plastic spatula from its wrapper (or the original spatula used for metals can be used).

7.3.3 Bring the 120 mesh (150 µm) stainless steel sieve to the first sampling location. Note that the same sieve used for metals sample dust collection may be used for the SVOC dust sample collection.

7.3.4 Attach the receiving pan part of the sieve assembly onto the sieve screen.

7.3.5 Use the spatula to scoop crumb rubber from the field onto the sieve screen, filling the sieve to approximately 50% capacity.

7.3.6 Place the lid on the sieve screen and shake laterally in a vigorous manner for approximately two minutes (shaking can be started and stopped as needed for rest, and more than one person may contribute to the shaking).

7.3.7 Avoid getting large rubber pieces or other materials into the pan; if large pieces enter the pan, remove them with tweezers.

7.3.8 Remove the lid and sieve screen and dump the bulk rubber from the screen back onto the field from where it was collected; do not dump the dust collected in the pan.

7.3.9 Re-distribute the rubber back into the field (this may be done after collection at each location or after all sampling has been completed).

7.3.10 Move to field sample location #2 on the field and repeat steps 7.2.4 through 7.2.8.

7.3.11 Move to field sample location #5 on the field and repeat steps 7.2.4 through 7.2.8.

7.3.12 Following sieving at the final location, remove the pan from the screen and while tilting the pan slightly use one hand to bang the side of the pan to bring as much dust as possible to one edge of the pan (note that due to static electricity it will not be possible to dislodge all dust adhering to the pan).

7.3.13 Place the metal or glass funnel above the open glass sample jar and carefully tap as much dust as possible from the pan, through the funnel, and into the jar (note that due to static electricity it will not be possible to dislodge all dust adhering to the pan). A stand or tube holder may be used to hold the tube during this process, or a second person may assist in the operation. Tightly cap the jar after as much dust is transferred as possible.

7.3.14 If visible dust remains adhered to the pan, a synthetic bristle brush may be used to brush collected dust into one edge of the pan, and then through the funnel into the glass sample jar.

7.3.15 Visually evaluate the amount of dust collected. The goal is to collect approximately 300 mg or more of dust, with a minimum of approximately 200 mg. Sample amounts will be estimated by visual comparison to containers holding approximately 200, 300, and 400 mg of dust.

7.3.16 If the amount of collected dust is not 200 mg or greater based on visual comparison to the standards, it will be necessary to repeat sample collection across the three locations until at least 200 mg is collected.

7.4 HANDLING AND PRESERVATION

7.4.1 Complete the sample collection records and COC records for the samples (Appendix A).

7.4.2 After collection and during transport from the collection site, store the dust samples in a cooler with frozen ice packs.

7.4.3 Store the samples on frozen ice packs or in a refrigerator or freezer until shipment. Ship samples by overnight delivery service in a shipping cooler with frozen ice packs.

7.4.4 Ship samples and their sample collection data/COC sheets to:

US EPA Chemical Services
Kent Thomas or Scott Clifton
109 T.W. Alexander Drive
Building E Loading Dock, Rm E178
Research Triangle Park NC 27709-0002
Telephone:919-541-7939

8.0 RECORDS

A data collection system will be used to capture information associated with the collection of all samples. For the dust samples, the sample collection information to be recorded will include the following, as a minimum: the sample ID, the date and time of the sample collection, the sampling location, initials or ID number of the field staff member responsible for the sample collection, and any comments regarding collection (Appendix A). Other information shall be collected as needed to ensure successful collection and interpretation of data. Please see D-SED-IEMB-030-SOP-01 for recording sample collection locations on the proper field diagram.

Section B3 in the QAPP addendum details the sample code information. Sample codes used for the EPA Tire Crumb Rubber Research Study will follow the general naming scheme used by the EPA for the tire crumb rubber characterization study.

The specific coding information for field dust samples is extracted from the QAPP addendum:

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

Where:

TCRS
Designates the tire crumb rubber research study

R – Participant identification number
1 – 8; each number assigned to a unique participant where participant-specific ID is required (personal air, dermal)
0 for all samples not associated with a specific participant

VV – Field ID number
Two-digit code unique to each field (We will use a different unique code for each field/participant group combination. We will not try to match to any previous field numbers so we can pre-print all labels. Numbers will go from 70 to 79).

W - Sample type designator
F = sample
D = duplicate sample
B = field blank
C = field control (spike)

X – Method type designator
I = field dust metals
K = field dust SVOCs

Y - Sample collection location character
C for composite dust samples collected over locations 1, 2, and 5

Z – Parent/subsample designation character

We will use a value of zero (0) for all parent samples.
We will use the character L to designate laboratory QC samples.
Additional digits may be assigned if any sub-samples are generated.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Field blank (FB) and field control spikes (FC) samples will be prepared and used according to the schedule outlined in the QAPP addendum Table B-3. For storage, shipping, analysis and quantitation procedures, FB and FC samples will be prepared and treated in the same manner as the field dust samples. (This step assumes that suitable dust surrogate material can be found for metals blanks and spiked controls. Diatomaceous earth will be used for SVOC blanks and spiked controls).

9.2 FB will be deployed to monitor background contamination during storage and analysis. FB samples will be shipped to the field, their caps will be opened and then immediately closed, and returned to the laboratory with the samples. FB samples shall otherwise be treated in the same manner as the field dust samples.

9.3 FC will be deployed to assess recovery of target analytes from a dust surrogate medium under the same storage and transportation conditions as the field samples. FC will be prepared by adding known amounts of target compounds to surrogate dust material which is stored in a sealed container. The container is shipped to the field and returned without opening/handling. It is stored under the same conditions as field collected samples.

9.4 At least one FB and FC should be included with each batch of dust samples shipped to the EPA laboratory.

9.5 No duplicate samples will be collected.

Appendix A.

Sample Collection and COC Records for Field Dust Samples

TIRE CRUMB EXPOSURE STUDY-- FIELD DUST SAMPLING DATA AND COC SHEET

COC-50 — **Field Locations -- Metals & SVOC Field Dust Samples**

Study Name:		**Collection**	
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID	**1 & 2 & 5**		

Sample Chain of Custody

					Shipped to EPA,RTP		**Received & Stored at EPA, RTP**		**Analyzed**		
Metals				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
		Field notes:									
Sample ID		**Receipt notes:**									
		Analysis notes:									
SVOC				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
		Field notes:									
Sample ID		**Receipt notes:**									
		Analysis notes:									

TIRE CRUMB EXPOSURE STUDY-- FIELD DUST SAMPLING DATA AND COC SHEET

COC-51

Metals Field Dust Field/Lab Blanks and Controls

Study Name:		**Collection**	
TCRS	**Date**		
Field ID No:			
	Operator		
Field Location ID			

Sample Chain of Custody

					Shipped to EPA,RTP		**Received & Stored at EPA, RTP**		**Analyzed**	
Metals				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Metals				**Collect Time**	**Initials**	**Date**	**Initials**	**Date**	**Initials**	**Date**
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								
Sample ID		**Field notes:**								
		Receipt notes:								
		Analysis notes:								

TIRE CRUMB EXPOSURE STUDY-- FIELD DUST SAMPLING DATA AND COC SHEET

COC-52

SVOC Field Dust Field/Lab Blanks and Controls

			Collection							
Study Name:		Date								
TCRS										
Field ID No:										
		Operator		Sample Chain of Custody						
Field Location ID				Shipped to EPA,RTP			Received & Stored at EPA, RTP		Analyzed	
SVOCs				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
SVOCs				Collect Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

U.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurement Division
Sensing and Spatial Analysis Branch

STANDARD OPERATING PROCEDURE

SOP Title: PROCEDURE FOR THE COLLECTION OF PARTICULATE MATTER (PM) AIR SAMPLES AT ACTIVITY FIELDS CONSTRUCTED USING CRUMB RUBBER

SOP ID: D-EMMD-SSAB-007-SOP-01	Effective Date: 08/21/17

SOP was Developed ☒ In-house ☐ Extramural

SOP Discipline: Field Collection

Alternative Identification:

SOP Contact Signature

Name: Ron Williams

Title: Author

Signature/Date: RONALD WILLIAMS

Digitally signed by RONALD WILLIAMS
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=RONALD WILLIAMS, dnQualifier=0000013874
Date: 2017.08.22 08:11:26 -04'00'

Management Signature

Name: Chandra Giri

Title: SSAB Chief

Signature/Date: CHANDRA GIRI

Digitally signed by CHANDRA GIRI
Date: 2017.08.21 13:50:36 -04'00'

QA Signature

Name: Margie Vazquez
Title: EMMD QA manager

Signature/Date: MARGARITA VAZQUEZ

Digitally signed by MARGARITA VAZQUEZ
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=MARGARITA VAZQUEZ, dnQualifier=0000018558
Date: 2017.08.21 11:39:22 -04'00'

TITLE: **PROCEDURE FOR THE COLLECTION OF PARTICULATE MATTER (PM) AIR SAMPLES AT ACTIVITY FIELDS INVOLVING TIRE CRUMB RUBBER**

SOURCE: USEPA
NERL/EMMD/SSAB
109 TW Alexander Dr., MD-E205-04
Research Triangle Park, NC 27709

AUTHOR(s):

for Williams Date: 8/21/2017
Ron Williams

Approved BY:

SSAB Branch Chief _____ Date:_____
Chandra Giri

QA Manager _____ Date:_____
Sania Tong-Argao

Notice

This Analytical Procedure has been prepared for use by the Sensing and Spatial Analysis Branch of the U.S. Environmental Protection Agency, Research Triangle Park, North Carolina and may not be specifically applicable to the activities or objectives of other organizations. This procedure has not been fully validated and should be used for research purposes only. Adequate QA/QC measures must be implemented with this procedure to allow assessment of data quality.

Revision History

Version No.	Name	Date of Revision	Description of Change(s)
1	Ron Williams	08/21/17	Original version

PROCEDURE FOR THE COLLECTION OF PARTICULATE MATTER (PM) AIR SAMPLES AT ACTIVITY FIELDS CONSTRUCTED USING CRUMB RUBBER

TABLE OF CONTENTS

1.0 Scope and Application

This method applies to the collection of particulate matter (collected as total suspended particulate or TSP) from outdoor air at activity fields that utilize crumb rubber and/or artificial turf. Samples will be analyzed for particle mass and metals.

2.0 Summary of Method

Portable battery operated air sampling pumps equipped with Harvard Impactor (HI) particulate matter (PM) without size selective inlets will be used to collect particle phase outdoor ambient air samples. Particulates will be collected on pre-weighed Teflon filters.

3.0 Prerequisites

- Tared Teflon filters (37 mm, 2.0 μ) prepared in advance of their needed deployment time period

3.1 Equipment and Supplies

NERL (National Exposure Research Laboratory) prepared/supplied items:

- Clean lab supplies (e.g. forceps, plastic Petri dishes, Harvard Impactors, SV-30 pumps, calibrated BIOS flow meters etc.)

- Harvard Impactors (HI), 20 LPM, pre-cleaned, pre-oiled
- HI filter cassettes with tared Pallflex 37 mm 2 μm porosity Teflon filter and Millipore AP 1003700 cellulose backing filter
- 12V DC, 18 Ah sealed lead acid battery or equivalent
- SKC HV-30 air sampling pump with inlet stand and security box
- Protective enclosure
- BIOS International Dry Cal-DC Lite air flow calibrator or equivalent, high flow detection cell
- Powder-free nitrile gloves
- Tygon flexible plastic vacuum tubing (½ inch OD, 5/16 inch ID, 3/16 inch wall)
- Sampling labels
- Screwdriver for adjusting air pump flow rate
- Zip lock bags for storage/shipment of new or used filter cassettes
- Teflon tape
- HI flow calibration cap
- Data collection forms

Field Personnel supplied items:

- Time piece, synchronized to local cell towers
- Pen (Black, Permanent Ink)

3.2 Training Requirements

All training required is provided by the US EPA. Sample collection should be conducted by qualified scientific staff trained in the use of the specific field monitoring equipment. If sampling is conducted by non-scientists, a scientist should oversee the monitoring effort. Training should include a demonstration and hands-on training by qualified persons with air sampling expertise. At a minimum, all standard operating procedures (SOPs) and operating instructions should be reviewed, understood, and followed exactly by the field staff.

4.0 Cautionary Notes or Special Considerations

The sampling systems are powered by sealed lead acid batteries (gel cell). The battery and pump system should be protected from excessive heat or cold (<50 °F, >104 °F). Pumps and batteries are shielded from direct exposure by means of an outer protective enclosure. This enclosure should be used at all times. Precautions must be taken against shorting across the battery terminals or reversing polarity of the power leads. Shorting of the battery may cause a rapid discharge that will generate excessive heat and may result in a fire or severe burns. Reversing the polarity (attaching positive to negative) may damage the pump or battery. Red (+) wires should be attached to the red (+) battery terminal and black (-) wires should be attached to the black (-) terminal.

Sampling will take place outside and equipment should be shielded from possible weather events. Sampling stands should to be set-up so that they are provided protection from tampering. Sampling stands must also be placed and secured such that no harm will come to children or others playing in the vicinity of the equipment. Protective enclosures are provided and must be used to insure safety.

5.0 Procedures

5.1 Loading Filters in the Harvard Impactors for use

5.1.1 In a clean laboratory space, unpack the materials shipped by the NERL-EMMD. Contact Ron Williams (919-541-2957) if any of the materials defined in the supplies list are missing or if you wish to use an alternative supply.

5.1.2 While wearing powder-free gloves, install one HI cassette into each HI sampler as defined by the HI Research Operating Procedure (ROP #07-DEARS).

Note: Since the filters are pre-weighed (tared) it is imperative that the filter Ids be maintained throughout the handling process so filter loadings can be accurately determined. See Section 5.2 for labeling instructions.

- This must be done in a low traffic area, where dust is kept to a minimum. A laminar flow hood would fit this requirement.

- Install the cassette with the indicated "top" or "up" side facing the top inlet of the sampler. Be sure that all of the red rubber seals securing the cassette are in their proper positions.
- Close and seal the HI units by use of the two metal catches located on each unit. Insure that the alignment pins are engaged and that the base and inlet sections are parallel prior to snapping the latches closed.
- Keep the zip lock bag that the HI cassette came in.

Note: These bags are labeled with the Id number of the tared filter. The filter and cassette must be returned to the appropriately labeled zip lock bag after monitoring.

5.2 Labeling the Sample

Note: Since the filters are pre-weighed (tared) it is imperative that the filter Ids be maintained throughout the handling process so filter loadings can be accurately determined when post-weighed.

- Prepare three labels for each sample using the coding system described below:

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

Where:

TCRS
Designates the tire crumb rubber research study

R – Participant identification number
1 – 8; each number assigned to a unique participant where participant-specific ID is required (personal air, dermal)
0 for all samples not associated with a specific participant

VV – Field ID Number

Two-digit code unique to each field (We will use a different unique code for each field/participant group combination. Previously used field numbers will not be duplicated so we can pre-print all labels. Numbers will go from 70 to 79).

W - Sample type designator
F = sample
D = duplicate sample
B = field blank
C = field control (spike)

X – Method type designator
A = field air PM/metals

Y - Sample collection location character
1 or 2 for on-field air location; 8 for off-field air location

Z – Parent/subsample designation character
We will use a value of zero (0) for all parent samples.
We will use the character L to designate laboratory QC samples.

- Place one label on the zip lock bag and a second one on the HI unit base at the time of transfer of the filter cassette from the zip lock bag to the HI unit.
  Note: Make sure the three-digit filter code on the zip lock bag matches the three digit filter code in the full sample code. This number tracks the tared filter through the post weighing
- Place the assembled HI unit in a clean large zip lock bag for transport to the monitoring location.
- Place the third label on the data collection sheet that will be used to record the collection information. (See Appendix I for data collection sheet format). (Note: as an option, the TCRS sample code may be pre-entered on the sheet instead of using a label).

5.3 Pump Set-up

5.3.1 Transport the assembled HI unit along with their air sampling pump boxes to the monitoring location along with the data collection sheets, BIOS calibration unit, calibration cap, GPS unit and any materials needed to secure the monitoring site.

5.3.2 One pump will be set-up at each monitoring location associated with each site with one exception. A single location will have duplicate monitoring systems so that precision can be established. The pump set up will be identical for each sampling filter type.

5.3.3 Select a site that will enable you to place the two near-field sampling stations as near to the field as safety allows, on different sides of the field (possibly adjacent sides) and in a downwind position where possible or applicable.

- Because some fields might be irregular in shape, some compromises might have to be afforded to the siting of the stations relative to perfect placement.
- Each pump box should be placed as flat on the ground as possible, even so, the units will operate in any angle of deflection. An upright (0^{o} angle is best relative to the HI units built-in weather shielding).
- Draw the locations on the site map (using black permanent ink) (see D-SED-IEMB-005-SOP-01 meta-data collection SOP) with sufficient detail to

indicate relative positions of each sampling box, their distance from the field and any other details worth noting (position of any nearby trees or playground equipment, roadways, etc.).

- Secure the metal rod to the HI air pump system.
- Attach the ring to the rod at the indicated mark.
- Open the pump box, remove the tubing and connect one end to the hose barb found at the base of the HI unit and the other end to the barb on the right rear of the pump unit. Remove any kinks in the hose.
- Slide the hose sufficiently onto the barb so that it is secure (a minimum of 3/8th of an inch). It may be necessary to lightly dampen the tubing to aide with sliding the tubing onto the barbs.
- The attached hose should be snug and not easily removed without use of applied force.
- Secure the HI unit upside down on the metal rod by inserting the small end of the impactor head downward through the opening of the ring with the base resting upside down on the ring. Verify the inlet opening of the impactor is 1.0 ± .1 meter from the ground. Alternatively, a finger clamp may be used to secure the HI unit.
- Attach the battery power adapter to the pump unit by inserting the adapter into the 12V input jack on the right rear of the pump unit.
- Attach the battery adapter leads to the appropriate terminals of the battery. Attach the red lead to the positive (+) red terminal and the black lead to the negative (-) black terminal. Make sure good contact is made.
- Turn on pump by sliding the on/off switch located on the pump deck to the "on" position.

Note: The pump flow rate should be "roughed in" to 20 LPM prior to attaching the HI unit. This will minimize the amount of time required to fine adjust the pump flow after the HI unit is attached.

5.4 Initial Pump Flow Measurement for Sample Start

5.4.1 Allow the pump to run for 3-5 minutes to warm-up and stabilize.

5.4.2 Attach the HI calibration cap to the Bios DryCal Calibrator using the supplied tubing. Secure the tubing to the bottom (outlet) barb on the calibrator and to the barb on the calibrator cap.

5.4.3 Press the "on" button of the BIOS unit.

5.4.4 Press and hold the "Read" button for approximately 3 seconds. This will activate the unit to make continuous measurements and automatically average 3 replicate readings.

5.4.4 Remove the top inlet portion of the HI unit by firmly grasping the red center portion with one hand and with the other hand firmly grasp the top inlet portion and gently twist and pull the top off. Do not block the inlet openings during this process. Place

the inlet on a clean surface (zip lock bag) to avoid contamination.

5.4.5 Attach the HI calibration cap to the HI unit by gently pressing the top onto the red portion of the HI unit. A slight twisting motion may be necessary to allow the calibration cap to slide over the O-rings. Be careful not to cut or tear the O-rings during this process.

5.4.6 Observe the flow rate values being displayed.

5.4.7 Adjust the flow rate to 20.0 ± 1.00 LPM if necessary. While observing the calibrator display, use a screwdriver to adjust the flow adjustment screw to adjust the flow rate. Adjustment should be made by gently turning the screw clockwise or counterclockwise to increase or decrease the pump flow rate. Make adjustments in partial turns.

5.4.8 Once adjusted to 20.0 ± 1.0 LPM allow the flow to stabilize for one minute, reset the calibrator by pressing the "Stop" button followed by holding the "Read" button for three seconds. After three readings, record the average flow rate on the datasheet.

5.4.9 Without turning the pump off, immediately remove the calibration cap.

5.4.10 Re-attach the HI inlet to the HI unit.

5.4.12 Record the time and date of the flow measurement in the appropriate section of the datasheet.

5.4.13 Record the Teflon filter ID number, attached to the outside of the HI unit, in the appropriate section of the datasheet

5.4.14 Secure pump and place protective enclosure around pump system to prevent tampering and for safety reasons.

5.5 Final Pump Flow Measurement for Sample End

5.5.1 After sampling for the desired time period, presumed to be ~ three-hour duration, attach the BIOS calibrator to the HI unit and measure the flow rate at the end of monitoring period.

5.5.2 Repeat steps 5.4.1through 5.4.6

5.5.3 Do not adjust the pump flow rate at the end of monitoring, simply record the average value of three measurements as the ending flow rate on the datasheet.

5.5.4 Immediately turn off the HV-30 pump using the on/off switch on the pump deck.

5.5.5 Record the date and time that the pump was turned off on the datasheet.

5.5.6 Remove the BIOS calibration cap from the HI and replace it with its HI inlet.

5.5.7 Remove the tubing from the HI unit and place the HI unit in a zip lock bag and seal. Ensure that the HI unit is returned to the zip lock bag with the correct sample code label.

5.5.8 Transport the HI unit to a clean environment for removal of the filter cassette.

5.6 Pump/Equipment Take Down

5.6.1 Disconnect the tubing from the pump unit.

5.6.2 Disconnect the battery leads from the battery and remove the adapter from the pump.

5.6.3 Pack all supplies and prepare for departure.
5.6.4 Transport/ship all supplies to the laboratory.

5.7 Removal of Filters from HI Units

5.7.1 In the same clean environment used in **5.1**, remove the HI units from their zip lock bags.
5.7.2 Wearing the powder-free gloves, open the HI units and remove the filter cassette, returning it to the previously-assigned zip lock bag.

Note: The bags are labeled with the Id number of the tared filter. The filter and cassette must be returned to the appropriately labeled zip lockbag after monitoring.

5.7.3 Wipe down all of the equipment that went to the field using lint-free laboratory wipes that are just slightly moistened to remove immediately transferrable dust. Repackage all of the materials into their original shipping containers and prepare the necessary shipping paperwork. All materials should be returned to: Ron Williams, US EPA, 4930 Page Road, Durham, NC, 27703 (919-541-2957) using next day Fed Ex or similar overnight delivery service. The kits will contain sealed gel cell batteries and the NERL Safety and Health Office (541-4307) can assist with any specialized shipping papers.

5.8 TSP (Total Suspended Particulate) Option

In the event that TSP (total suspended particulate) measurements are being performed (no $PM_{2.5}$ size fractionation required), the sampler shall be operated with the oiled impactor stage removed from the HI. This is how the sampler will be used in support of the Tire Crumb Rubber Study. This is accomplished by separating the inlet from the impactor stage, removing the oiled impactor insert and reassembly of the HI. All other aspects of the sampling protocol remain as for the $PM_{2.5}$ sample collection (filter selection, duration, gravimetric analysis, etc). It should be noted that TSP mass collection is not dependent upon a 20 LPM flow rate. It is important to establish an initial flow rate and a final flow rate with the average being the flow rate used to calculate total collected air volume. The HI should be operated in a vertical orientation (inlet facing skywards) if TSP operation is to be used. No inlet is needed under TSP sampling conditions. An initial flow rate in the range of 15-25 LPM is acceptable. The HV-30 pumps have an upper limit of 30 LPM so operating the TSP version of the HIs at 20 LPM is suggested but not a requirement.

6.0 Quality Control

The quality control requirements will allow assessment of the quality of the samples collected. Determination of possible contamination and reproducibility of the method will be targeted as data quality indicators.

6.1 Field Blanks

- There should be at least one field blank performed for each day of air monitoring. All field blanks should be assigned as a sample type code B. This sample uses the same labeling code as described in Section 5.2.
- These should be installed within a HI, carried to the field (but not connected to the sampling box nor having any air pulled through them).

6.2 Duplicate Samples

Duplicate samples shall be taken at a single location during each day of field measurement. Two sampling systems (pump/inlets) shall be positioned within 2 meters of each other and operated as specified in Section 5. The purpose of duplicate samples being the determination of precision for the sampling method in its entirety.

6.3 BIOS Flow Calibrator Calibration

The BIOS calibrator, as previously described, will be used to determine initial and final flows so that an average flow rate can be determined. This device has been calibrated annually by the manufacturer and no adjustment of this device is needed. Review the calibration date on the BIOS to ensure it has been calibrated within the last one year period.

7.0 General Sampling Precautions

Samplers must be placed in an area representative of the average ambient conditions. HI units should be kept in sealed bags until just prior to the start of the sampling period. Pumps should never be operated without a particulate filter in-line or with the sampling train sealed. Pumps should be calibrated against the reference BIOS. Calibration should take place just prior to and immediately after sampling. The average of initial and final flow rates will be used in conjunction with the total elapsed sampling time to calculate total air volumes sampled and integrated analyte concentrations available during the capture period. Air volume calculations will be performed at a later stage and will not be performed as part of the field sample collection.

8.0 Possible Corrective Actions for Observed Problems During Sampling

8.1 Pump Failure

- If a pump fails, correct any obvious errors such as kinked lines, battery not fully charged. If possible, replace the pump.
- If a replacement pump is unavailable, stop data collection immediately and contact the EPA's tire crumb rubber study lead (Kent Thomas @ 9195417939) for instructions. Examples of pump failure include: failure to reach desired flow rate; or failure to maintain the desired flow rate.

8.2 Possible Contamination of Filters or Supplies

Contact the EPA's tire crumb rubber study lead (Kent Thomas @9195417939) for instructions concerning possible replacement items or directions concerning decontamination procedures.

9.0 Recordkeeping

9.1 Data Sheets

All data concerning the data collection will be recorded by the appropriate operator on the Sample Collection and COC Data Sheets shown in Appendix I. After the data is collected, copies of the original shall be made and these copies will be labeled and stored in a separate notebook for safekeeping. The originals are to be returned to the NERL-EMMD supervising scientist along with the collected samples.

9.2 Calculations

Flow rates are direct readings from the BIOS. Average flow rate is the sum of the initial and final flow rate divided by 2. The elapsed minutes is the sum total of minutes the pump operated during the sampling episode. A normal 3 hour run period should have approximately 3 hours X 60 min/hour = 180 minutes.

9.3 Chain-of-Custody

Chain of Custody (COC) record forms are shown in Appendix I for samples and QC samples. The instrument operator will sign-off that the filter is being utilized in the field. The original of the COC will accompany the filter back to the NERL-EMMD (the originator should retain a copy for their own records).

Subsequent analysis (i.e. gravimetric analysis, etc.) will be indicated on the sample form by responsible parties. Original copies of all data forms will be maintained in the NERL EMMD project files.

10.0 References

Research Operating Procedure 07 (version 3) for Use and Preparation of the Harvard Impactor for Collection of Particulate SVOC Matter in the DEARS. 12/17/2005.

D-SED-IEMB-005-SOP-01. Standard Operating Procedure for the Collection of Field and Activity Metadata During Exposure Characterization Pilot Study Field Sampling. 2017

Appendix I. Air PM/Metals Sample Data Collection Sheet and Chain of Custody Form

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

COC-08 — Field Locations -- Active PM & SVOC Air Samples

		Deployment	Recovery
Study Name: TCRS	Date		
Field ID No:	Operator		
Field Location ID: 1			

Sample Chain of Custody

PM Filter ID	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA, RTP: Initials	Shipped to EPA, RTP: Date	Received & Stored at EPA, RTP: Initials	Received & Stored at EPA, RTP: Date	Analyzed: Initials	Analyzed: Date

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

SVOC	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA, RTP: Initials	Shipped to EPA, RTP: Date	Received & Stored at EPA, RTP: Initials	Received & Stored at EPA, RTP: Date	Analyzed: Initials	Analyzed: Date

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

Sample ID	Field notes:
	Receipt notes:
	Analysis notes:

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

COC-09

Field Locations -- Active PM & SVOC Air Samples

		Deployment	Recovery
Study Name: TCRS	**Date**		
Field ID No:	**Operator**		
Field Location ID 2			

Sample Chain of Custody

PM Filter ID	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA, RTP: Initials	Shipped to EPA, RTP: Date	Received & Stored at EPA, RTP: Initials	Received & Stored at EPA, RTP: Date	Analyzed: Initials	Analyzed: Date

Sample ID	**Field notes:**
	Receipt notes:
	Analysis notes:

Sample ID	**Field notes:**
	Receipt notes:
	Analysis notes:

SVOC	Start Time	Start Flow	Stop Flow	Stop Time	Shipped to EPA, RTP: Initials	Shipped to EPA, RTP: Date	Received & Stored at EPA, RTP: Initials	Received & Stored at EPA, RTP: Date	Analyzed: Initials	Analyzed: Date

Sample ID	**Field notes:**
	Receipt notes:
	Analysis notes:

Sample ID	**Field notes:**
	Receipt notes:
	Analysis notes:

TIRE CRUMB EXPOSURE STUDY-- VOC MONITORING DATA AND COC SHEET

COC-10

Field Locations -- Active PM & SVOC Air Samples

			Deployment	Recovery
Study Name:				
TCRS		Date		
Field ID No:				
		Operator		
Field Location ID	8			

					Sample Chain of Custody					
					Shipped to EPA, RTP		Received & Stored at EPA, RTP		Analyzed	
PM Filter ID	Start Time	Start Flow	Stop Flow	Stop Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

SVOC	Start Time	Start Flow	Stop Flow	Stop Time	Initials	Date	Initials	Date	Initials	Date
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								
Sample ID		Field notes:								
		Receipt notes:								
		Analysis notes:								

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 Collecting and Using Extant Publicly-Available Video

Number: D-SED-EHCAB-001-SOP-01	Effective Date: November 14, 2016

SOP was Developed S In-house £ Extramural

Alternative Identification: EHCAB-001-01

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

Standard Operating Procedure for Collecting and Using Extant Publicly-Available Video

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Standard Operating Procedure for Collecting and Using Extant Publicly-Available Video

1.0 SCOPE AND APPLICATION

Using publicly available videos (e.g. content posted on YouTube), videography is being used to collect activity pattern data on children and adults while playing/practicing on artificial turf fields that contain tire crumb infill at athletic facilities. The purpose of the extant videography is to provide an objective assessment of user activity patterns potentially impacting exposure to chemicals found in crumb rubber infill that are difficult to capture consistently using questionnaires.

2.0 SUMMARY OF THE METHOD

Using study acceptance criteria, trained EPA technicians shall identify and download publicly-available internet videos (e.g., YouTube) of 30 children and 30 adults engaging in activities on artificial turf fields that contain tire crumb infill. EPA technicians shall document from each downloaded video a description and image of the selected subject, the type of activity/sport, the type of field (e.g., indoor or outdoor), and the duration of the activity/sport.

EPA technicians shall record the selected activities of each person on tape for a total of 15 minutes for field hockey and soccer and 10 minutes for football using three types of paper templates. These activities include frequency of hand-to-mouth, object-to-mouth, hand-to-turf, and body-to-turf events; number of fingers in mouth per hand-to mouth event; and activity level duration (i.e., time spent at resting, low, medium, or high). The activities of each person recorded on the paper templates shall be manually transferred (using double key entry) into a spreadsheet in MS Excel and a copy of these files made using a thumb drive.

3.0 DEFINITIONS

SOP – Standard operating procedure

COC – Chain-of-custody

VID – Video identification number

4.0 CAUTIONS

The videography requires the collection of images that may be considered to be personally identifiable participant data. Also, it is likely that the video footage will include other players and bystanders that are not the focus of activity data collection, as well as inclusion of field or geographic features that may make the video location discernible. No personal or site identifying information shall be recorded as research data as part of the data activity transcription. Any and all human subjects protections requirements as specified by the Institutional Review Board shall be followed.

5.1 RESPONSIBILITIES

5.2 EPA laboratory technicians shall be responsible for using video websites (e.g. YouTube) or

general search engines (e.g. Google) to identify, download, and securely save acceptable extant videos of children or adults playing or practicing on artificial turf fields with crumb rubber infill. They shall also be responsible for extracting data from the downloaded extant videos and recording selected activities onto three types of paper templates (see Appendices D-F). They shall be responsible for manually transferring these data into a Microsoft Excel spreadsheet (using double key entry) and making a copy using a thumb drive.

5.3 The EPA Principal Investigator shall be responsible for maintaining custody of the EPA non-networked laptop computer and one copy of the extant video files. He or she shall also be responsible for signing out the non-networked laptop computer and video files to laboratory technicians to code people's selected activities while playing on synthetic turf fields. In addition, he or she will be responsible for providing the EPA Database Manager with a copy of the activity pattern data recorded from the extant video files in an MS Excel file.

5.4 The EPA Database Manager shall be responsible for maintaining one copy of the extant video files. He or she shall also be responsible for converting the activity data in the MS Excel file into a SAS database.

6.1 MATERIALS

6.2 Networked, password-protected laptop computer

6.3 Non-networked, password-protected laptop computer at EPA-RTP facility

6.4 Microsoft Internet Explorer or Google Chrome (most recent version of either Web browser)

6.5 Windows Media Player, version 12

6.6 Microsoft Excel software, version 2007 or newer

6.7 Microsoft Word software, version 2007 or newer

6.8 Printer

6.9 Hardcopy of example standardized list of search terms (Appendix A)

6.10 Hardcopies of activity templates (Appendices B-F)

6.11 Individual digital copies of Appendices A-F as Word file templates (e.g. Appendix A.docx)

6.12 Pen

6.13 Adobe Acrobat XI Pro

6.14 Encrypted, portable USB thumb drives (see Reference section)

6.15 Hardcopy file folder

6.16 Scanner

6.17 External hard drive

7.1 PROCEDURES

7.2 Collection of Extant Publicly-Available Videos

7.2.1 Sign out the non-networked laptop computer (for up to 7 days) from the EPA Principal Investigator by printing with a pen your name, date, and time on the hard copy sign out sheet.

7.2.2 Turn on and log onto your networked laptop computer using your LAN ID and password. Insert an encrypted thumb drive and create a folder on it called Tire Crumb Extant Video. Open Appendix A (List of Example Search Terms), Appendix B (Participant Activity Information on Extant Video), and Appendix C (List of VIDs by Scenario and Age Group) using Microsoft Word.

7.2.3 Open Internet Explorer or Chrome on the laptop computer. Use the address bar to navigate to a video site (e.g. YouTube) or a general search engine (e.g. Google). Using the Example List of Search Terms (Appendix A), select and type a phrase (e.g., "children's soccer game turf") into the search area of this website to find a target video. (Note that the search terms will be refined as experience is gained in determining the types and quality of extant synthetic field-related video. For example, different sport, practice, or training activities may be identified for searching).

7.2.4 Select a video from among the search results of 30 minutes or greater length, watch the entire video, and determine whether it meets the study's acceptance criteria for extant video data.

Acceptance criteria include 1) resolution of taped activity (which must be high enough in quality to discern when a participant makes contact with the field), 2) continuity of person on tape (viewer must be able to see 1 participant on video for a minimum of 1 5 minutes for field hockey and soccer and 10 minutes for football), and 3) applicability for research goals (the participant and activity must correspond with the scenarios and age groups targeted for high-end exposure characterization, per the study QAPP). Note: If all acceptance criteria are obtained (above), up to three individuals can be used per videotape.

7.2.5 If the selected video meets all of the acceptance criteria, type the letters "ss" following the HTTP text and in front of the website name (e.g. "https://www.ssyoutube.com/watch_") on the address bar. Press the Enter key to initiate a download of the video as an MP4 audio and video file. Click on the MP4 file and save it in the Tire Crumb Extant Video folder on the thumb drive. Label the filename and description as e.g. "Tire Crumb Extant Video_*VID*.mp4," using a number 01 through 60 as the VID based on the sequence in which the videos are retrieved.

Save a copy of Appendices A and C in the Tire Crumb Extant Video folder on the thumb drive. *(Note: The thumb-drive versions of Appendices A and C can be used for every video downloaded and VID assigned during the extant videography.)* On Appendix B, type the VID in the top cell where indicated and save a copy of the file in the Tire Crumb Extant Video Folder on the thumb drive as e.g. "Appendix B_*VID*.docx" and continue to step 7.1.6.

If the selected video does not meet the acceptance criteria, repeat steps 7.1.2 through 7.1.4. Apply the standardized search terms on other video sites or search engines if desired or necessary.

7.2.6 Safely eject the thumb drive containing the saved video file and appendices. Navigate to the Downloads folder on the networked laptop and delete the MP4 file downloaded in step 7.1.5.

Empty the laptop's Recycle Bin folder to eliminate the file from the networked computer. Log off and shut down the networked laptop.

7.2.7 Turn on and log onto the non-networked laptop computer by clicking on the "Administrator" icon, and typing in the generic password "Welcome 1", then insert the thumb drive containing the saved video file. On the external hard drive, create or locate a folder called Tire Crumb Extant Video. Copy the downloaded MP4 file, along with Appendices A, B, and C, from the thumb drive to the Tire Crumb Extant Video folder on the C drive. Safely eject the thumb drive.

7.2.8 Using Windows Media Player, open and play the transferred video file from the Tire Crumb Extant Video folder. Using Microsoft Word, open the participant information file (e.g. "Appendix B_*VID*.docx").

Type the following information on the participant information file (Appendix B) where indicated: the duration of the video file in hours, minutes, and seconds; the start and stop times (in hours, minutes, and seconds from the beginning of the video) for the 15 or 10-minute period, respectively, to be viewed for activity coding; a description of the video subject being followed (i.e., gender and jersey color and number); and the type of sport or activity (e.g. football, soccer). Take a screen capture of the videotaped participant using Windows Media Player by pausing the video on a frame showing the subject, pressing Alt + Print Screen on the keyboard, and pasting the image into the Appendix B table where indicated. Record the time of the screen shot on the videotape in Appendix B.

Based on the subject's visual age group (based on other players) and the sport or activity filmed, type the VID number in a cell under the appropriate column on the List of VIDs by Scenario and Age Group sheet (Appendix C). *(Note: At the conclusion of the extant videography, each of the three exposure scenarios should have 20 VIDs associated with them, 10 with child subjects and 10 with adult subjects.)*

Close Windows Media Player.

7.2.9 Repeat Steps 7.1.2 to 7.1.8 to locate and obtain additional video files.

When extant video collection is ceased at the end of each workday, and also once all study requirements for extant video collection are fulfilled, print out hardcopies of Appendices B, and C. Place the printed sheets in a file folder, label the folder "Tire Crumb Extant Video" along with the current date (e.g. Tire Crumb Extant Video, May 2, 2016), and log off and shut down the non-networked laptop. Place the thumb drive, laptop computer, and file folder in a locked cabinet when you are away from your office during the day or at the end of the workday. After searching for videotapes for a three day period, return the thumb drive, laptop computer, and file folder to the EPA Principal Investigator and print with a pen your name, date, and time on the sign out sheet.
**Once all 60 video files have been selected, place all of the video files onto a thumb drive and print out final hardcopies of Appendices B and C and give them to the EPA Principal Investigator.

7.3 Data Extraction from Extant Publicly-Available Video

7.3.1 Sign out the non-networked laptop computer from the EPA Principal Investigator. Turn on and log onto the laptop computer. Open the Tire Crumb Extant Video folder on the C drive. Print out a hardcopy of each of the 3 Activity Templates for frequencies, number, and duration of selected

subject activities on extant video (Appendices D-F). Select a completed Appendix B document with a VID, participant description, sport/activity type, and duration listed.

7.3.2 Open the MP4 file with the corresponding VID in the filename, saved in the Tire Crumb Extant Video folder, using Windows Media Player. Before playing the video, write the VID of the video on the upper-left of each activity template.

7.3.3 Watch the entire video and familiarize yourself with the participant's activities on videotape.

During the second playback, code the person's activities using Appendix D. On this paper template, with a pen record with tally marks how many times the participant made hand-to-mouth, object-to-mouth, hand-to-turf, and body-to-turf contact during the 30-minute time period Write the date of the analysis on the upper-right of the template where indicated.

7.3.4 Log off and shut down the non-networked laptop. Scan the completed activity templates and email them as PDF files to the EPA Principal Investigator. Return the laptop and give the completed hardcopy activity templates to the EPA Principal Investigator. *(Note: This procedure will be completed for all 60 video files before other selected activities on videotape will be coded using the two other paper templates, Appendices E and F.)*

7.3.5 Repeat steps 7.2.1 through 7.2.4 using the Appendix E Activity Template. For each hand-to-mouth event seen on the video, make a tally mark in the bottom row of cells under the column which indicates the number of fingers in mouth (one through five). At the conclusion of the playback, add the tally marks to determine the total number of times the videotaped participant was seen with one finger in mouth, two fingers in mouth, etc. in the same cells used for tallying.

7.3.6 Repeat steps 7.2.1 through 7.2.4 using the Appendix F Activity Template. For each of the four activity levels listed (resting, low, moderate, and high), record the time intervals that the subject spends at each activity level. For example, if the subject is seen sprinting from 0:15:00 to 0:15:30, write "0:00:30" to indicate 30 seconds of high activity. At the conclusion of the video, add the time intervals for each activity level to determine the total duration of time spent by the subject at each level.

7.4 Electronic Tabulation of Data Extraction Results

7.4.1 Sign out (via sign out sheet) the non-networked laptop computer and the file folder containing the completed paper Activity Templates (Appendices D-F) from the EPA Principal Investigator.

7.4.2 Turn on and log onto the non-networked computer. Insert a portable thumb drive. Create a MS Excel spreadsheet and format the spreadsheet according to the template provided in Appendix G for all VIDs, 01 through 60. Use the keyboard to enter the handwritten activity data from the paper templates for each person followed on extant video.

7.4.3 Save the spreadsheet as "ExtantActivityData" in the Tire Crumb Extant Video folder on the C drive. Include the EPA technician's name and the date of data entry at the top of the spreadsheet above the column headings. Copy the spreadsheet onto the thumb drive in the Tire Crumb Extant Video folder.

7.4.4 Safely eject the thumb drive. Log off and shut down the non-networked computer. Return the computer and thumb drive to the EPA Principal Investigator and check-in the materials using sign out sheet.

7.4.5 The EPA Principle Investigator will upload the MS Excel spreadsheet via the thumb drive onto a secured EPA server (private L drive folder: TCRS/Activity Data).

7.4.6 The EPA Database Manager will convert the MS spread sheet into a SAS database. The SAS database will be housed at this location: private L drive folder: TCRS/ActivityDatabase.

8.0 RECORDS

Unique VIDs for each downloaded video are recorded on the video filenames and on the activity templates for video data extraction. No names or personal information shall be collected if they can be discerned from the video. No data extraction shall be performed for non-participants. No organization name, team name, or location information shall be collected in the information extraction. The electronic video files shall be treated as personally identifiable data and will be managed and secured to allow access and use only by trained study staff for the intended purpose of turf field activity data collection. No video or still images shall be publicly released as part of the research effort and research reporting.

Double key data entry, in which the EPA Principal Investigator shall compare the technician's transcriptions of the same MS activity data file, will be used to verify the accuracy of the electronic data acquired from the extant videography. Any discrepancies between the two data files will be resolved by the EPA Principle Investigator by reviewing the original hardcopy version and making necessary changes as needed to the data file. The EPA Principal Investigator or equivalent will also copy the downloaded extant video files onto a portable USB drive or external hard drive to be kept in a locked cabinet.

9.1 QUALITY CONTROL AND QUALITY ASSURANCE

9.2 *Extant video quality checks:* See section 7.1.4.

9.3 *Data and records management:* About 10% of the downloaded videos shall be re-coded by the same trained laboratory technician. In addition, this same subset of videos (10%) shall also be coded by a second trained staff member or contractor. The EPA Principal Investigator will use this double key data entry to assess comparability and intra/inter-reviewer consistency for the subjects' activity data recorded using each type of paper template. The goal is 90% intra-reviewer and 85% inter-reviewer accuracy of activity data coding for laboratory technicians. If the coder fails the intra- and/or inter-reviewer accuracy test(s), he/she will recode a person's activity data on a video file until they can pass the test.

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.

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: Example Standardized List of Extant Video Search Terms

Scenario 1 (Soccer)	Scenario 2 (Football)	Scenario 3 (Field hockey/P.E.)
Children soccer game artificial turf	Children football game artificial turf	Children field hockey game artificial turf
Children soccer full practice artificial turf	Children football practice artificial turf	Children field hockey practice artificial turf
Adult soccer game artificial turf	Adult football game turf	Adult field hockey game turf
Adult soccer full practice artificial turf	Adult football practice artificial turf	Adult field hockey practice artificial turf
MLS soccer game artificial turf	NFL football game turf	NCAA field hockey game turf
MLS soccer practice artificial turf	NFL football full practice artificial turf	NCAA field hockey practice artificial turf
NASL soccer game artificial turf	NCAA football game turf	USA Field Hockey game turf
NASL soccer practice artificial turf	NCAA football full practice artificial turf	USA Field Hockey practice artificial turf
USL soccer game artificial turf	Arena Football League game artificial turf	High school men's field hockey game artificial turf
USL soccer practice artificial turf	Arena Football League practice artificial turf	High school women's field hockey game artificial turf
NWSL women's soccer game artificial turf	Women's football league game artificial turf	Indoor field hockey game artificial turf
NWSL women's soccer practice artificial turf	Women's football league practice artificial turf	Indoor field hockey practice artificial turf
NCAA men's soccer game artificial turf	Indoor football game artificial turf	US men's international field hockey game artificial turf
NCAA men's soccer practice artificial turf	Indoor football practice artificial turf	US women's international field hockey game turf
NCAA women's soccer game artificial turf	Flag football game artificial turf	Field hockey goalkeeper game artificial turf
NCAA women's soccer practice artificial turf	Flag football practice artificial turf	Field hockey goalkeeper practice artificial turf
Indoor soccer game artificial turf	Touch football game turf	Ultimate Frisbee game turf
Indoor soccer practice turf	Touch football practice turf	Ultimate Frisbee practice turf
Soccer goalkeeper game turf	Boys football game turf	Athletic drills artificial turf
Soccer goalkeeper practice artificial turf	Girls football game artificial turf	Boys field hockey game or practice artificial turf
US men's international soccer game artificial turf	Boys football practice artificial turf	Girls field hockey game or practice artificial turf
US women's international field hockey game artificial turf	Girls football practice artificial turf	Physical education artificial turf

Appendix B: Participant Activity Information on Extant Video

Technician Name	
VID	
Video Duration	
Start and stop times for 30-minute activity coding period	Start: 0:00:00 Stop: 0:00:00
Child or adult Sex Type/color of clothing Main position or activity on field	
Screen capture of participant and record time of screen shot	Time 0:00:00
Type of field (indoor or outdoor)	
Type of sport or activity (e.g., soccer, football)	
Notes:	

Appendix C: List of VIDs by Scenario and Age Group

Number	Scenario 1 (Soccer)		Scenario 2 (Football)		Scenario 3 (Field hockey/P.E.)	
	Child	Adult	Child	Adult	Child	Adult
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Appendix D: Activity Template - Frequencies of selected subject activities on extant video

Technician Name:_____

VID:_____ Analysis Date:___-____-_____

Hand-to-mouth	**Object-to-mouth**	**Hand-to-turf**	**Body-to-turf (excludes hands)**

Appendix E: Activity Template - Number of selected subject activities on extant video

Technician Name: _____

VID:_____ Analysis Date:___-____-____

Fingers in mouth (per hand-to-mouth event)				
One	Two	Three	Four	Five

Appendix F: Activity Template - Duration (minutes) of selected subject activities on extant video

Technician Name: _____

VID:_____ Analysis Date:___-____-_____

Resting (e.g., sitting/standing)	**Low activity (e.g.., walking)**	**Moderate activity (e.g., jogging)**	**High activity (e.g., running)**

Appendix G: Template for Tabulation of Extant Video Activity Data

VID	Scenario (1, 2, 3) or sport	Adult or Child	Sex	Indoor or Outdoor Field	Hand-to-mouth events	Object-to-mouth events	Hand-to-turf events	Body-to-turf events (excl. hands)	Times with 1 finger in mouth	Times with 2 fingers in mouth	Times with 3 fingers in mouth	Times with 4 fingers in mouth	Times with 5 fingers in mouth
01													
02													
03													
04													
05													
06													
07													
08													
09													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													

Appendix G: Template for Tabulation of Extant Video Activity Data (continued)

VID	Duration at rest (min.)	Duration at low activity (min.)	Duration at moderate activity (min.)	Duration at high activity (min.)
01				
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				

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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 Videography of Activity Characterization Study Participants

Number: D-SED-EHCAB-005-SOP-01	Effective Date: August 21, 2017

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification: EHCAB-005-01

SOP Steward

Name: Marsha K. Morgan

Signature Date:

Approval

Name: Kent W. Thomas
Title: Tire Crumb Leader

Signature Date:

Concurrence

Name: Christine Alvarez
Title: QA Manager, Systems Exposure Division
Signature Date:

Standard Operating Procedure for Videography of Activity Characterization Study Participants

TABLE OF CONTENTS

Standard Operating Procedure for Videography of Activity Characterization Study Participants

1.0 SCOPE AND APPLICATION

Videography is being used to collect activity pattern data on child or adult participants who routinely play on artificial turf fields that contain tire crumb infill at athletic facilities. The purpose of the video data collection is to quantify selected activity patterns of children and/or adults while they play/practice on artificial turf fields to provide objective information on potential exposures to tire crumb rubber constituents through inhalation, dermal, and ingestion pathways.

2.0 SUMMARY OF THE METHOD

Field technicians shall record child and adult participants that participate in the exposure characterization component of the Tire Crumb Research Study (TCRS). Field technicians shall videotape these participants playing/practicing on artificial turf fields for 1.5-hours using a video camera (containing two video data (SD) cards) that is attached to a monopod.

Laboratory technicians shall translate the selected micro-activities of participants by using a non-networked laptop computer and a large computer monitor. The technicians shall record the selected activities of each person on tape for 1.5-hours using three types of paper templates. These micro-activities include frequency of hand-to-mouth, object-to-mouth, hand-to-turf, and body-to-turf events; number of fingers in mouth per hand-to-mouth event; and activity level duration (i.e., time spent at resting, low, medium, or high). The activities of each person recorded on the paper templates shall be manually transferred (using double-key entry) into a spreadsheet in MS Excel and a copy of these files made using a thumb drive.

3.0 DEFINITIONS

SOP – Standard operating procedure

COC – Chain-of-custody

PID – Participant identification number

CDC – Centers for Disease Control and Protection

4.0 CAUTIONS

The videography requires the collection of images that may be considered to be personally identifiable participant data. Also, it is likely that the video footage will include other players and bystanders that are not videography participants, as well as inclusion of field or geographic features that may make the video location discernible. Any and all human subjects protections and training requirements as specified by the Institutional Review Board shall be followed (currently stated in the Work Assignment).

5.1 RESPONSIBILITIES

5.2 CDC research staff shall be responsible for recruiting eligible children and/or adults and obtaining their assent/informed consent before videotaping their activities while playing/practicing on artificial turf fields.

5.3 The contractor staff shall be responsible for attending an EPA pre-pilot testing activity and training their field technicians to videotape study participants while playing/practicing on artificial turf fields as well as code selected micro-activity data from the videotapes of participants.

5.4 Contractor field technicians shall be responsible for videotaping the study participants while playing/practicing on the artificial turf fields for up to 1.5 hours (target time is 1.5 hour per participant) at the enrolled facilities.

5.5 The contractor staff shall be responsible for coding selected activity data from the participant SD cards using three types of paper templates (Appendices B-D). They shall be responsible for manually transferring and checking these data into a Microsoft Excel spreadsheet (using double-key entry) and making a copy using a thumb drive. In addition, contractor staff shall be responsible for providing an electronic version of the selected micro-activity data (in MS Excel files) to the EPA Principle Investigator.

5.6 The EPA Principal Investigator shall be responsible for signing in/out the SD cards to laboratory staff. In addition, he or she will be responsible for providing the EPA Database Manager with a copy of the activity pattern data recorded from the study participant video files in an MS Excel file.

5.7 The EPA Database Manager shall be responsible for maintaining one copy of the study participant video files. He or she shall also be responsible for converting the activity data in the MS Excel file into a SAS database.

6.1 MATERIALS

6.2 Two 27-inch computer monitors

6.3 One networked, password-protected laptop or desktop computer

6.4 Two non-networked, password-protected laptop computers

6.5 Videotaping equipment (see Appendix A)

6.6 COC sheets (see Appendix F)

6.7 Microsoft Word software, version 2007 or newer

6.8 Microsoft Excel software, version 2007 or newer

6.9 Hardcopies of Activity Templates (Appendices B-D)

6.10 Individual digital copies of Appendices B-E as Word file templates (e.g. Appendix B.docx)

6.11 Pens (acid-free)

6.12 Windows Media Player, version 12

6.13 Adobe Acrobat XI Pro

6.14 Scientific calculator (physical or virtual)

6.15 Encrypted, portable USB thumb drives (see Reference section)

6.16 Hardcopy file folder

6.17 Paper scanner

6.18 White dry erase board (8.5 x 11 inch) with pen

6.19 Aluminum video monopod

7.0 PROCEDURES

7.1 Videotaping Study Participants at Enrolled Facilities

7.1.1 CDC research staff shall obtain assent/informed consent to videotape a subset of study participants that are participating in the activity characterization and exposure measurement component of the TCRS. This assent/informed consent shall be requested as part of the eligibility screening interview for users of TCRS-enrolled facilities. When CDC research staff is not present at a field site, they shall provide contractor field technicians with written hardcopy documentation and contact information on which participants (by field location) have consented to have their activities videotaped for 1.5 hours. Note: If CDC research staff is present at the field site when the participant activity is scheduled, they shall provide the contractor field technicians with contact information (verbally) on which participants (by field location) have consented to have their activities videotaped for 1.5 hours.

7.1.2 Contractor field technicians shall attend pre-pilot testing activities to observe a demonstration on how EPA would like to videotape the selected micro-activities of individual participants while playing/practicing on synthetic turf fields. The signed names of the field technicians, including attendance date, shall be recorded onto a sheet of white paper and a copy provided to the EPA Principal Investigator. This document will be stored in a locked cabinet in the EPA Principal Investigator's office located in D-576.

7.1.3 EPA staff and/or contractors shall contact study participants who have given informed consent (via CDC) to be videotaped and arrange the scheduled times to videotape the adults and/or children while playing/practicing on artificial turf fields.

7.1.4 Contractor field technicians shall videotape individual study participants during the scheduled appointment times. Perform equipment checks on the camcorder to assure that it is functioning properly before entering the field. Turn on the camcorder and verify that it works and then check that the inserted battery is sufficiently charged ($\geq$ 75%). Place two new SD cards into the camcorder. Verify the date and time is accurate and is being recorded on the two SD files. Remove the lens cap and then open the LED screen. Select the recording option to write a real-time copy of the video files on both SD cards inserted into the camera (i.e. "Rec Set - Simultaneous Rec"). Attached the camcorder to the monopod. Using a dry erase board (8.5" x 11") with attached pen, write the participant's PID on it (2-inch size).

7.1.5 Set up the monopod (with camcorder) at one end of the playing field (nearest to the participant being filmed). Use zoom as needed when videotaping the participant (use 50% frame height). Move the monopod a few feet (left or right) as needed to obtain unobstructed views of the participant. To start videotaping the participant, press the Record button on the video camera. At

the beginning of the video, place the dry erase board (with the participant's PID) in front of the camera lens for five seconds. *(Note: If the participant spends less than 1.5-hours playing/practicing on artificial turf fields at the enrolled facilities then videotape for the entire available playing practice period).* Videotape the study participant for 1.5 total hours while they are playing/practicing on artificial turf fields at the enrolled facilities. This time period may include on-field breaks for water and/or food. Throughout the videotaping period, be as quiet as possible and keep a low profile. Concentrate on filming the participant's selected micro-activities including hand-to-mouth, hand-to-turf, object-to-mouth, and body-to-turf contacts. Do not videotape the participant at inappropriate times (e.g., changing clothes, bathing, using the restroom, going to the locker room, leaving the facility); stop the video camera and wait until the participant has returned to the field and then resume videotaping their activities.

7.1.6 After videotaping the participant for 1.5 total hours, press the Record button on the camcorder to stop recording. Put on the lens cap. Remove the camcorder from the monopod. Using the camcorder, review both SD cards to verify that the participant was recorded for the entire time period (including that the date and time of filming is correct) and that the selected micro-activities can be clearly observed on these video files (press the "Thumbnail" button and navigate the contents of each SD card, "A" and "B" to find the files; fast-forward through the playback to minimize time spent on-site). Then, remove the two SD cards and place them into the SD card cases. Affix a white label to the SD card case (*not directly to the SD card*) and write on it with a pen the PID, field ID, and date. Place the camcorder, SD cards cases containing the SD cards, and other accessories into the camera bag. Do not leave the video camera, SD cards, and other accessories in an unlocked car or in a car if the weather is hot (i.e., temperature is greater than 90°F). Repeat sections 7.1.4 – 7.1.6 for each scheduled participant. Leave the facility and return to the contractor duty station (keep all items secured in a locked room or cabinet).

7.1.7 After returning to the office in RTP, NC, contractor field technicians shall turn on and log onto the non-networked, password-protected laptop computer using your LAN ID and password. For each study participant, remove the two SD card cases from the camera bag. Store one of the SD cards (in a plastic case) in a locked cabinet. For the other one, remove the SD card and insert in into the laptop.

7.1.8 Locate the participant's video files on the SC card (SD/Private/AVCHD/BDMV/STREAM/mts). Use Windows Media Player to play the video files on the inserted SD card. Rename the video files to include the participant's PID e.g. "Tire Crumb Field Video *(PID)*.mts and place a copy of the participant's video files on the C drive, under the folder name: TCRS Video Files." Obtain a blank COC form (Appendix F) and complete the form using information on stop/start times from the video player. Eject the SD card from the laptop after all video files have been appropriately renamed. Place the SD card back into the plastic case. Give this SD card and filled out/signed COC to the EPA principal investigator (D-576). Repeat section 7.1.7-7.1.8 for each filmed participant.

7.2 *Data Extraction from Study Participant Videos*

7.2.1 To code the participant activities on the video files, turn on and log onto the non-networked laptop computer. Open the TCRS Video Files folder on the C drive. For each participant, do the following procedures. Print out a hardcopy of the Activity Template - Frequencies of selected micro-activities on study participant video (Appendix B). Open one of the video files saved in the TCRS Video Files folder using Windows Media Player. Before playing the video, write the PID of the video on the upper-left of each activity template. Write the date of the analysis on the upper-right of the template.

7.2.2 Watch the entire video and familiarize yourself with the participant's micro-activities on videotape.

7.2.3 During the second playback, code the person's micro- activities using Appendix B. On this paper template, with a pen record with tally marks how many times the participant made individual hand-to-mouth, object-to-mouth, hand-to-turf, and body-to-turf contacts during the 1.5-hour time period. Also provide the specific time that each selected micro-activity occurred by the participant on this template e.g. 00:45:26 (hours, minutes, and seconds after the start). In addition, record whether the participant was using a mouthguard or wearing gloves (one or two) on this template. The video may be paused or rewound as necessary to verify types of contact and specific times. At the conclusion of the video, record the total counts of each micro-activity at the bottom of the same cells used for tallying. Log off and shut down the non-networked laptop. Scan the completed activity templates and place them onto an encrypted thumb drive. Return the thumb drive, and give the completed hardcopy paper activity templates to the EPA Principal Investigator; The EPA principle investigator will store these items in a locked cabinet in his/her office. *(Note: This procedure will be completed for all 24 video files before other selected activities on videotape will be coded using the two other paper templates, Appendices C and D.)*

7.2.4 Repeat steps 7.2.2 through 7.2.4 using the Appendix C Activity Template. For each hand-to-mouth event seen on the video, make a tally mark in the bottom row of cells under the column which indicates the number of fingers in mouth (one through five). At the conclusion of the playback, add the tally marks to determine the total number of times the videotaped participant was seen with one finger in mouth, two fingers in mouth, etc. Record the totals at the bottom of the same cells used for tallying.

7.2.5 Repeat steps 7.2.2 through 7.2.4 using the Appendix D Activity Template. For each of the four activity levels listed (resting, low, moderate, and high), record the time intervals that the subject spends at each activity level. For example, if the subject is seen sprinting from 0:15:00 to 0:15:30, write "0:00:30" to indicate 30 seconds of high activity. At the conclusion of the video, add the time intervals for each activity level to determine the total duration of time spent by the subject at each level. Record the total duration of time (in hh:mm:ss format) at the bottom of the appropriate column for each of the four listed activity levels.

7.3 *Electronic Tabulation of Data Extraction Results*

7.3.1 Sign out (via the sign-out sheet) the file folder in a binder containing the completed paper Activity Templates (Appendices B-D) from the EPA Principal Investigator.

7.3.2 Turn on and log onto the non-networked laptop computer. Insert a portable thumb drive. Create a MS Excel spreadsheet and format the spreadsheet according to the template provided in Appendix E for all PIDs referenced in the Activity Templates. Use the keyboard to enter the handwritten activity data from the paper templates for each person followed on video. Perform a 100% check that all the data entered from individual activity templates into the spreadsheet are correct.

7.3.3 Save the spreadsheet as "FieldActivityData" in the Tire Crumb Participant Video folder on the C drive. Include the EPA technician's name and the date of data entry at the top of the spreadsheet above the column headings. Copy the Tire Crumb Participant Video folder and all its contents from the C drive onto the inserted thumb drive.

7.3.4 Safely eject the thumb drive. Log off and shut down the non-networked computer. Return the

thumb drive to the EPA Principal Investigator and check-in the materials using the sign-out sheet.

7.3.5 The EPA Principal Investigator will upload the MS Excel spreadsheet via the thumb drive onto a secured EPA server (private L drive folder: TCRS/Activity Data).

7.3.6 The EPA Database Manager will convert the MS Excel spreadsheet into a SAS database. The SAS database will be housed at this location: private L drive folder: TCRS/Activity Database.

8.0 RECORDS

Unique PIDs previously assigned to each participant and his/her activity video are recorded on the video filenames (as transferred via SD card) and on the Activity Templates. The videographer shall attempt to minimize videotaping other players or bystanders as much as possible. No names or personal information will be collected from non-participants. No data extraction will be performed for non-participants. The electronic video files will be treated as personally identifiable data and will be managed and secured to allow access and use only by trained study staff for the intended purpose of field-related activity data collection. No video or still images will be publicly released without the written consent of the study participant.

Contractor staff shall provide the EPA Principal Investigator or equivalent with electronic video files (SD cards) of each study participant. The EPA Principle Investigator will also make a copy of the participant's video files by placing them on an external hard drive and storing them in a locked cabinet in his/her office. Individual contractor technicians shall perform double-key data entry of each Activity Template (B-D) onto separate FieldActivityData electronic files (a and b). Any discrepancies between the two data files will be resolved by the EPA Principal Investigator by reviewing the original hardcopy version and making necessary changes as needed to the electronic data files. The EPA Principal Investigator or equivalent will also make a copy of the participant's data files by placing them on an external hard drive and storing them in a locked cabinet in his/her office. All records will be archived by EPA for a minimum of 10 years.

9.1 QUALITY CONTROL AND QUALITY ASSURANCE

9.2 *Video camera and accessories checks:* See section 7.1.4.

9.3 *Data and records management:* About 10% of the downloaded videos shall be viewed again and the selected micro-activities will be quantified (re-coded) onto the hardcopy Activity Template by the same trained laboratory technician. In addition, this same subset of videos (10%) shall also be coded by a second trained staff member or contractor. The EPA Principal Investigator will use this double-key data entry to assess comparability and intra/inter-reviewer consistency for the subjects' activity data recorded using each type of paper template. The goal is 90% intra-reviewer and 85% inter-reviewer accuracy of activity data coding for laboratory technicians. If the coder fails the intra- and/or inter-reviewer accuracy test(s), he/she will recode a person's activity data on a video file until they can pass the test.

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.

Standard Operating Procedure for Administering the Facility Adult User Questionnaire, Activity Characterization for the Tire Crumb Research Study, National Exposure Research Laboratory, Research Triangle Park, N.C., 2016.

Standard Operating Procedure for Administering the Facility Child User Questionnaire, Activity Characterization for the Tire Crumb Research Study, National Exposure Research Laboratory, Research Triangle Park, N.C., 2016.

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: List of Required Videotaping Equipment

Item	**Manufacturer**	**Quantity**
Sony HXR-NX100 Full HD NXCAM Camcorder	Sony	1
Sony 32GB High Speed UHS-I SDHC U3 Memory Card (Class 10)	Sony	34
Pearstone Microfiber Cleaning Cloth, 18% Gray (7 x 7.9")	Pearstone	1
Arco Video Dr. Bag 30	Arco	1
Ruggard Desiccant Silica Gel Pack - Metal Case (40 g)	Ruggard	1
Ruggard Memory Card Case for 12 SD Cards and 12 microSD Cards	Ruggard	1
Ruggard Memory Card Case for 2 SD cards	Ruggard	10
Sony NP-F970 L-Series Info-Lithium Battery Pack (6300mAh)	Sony	1
White labels (for SD Card Cases)	----	1
White dry erase board with black pen	Sparco	1
Manfrotto aluminum video monopod	Manfrotto	1

Appendix B: Activity Template - Frequencies of selected micro-activities on study participant video

Technician Name: _____

VID:_____ Analysis Date:___-_____-___

Wearing Mouthguard: Yes or No (circle one) Wearing Gloves: None 1 2 (circle one)

Hand-to-mouth	**Object-to-mouth**	**Hand-to-turf**	**Body-to-turf (excludes hands)**

Appendix C: Activity Template - Number of selected micro-activities on study participant video

Technician Name:_____

VID:_____ Analysis Date:___-_____-___

Fingers in mouth (per hand-to-mouth event)				
One	Two	Three	Four	Five

Appendix D: Activity Template - Duration (minutes) of selected micro-activities of study participant on video

Technician Name: _____

PID: _____ Analysis Date:___-_____-___

Resting (e.g., sitting/standing)	**Low activity (e.g.., walking)**	**Moderate activity (e.g., jogging)**	**High activity (e.g., running)**

Appendix E: Template for Tabulation of Study Participant Activity Data

PID	Sport	Adult or Child	Sex	Indoor or Outdoor Field	Hand-to-mouth events	Object - to-mouth events	Hand-to-turf events	Body-to-turf events (excl. hands)	Times with 1 finger in mouth	Times with 2 fingers in mouth	Times with 3 fingers in mouth	Times with 4 fingers in mouth	Times with 5 fingers in mouth

Appendix E: Template for Tabulation of Study Participant Activity Data (continued)

PID	Duration at rest (min.)	Duration at low activity (min.)	Duration at moderate activity (min.)	Duration at high activity (min.)

Appendix F. Chain-of-custody form for the participant's videotapes

Tire Crumb Research Study			
Site location: Type of sport, adult or child, and sex:		Site Information (field number, indoor or outdoor field): (Do not include facility name or location here):	
Videotape ID	Description: Total recording time on videotape and recording stop/start times	Comments: Weather conditions, problems with videotaping equipment, reasons for stopping subject recoding (e.g., bathroom break, injuries)	
Collected by (Full name):	Date collected:	Collection start time:	Collection end time:
Shipped by (Full name):	Date shipped:		
Received by (Full Name):	Date received:	Storage:	Relinquished by (Full name):

U.S. Environmental Protection Agency
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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 Facility Child User Questionnaire

Number: D-SED-EHCAB-004-SOP-01	Effective Date: August 15, 2016

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification: EIB-004-01

SOP Steward

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Approval

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Signature: KENT THOMAS Digitally signed by KENT THOMAS DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=KENT THOMAS, dnQualifier=0000015373 Date: 2016.08.10 07:53:09 -04'00' Date: August 10, 2016

Concurrence*

Name: Brittany Stuart
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1.0 SCOPE AND APPLICATION

The Facility Child User Questionnaire is used to collect data on children (< 13 years old) who routinely play on artificial turf fields that contain tire crumb infill at athletic facilities. The purpose of the questionnaire is to collect information on the child's activity patterns that may impact the magnitude and frequency of their exposures to chemicals found in crumb tire infill.

2.0 SUMMARY OF THE METHOD

Research staff administer the Facility Child User Questionnaire to the parents of child participants who routinely play on artificial turf fields using a computer-assisted interview (CAI) method. The questionnaire is used to record specific types of data about the participating children including demographics (i.e., age, gender, and race), education levels, activity-levels, types and frequency of sports played on fields, frequency and duration of field use, hygiene practices (e.g., hand washing and eating events), types of clothing worn, and contact rates on turf for different types of activities. Using a laptop computer, research staff open the Facility Child User Questionnaire via the Epi Info software program and ask parents each question about their children and record their responses. Research staff save all 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

PID – Participant identification number

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 responsible for questionnaire administration.

5.3 The field technicians 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) forms, 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 sheets

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 Child Questionnaire folder located under Epi Info 7 Projects Folder. In this folder, select the ChildInfo.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 20 questions. Record the child participant identification number (PID; obtained from the EPA Tire Crumb Research Leader), facility name, interview date, study ID number, facility location, and interviewer ID number at the top of the questionnaire form.

7.1.4 Begin the questionnaire. Ask the child's parent 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" and click on the "Save" button and save the file as "ChildQuestionnairePIDXX (example: ChildQuestionnaire01).

7.1.6 For the next child 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 Child Questionnaire folder using only the study designated portable flash drive. Label the flash drive with a unique identifier (example: TCRS:Child Questionnaire). *All questionnaire files will be uploaded to this one flash drive.

8.0 RECORDS

COC forms (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://wwwn.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: Facility Child User Questionnaire

Form Approved
OMB No. 0923-xxxx
Exp. Date xx/xx/201x

Youth/Child Field User Questionnaire

PID		Site ID Number	
Facility Name		Facility Location	
Interview Date		Interviewer ID	

Interviewer: I would like to ask you some questions about activities that may affect your child's exposures to, and contact with synthetic turf fields that contain crumb rubber materials.

Field Contact Frequency and Duration Questions

Interviewer: I have several questions about the time your child spends on synthetic turf fields at this facility

B1. How long has your child been coming to this facility?

	(years)
	(months)

B2. Specifically on the synthetic fields at this facility, what sports, physical education classes, or other activities has your child actively participated in by season (specify) over the past year?

Season	Sport	Specify Other

B3. Over the past year, how many days per week by season has your child typically spent **on synthetic fields at this facility**?

Spring		(days per week)
Summer		(days per week)
Fall		(days per week)
Winter		(days per week)

B4. Over the past year, how many hours per day by season has your child typically spent **on the synthetic fields at this facility**?

Spring		(hours per day)
Summer		(hours per day)
Fall		(hours per day)
Winter		(hours per day)

B5. Over the past year, what was the longest period of time that your child has spent **on the synthetic fields** at this facility during a single day?

	(number of hours)

Contact Types and Scenarios per Each Type of Field Use

Interviewer: I have several questions about the kinds of activities that your child takes part in specifically ***on synthetic turf fields installed at this facility****.*

For the following question, please use one of the three responses (often, sometimes, and rarely/never). "Often" means > 50% of the time and "sometimes" means < 50%.

B6. How frequently does your child do the following activities **on synthetic fields** at this facility each season?

	Dive on ground	Fall on ground	Sit on turf	Eat snacks	Drink
Spring					
Summer					
Fall					
Winter					

Inhalation Exposure-Related Questions

B7. When using **synthetic fields at this facility**:

What % of the time is your child highly active, for example, running?

What % of the time is your child moderately active, for example, jogging?

What % of the time does your child have low activity, for example, walking?

What % of the time is your child resting, for example, sitting or standing?

Dermal and Non-Dietary Ingestion Exposure-Related Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B8. When **using synthetic turf fields at this facility**:

	Every Time	Often	Some times	Rarely / Never
How often does your child chew gum?	3	2	1	0
How often does your child use a mouth guard?	3	2	1	0
How often does your child eat?	3	2	1	0
How often does your child drink?	3	2	1	0
How often does your child play in the rain?	3	2	1	0
How often does your child wipe their hands with a hand wipe before eating?	3	2	1	0
How often does your child sweat heavily?	3	2	1	0
How often does your child touch the turf (with their hand)?	3	2	1	0
How often does your child touch the turf with their body excluding hands?	3	2	1	0
How often does your child sit on turf with bare skin wearing shorts?	3	2	1	0
How often is your child barefooted on the turf?	3	2	1	0
How often does your child play with the turf materials or rubber granules?	3	2	1	0
How often does your child touch their mouth with their hands or fingers?	3	2	1	0
How often does your child place non-food objects in their mouth every time like toothpicks, or pens or use their mouth to hold an object? If rarely/never, skip next.	3	2	1	0
What type of object does your child most often places in their mouth while at this facility?				
How often does your child get cuts or abrasions from contact with the turf?	3	2	1	0

If rarely/never, skip next.

What is the body part that usually has the most cuts or abrasions: knee, elbow, hand, thigh, shin, or other?

B9. What clothing does your child typically wear in this facility during each season (check all that apply)?

	Spring	Summer	Fall	Winter
Shorts	☐	☐	☐	☐
Short-sleeve shirt	☐	☐	☐	☐
Long pants	☐	☐	☐	☐
Long-sleeve shirt	☐	☐	☐	☐
Gloves	☐	☐	☐	☐
Socks	☐	☐	☐	☐
Helmet	☐	☐	☐	☐
Hat	☐	☐	☐	☐
Pads	☐	☐	☐	☐

Tire Crumb Take-Home Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B10. After using this facility:

How often do you notice tire crumbs, dirt, or debris

	Every Time	Often	Sometimes	Rarely/Never
on your child‘s body?	3	2	1	0

in your car?	3	2	1	0
in your home?	3	2	1	0
In your laundry room/mudroom?	3	2	1	0
in living room?	3	2	1	0
in your child's bedroom?	3	2	1	0
in your bathroom(s) your child uses?	3	2	1	0

Post-Use Hygiene Practices Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B11. After using this facility:

	Every Time	Often	Sometimes	Rarely/Never
How often does your child shower and change clothes immediately after engaging in activities on the synthetic turf at this facility?	3	2	1	0
How often does your child's shoes/equipment get wiped or removed before entering your home?	3	2	1	0

For the following questions, please use one of the six responses (never, once a month, 2 to 3 times a month, once a week, 2-3 times a week, or four or more times a week).

B12. At other locations:

	Never	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 or more times a week
How often has your child played on any other synthetic turf fields during the past year?	0	1	2	3	4	5
How often has your child played on any synthetic turf fields in the last five years?	0	1	2	3	4	5

How often has your child played on any natural grass fields during the past year?	0	1	2	3	4	5
How often has your child played on any natural grass turf fields in the last five years?	0	1	2	3	4	5
How often has your child played on playgrounds with rubber mulch, mats or synthetic turf during the past year?	0	1	2	3	4	5
How often has your child played on playgrounds with rubber mulch, mats or synthetic turf during in the last five years?	0	1	2	3	4	5

General Hygiene Questions

B13. How many times in general does your child wash their hands per day? ☐

B14. How many times in general does your child bathe or shower per week? ☐

General Demographic Questions

D1. How old is your child? ☐

D2. Is your child male or female? ◉ Male ◉ Female ◉ Refused

D3. Do you consider your child to be Hispanic or Latino? ◉ Yes ◉ No ◉ Refused

D4. Which of the following categories best describes your child's race? (select one or more)

◉ Native American Indian or Alaska Native
◉ Black or African American
◉ White
◉ Don't know
◉ Asian
◉ Native Hawaiian or Other Pacific Islander
◉ Refused

D5. How tall is your child? ☐ (ft) ☐ (in)

D6. How much does your child weigh? _____ (lbs)

D7. What is your child's current grade in school?

- ◉ 2nd
- ◉ 3rd
- ◉ 4th
- ◉ 5th
- ◉ 6th
- ◉ 7th
- ◉ 8th
- ◉ 9th
- ◉ Other
- ◉ Refused

Specify other grade _____

That concludes the survey. Thank you 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 COC form[a]

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

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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 Facility Adult User Questionnaire

Number: D-SED-EHCAB-003-SOP-01	Effective Date: August 15, 2016

SOP was Developed ☒ In-house ☐ Extramural

Alternative Identification: EIB-003-01

SOP Steward

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Approval

Name: Kent W. Thomas
Title: Tire Crumb Leader

Signature: KENT THOMAS Digitally signed by KENT THOMAS DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=KENT THOMAS, dnQualifier=0000015373 Date: 2016.08.10 07:54:44 -04'00' Date: August 10, 2016

Concurrence*

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

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1.0 SCOPE AND APPLICATION

The Facility Adult User Questionnaire is used to collect data from adults and older children (≥ 13 years old) who routinely play on artificial turf fields that contain tire crumb infill at athletic facilities. The purpose of the questionnaire is to collect information on the participants' activity patterns that may impact the magnitude and frequency of their exposures to chemicals found in crumb tire infill.

2.0 SUMMARY OF THE METHOD

Research staff administer the Facility Adult User Questionnaire to adults/older children who routinely play on artificial turf fields using a computer-assisted interview (CAI) method. The questionnaire is used to record specific types of data about the participants including demographics (i.e., age, gender, and race), education levels, activity-levels, types and frequency of sports played on fields, frequency and duration of field use, hygiene practices (e.g., hand washing and eating events), types of clothing worn, and contact rates on turf for different types of activities. Using a laptop computer, research staff open the Facility Adult User Questionnaire via the Epi Info software program and ask the participants each question and record their responses. Research staff save all 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

PID – Participant identification number

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 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) forms, 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 sheets

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 Adult Questionnaire folder located under Epi Info 7 Projects Folder. In this folder, select the AdultInfo.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 21 questions. Record the participant identification number (PID; obtained from the EPA Tire Crumb Research Leader), facility name, interview date, study ID number, facility location, and interviewer ID number at the top of the questionnaire form.

7.1.4 Begin the questionnaire. Ask the participant 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" and click on the "Save" button and save the file as "AdultQuestionnairePIDXX (example: AdultQuestionnairePID01).

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 Adult Questionnaire folder using only the study designated portable flash drive. Label the flash drive with a unique identifier (example: TCRS:Adult Questionnaire). *All adult questionnaire files will be uploaded to this one flash drive.

8.0 RECORDS

COC forms (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://wwwn.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: Facility Adult User Questionnaire

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

Adult/Adolescent Field User Questionnaire

PID		Site ID Number	
Facility Name		Facility Location	
Interview Date		Interviewer ID	

Interviewer: I would like to ask you some questions about activities that may affect your exposures to, and contact with synthetic turf fields that contain crumb rubber materials.

•

Field Contact Frequency and Duration Questions

Interviewer: I have several questions about the time you spend on synthetic turf fields at this facility.

B1. How long have you been coming to this facility?

	(years)
	(months)

B2. Specifically on the synthetic fields at this facility, what sports, physical education classes, or other activities have you actively participated in by season (specify) over the past year?

Season	Sport	Specify Other

B3. Over the past year, how many days per week by season have you typically spent ***on the synthetic fields at this facility***?

Spring		(days per week)
Summer		(days per week)
Fall		(days per week)
Winter		(days per week)

B4. Over the past year, how many hours per day by season have you typically spent ***on the synthetic fields at this facility***?

Spring		(hours per day)
Summer		(hours per day)
Fall		(hours per day)
Winter		(hours per day)

B5. Over the past year, what was the longest period of time that you spent ***on the synthetic fields at this facility*** during a single day?

	(number of hours)

Contact Types and Scenarios per Each Type of Field Use

Interviewer: I have several questions about the kinds of activities that you take part in specifically **on synthetic turf fields installed at this facility**.

For the following question, please use one of the three responses (often, sometimes, and rarely/never). "Often" means > 50% of the time and "sometimes" means < 50%.

B6. How frequently do you do the following activities while **on synthetic fields** at this facility each season?

	Dive on ground	Fall on ground	Sit on turf	Eat snacks	Drink
Spring					
Summer					
Fall					
Winter					

Inhalation Exposure-Related Questions

B7. When using ***synthetic fields at this facility***:

What % of your time are you highly active, for example, running? ____

What % of your time are you moderately active, for example, jogging? ____

What % of the time do you have low activity, for example, walking? ____

What % of the time are you resting, for example, sitting or standing? ____

Dermal and Non-dietary Ingestion Exposure-related Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B8. When ***using synthetic turf fields at this facility***:

	Every Time	Often	Some times	Rarely /Never
How often do you chew gum?	3	2	1	0
How often do you use a mouth guard?	3	2	1	0
How often do you eat?	3	2	1	0
How often do you drink?	3	2	1	0
How often do you play in the rain?	3	2	1	0
How often do you wipe your hands with a hand wipe before eating?	3	2	1	0
How often do you sweat heavily?	3	2	1	0
How often do you touch the turf with your hand?	3	2	1	0
How often do you touch the turf with your other body parts excluding hands?	3	2	1	0
How often do you sit on the turf with bare skin wearing shorts?	3	2	1	0
How often are you barefooted on the turf?	3	2	1	0
How often do you play with the turf materials or rubber granules?	3	2	1	0
How often do you touch your mouth with your hands or fingers?	3	2	1	0
How often do you place non-food objects in your mouth like toothpicks, or pens or use your mouth to hold an object?	3	2	1	0

If rarely/never, skip next.

What type of object do you most often place in your mouth while at this facility?	

How often to you get cuts or abrasions from contact with the turf?	3	2	1	0

If rarely/never, skip next.

What is the body part that usually has the most cuts or abrasions: knee, elbow, hand, thigh, shin, or other?	

B9. What clothing do you typically wear in this facility during each season (check all that apply)?

	Spring	Summer	Fall	Winter
Shorts	☐	☐	☐	☐
Short-sleeve shirt	☐	☐	☐	☐
Long pants	☐	☐	☐	☐
Long-sleeve shirt	☐	☐	☐	☐
Gloves	☐	☐	☐	☐
Socks	☐	☐	☐	☐
Helmet	☐	☐	☐	☐
Hat	☐	☐	☐	☐
Pads	☐	☐	☐	☐

Tire Crumb Take-Home Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B10. After using this facility:

How often do you notice tire crumbs, dirt, or debris

	Every Time	Often	Sometimes	Rarely/Never
on your body?	3	2	1	0
in your car?	3	2	1	0
in your home?	3	2	1	0
In your laundry room/mudroom?	3	2	1	0
In your living room?	3	2	1	0
In your bedroom?	3	2	1	0
In your bathroom(s)?	3	2	1	0

Post-Use Hygiene Practices Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B11. After using this facility:

	Every Time	Often	Sometimes	Rarely/Never
How often do you take shower and change clothes immediately after engaging in activities on the synthetic turf at this facility?	3	2	1	0
How often do you take actions to prevent tire crumbs from getting into your car?	3	2	1	0
How often do you wipe or remove shoes/equipment before entering your home?	3	2	1	0

For the following questions, please use one of the six responses (never, once a month, 2 to 3 times a month, once a week, 2-3 times a week, or four or more times a week).

B12. At other locations:

	Never	*Once a month*	2 to 3 times a month	Once a week	2 to 3 times a week	4 or more times a week
How often have you played on any other synthetic turf fields during the past year?	0	1	2	3	4	5
How often have you played on any synthetic turf fields in the last five years?	0	1	2	3	4	5
How often have you played on any natural grass fields during the past year?	0	1	2	3	4	5
How often have you played on any natural grass turf fields in the last five years?	0	1	2	3	4	5
How often have you played on playgrounds with rubber mulch, mats or synthetic turf during the past year?	0	1	2	3	4	5

How often have you played on playgrounds with rubber mulch, mats or synthetic turf during in the last five years?	0	1	2	3	4	5

General Hygiene Questions

B13. How many times in general do you wash hands per day? ☐

B14. How many times in general do you bathe or shower per week? ☐

General Demographic Questions

D1. How old are you? ☐

D2. Are you male or female? ◉ Male ◉ Female ◉ Refused

D3. Do you consider yourself to be Hispanic or Latino? ◉ Yes ◉ No ◉ Refused

D4. Which of the following categories best describes your race? (select one or more)

◉	Native American Indian or Alaska Native	◉	Black or African American	◉	White	◉	Don't know
◉	Asian	◉	Native Hawaiian or Other Pacific Islander	◉	Refused		

D5. How tall are you? ☐ (ft) ☐ (in)

D6. How much do you weigh? ☐ (lbs)

D7. Are you still in school? ☐ yes ☐ no

If so, what is your current grade in school?

◉ 7th ◉ 8th ◉ 9th

◉ 10th ◉ 11th ◉ 12th

- ◉ Technical School
- ◉ College
- ◉ Graduate School
- ◉ Other
- ◉ Refused

Specify Other Grade

D8. If No, what is your highest education level?

- ◉ 11th or less
- ◉ High School Graduate/ GED
- ◉ Post High School Training
- ◉ Some College
- ◉ College Graduate School
- ◉ Post-graduate
- ◉ Other
- ◉ Refused

D9. What is your occupation?

This concludes the survey. Thank you 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 COC form[a]

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

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

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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

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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 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 D-EMMD-AQB-016-SOP-01, D-EMMD-AQB-015-SOP-01, or D-EMMD-AQB-007-SOP-01 and conditioned using 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	PAH	polycyclic aromatic hydrocarbon
DQO	data quality objective	ppbv	parts per billion by volume
ECC	electronic carrier control	pptv	parts per trillion by volume
GC	gas chromatograph	psig	pounds per square inch gauge
FB	field blank	PTFE	polytetrafluoroethylene
FS	field spike	SA	sample
ID	identification	SB	shipping blank
in.	inch	SOP	standard operating procedure
MDL	method detection limit	TCRS	tire crumb research study
MFC	mass flow controller	TD	thermal desorber
min	minute	TOF	time of flight
mL	milliliter	VOC	volatile organic compound
MS	mass spectrometer		

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 1 hour using the tube conditioning procedure described in 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 with 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 (D-EMMD-AQB-007-SOP-01) or the climate-controlled exposure chamber (D-EMMD-AQB-016-SOP-01). TO14A VOC Standards and internal standards may also be loaded using the active-loading system (see 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 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 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 BenchTOF-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 D-EMMD-AQB-007-SOP-01 or D-EMMD-AQB-016-SOP-01. Standards may also be prepared using the active loading system as detailed in 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).

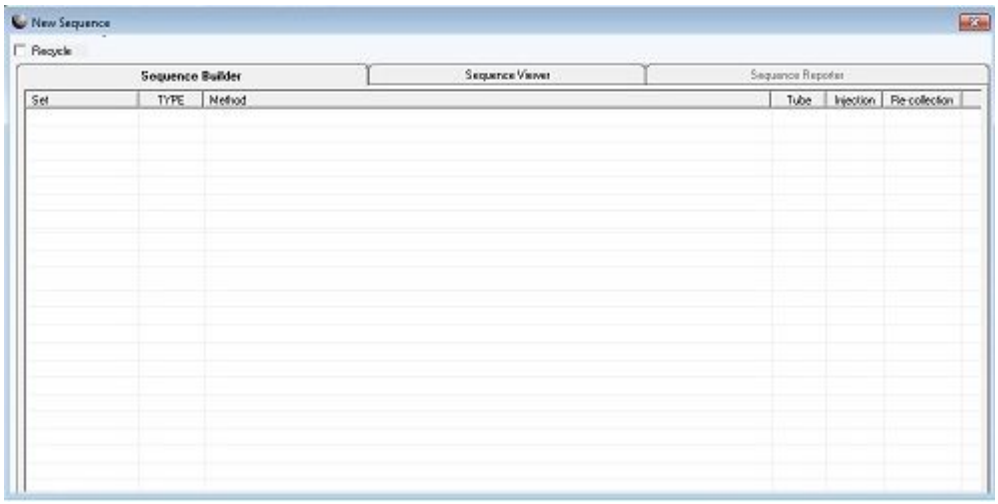

Figure 1. New sequence table.

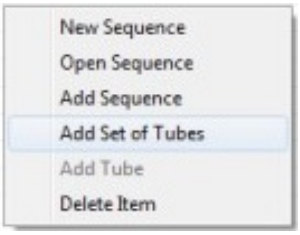

Figure 2. Adding a set of tubes.

5. When the **Select Method** dialog box (Figure 3) and "Add New Set" dialogue 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.)

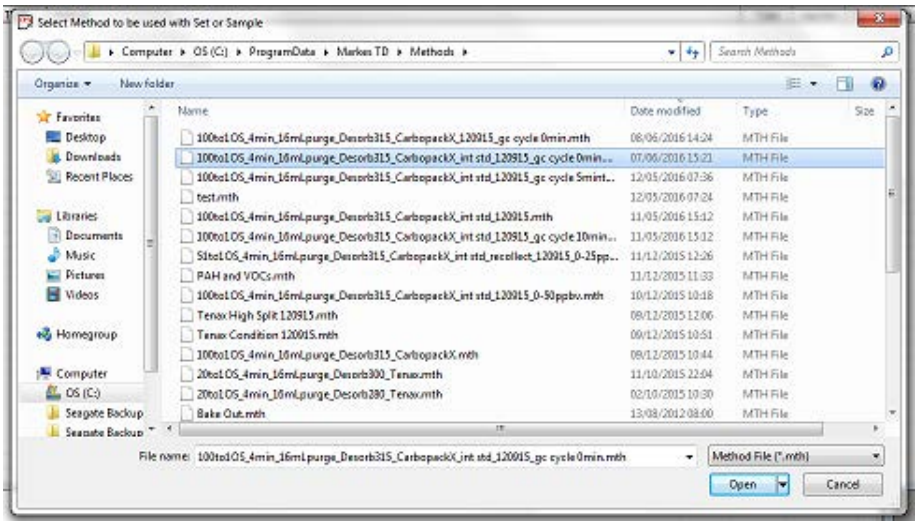

Figure 3. Selecting the desired method.

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).

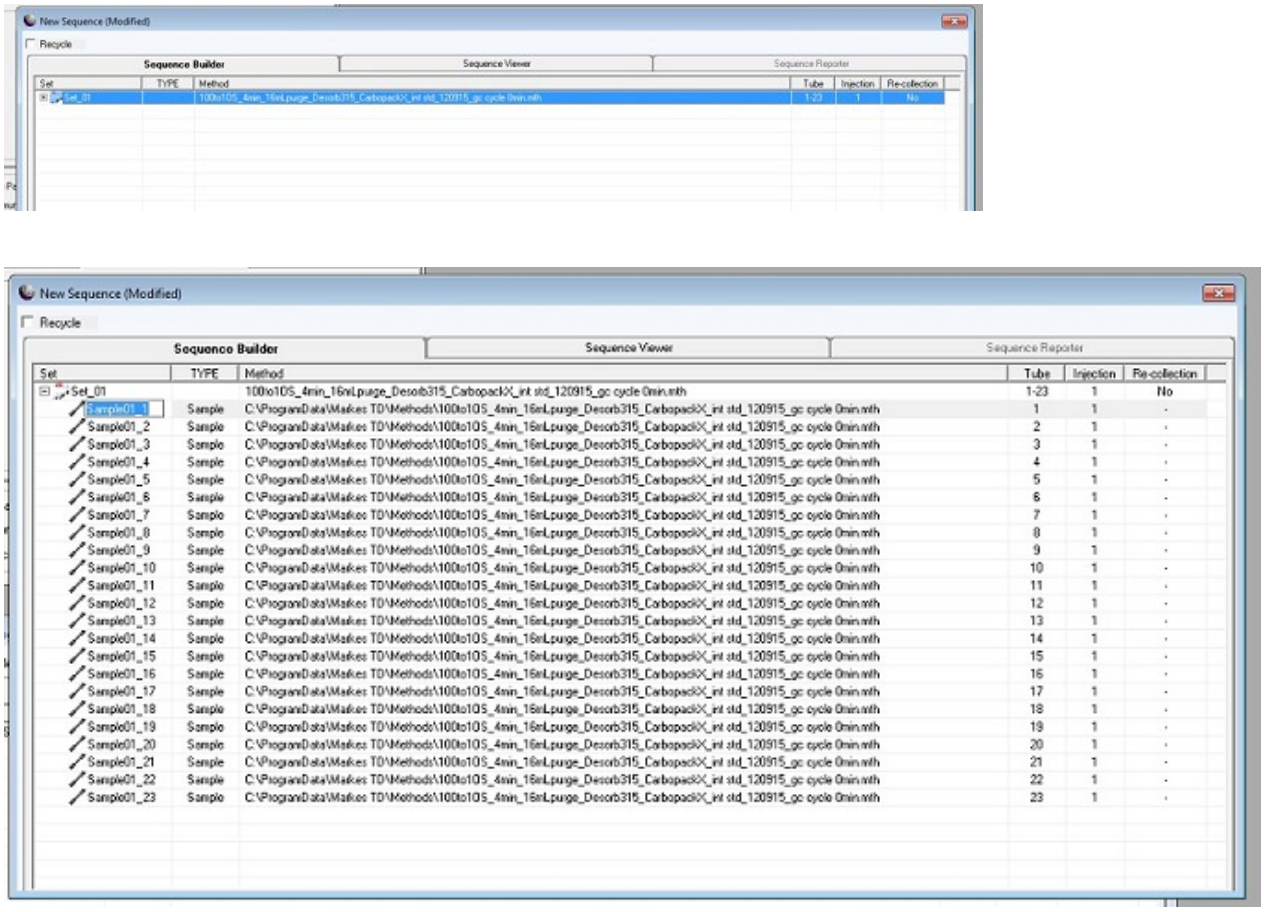

Figure 5. Expanding the sequence table.

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.

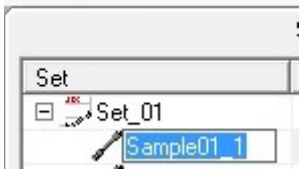

Figure 6a. Selecting the auto-generated tube ID.

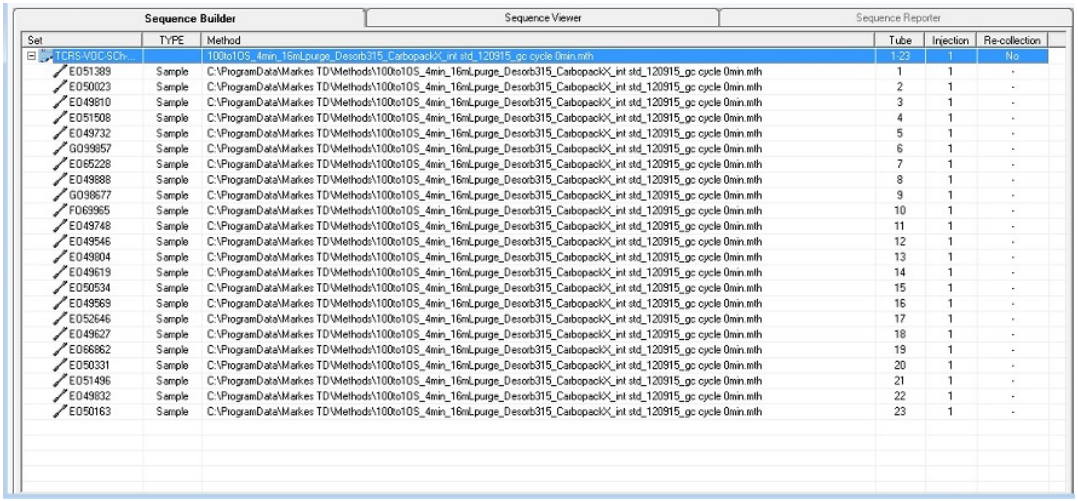

Figure 6b. Entering tube ID numbers into the sequence table.

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.

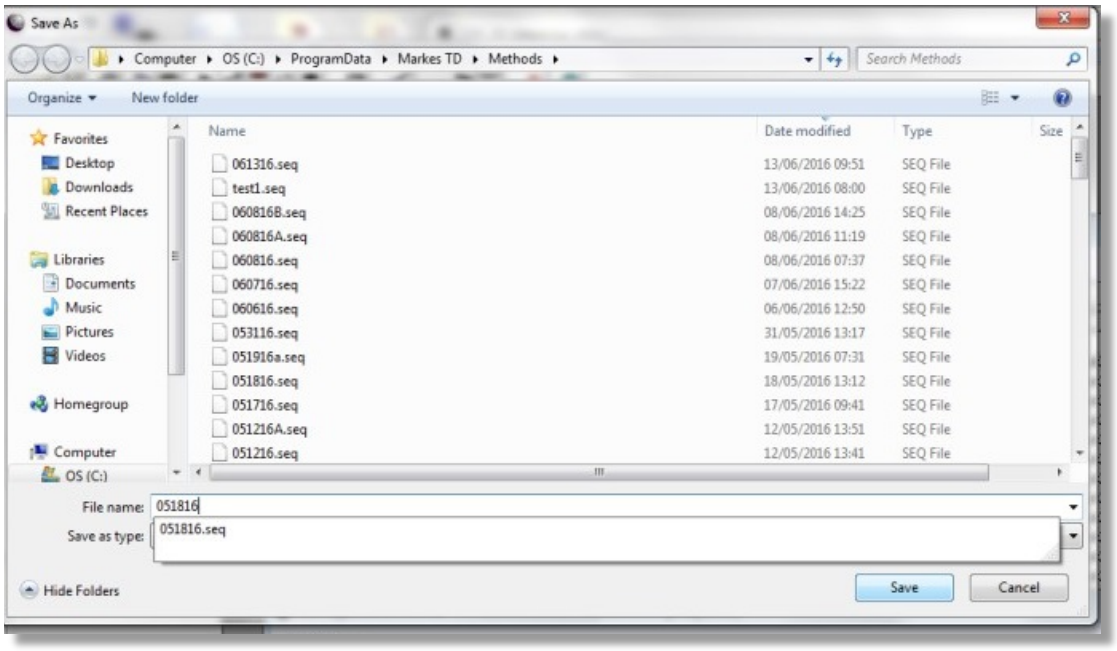

Figure 7. Saving the sequence name in the MMDDYY format.

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 (D-EMMD-AQB-007-SOP-01 or D-EMMD-AQB-016-SOP-01) actively loaded standards are also available for use (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 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 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).

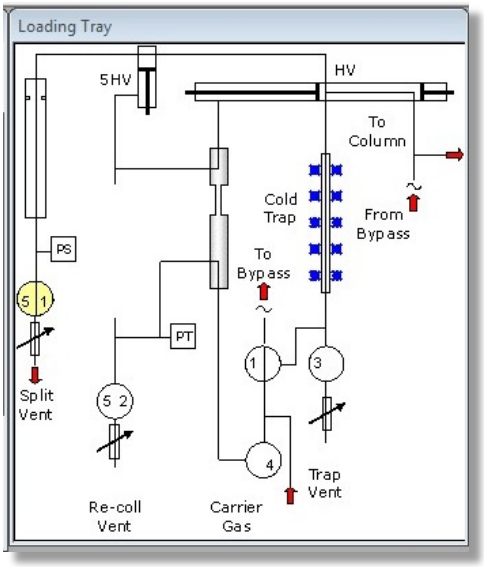

Figure 11. Unity 2 Schematic during desorption.

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.

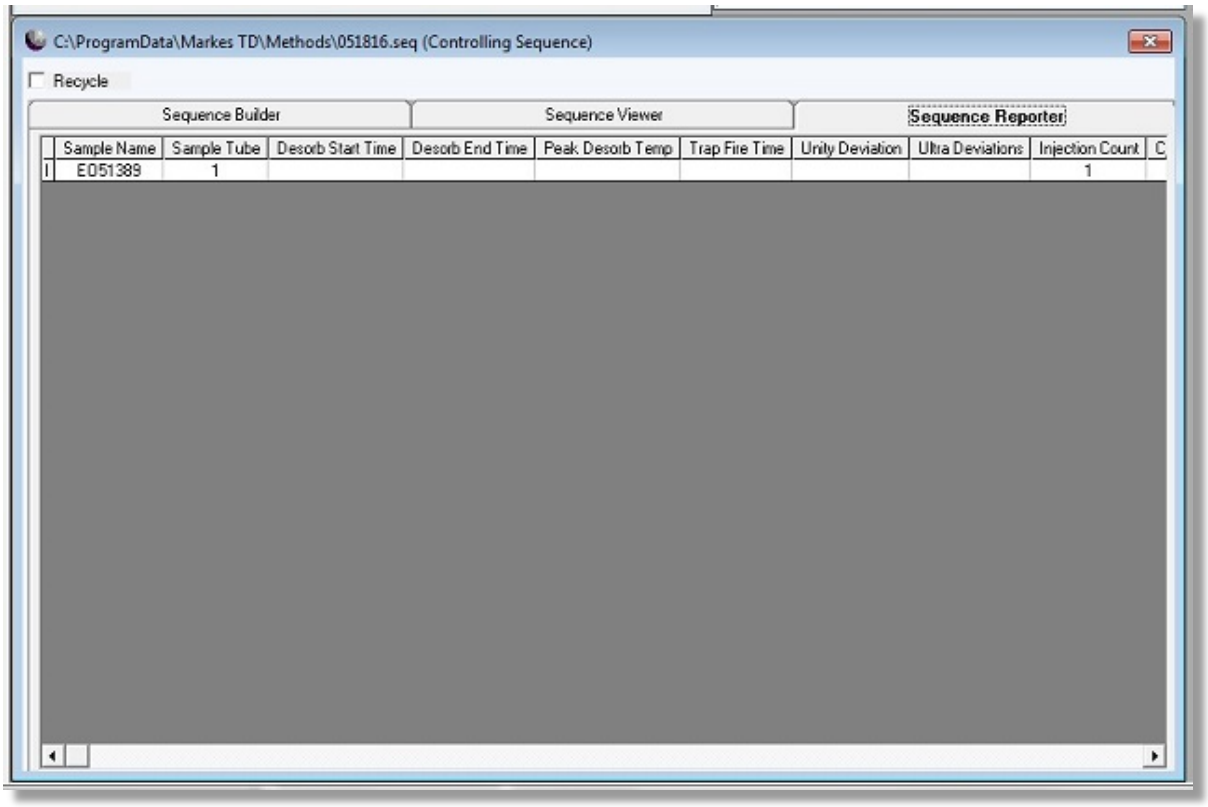

Figure 12. Sequence Reporter tab.

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 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.

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

Figure 14. Suggested maintenance schedule.

15.0 References and Supporting Documentation

15.1 References

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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.

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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
```

1.0 Scope and Application

1.1 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.

1.2 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."

1.3 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.

1.4 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

2.0 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 (D-EMMD-AQB-015-SOP-01) onto tubes conditioned using the Markes TC-20 (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.

3.0 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.

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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		

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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: [redacted] Date: 9/8/16

Approval

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

Signature: [redacted] Date: 09/08/2016

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

Section

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 studies QA Plan. If interferences not identified during method evaluation are discovered with study samples, they will be identified and evaluated as part of a studies ongoing 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 stabilty 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 (- 20º C) 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:

15 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 and 15 ºC 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 -20 ºC 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:

24 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 -20 ºC 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 studies 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.

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U.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurement Division
Public Health Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: Determination of Selected Organic Contaminants in Tire Crumb Rubber Subsamples for Multi-Residue Characterization by Ultra Pressure Liquid Chromatography/ Tandem Mass Spectrometry (UPLC-MS/MS)

SOP ID: D-EMMD-PHCB-SOP-2327-0	Effective Date: 01/29/2018

SOP was Developed: ☒ In-house

SOP Discipline*: Organic Chemistry

Alternative Identification:

SOP Contact Signature

Name: Elin Ulrich

Signature/Date: ELIN ULRICH Digitally signed by ELIN ULRICH Date: 2018.03.27 16:28:58 -04'00'

Management Signature

Name: Myriam Medina-Vera

Title: PHCB Chief

Signature/Date: MYRIAM MEDINA-VERA Digitally signed by MYRIAM MEDINA-VERA Date: 2018.03.28 07:04:14 -04'00'

QA Signature

Name: Margie Vazquez

Title: EMMD QA 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)
0	Larry McMillan	03/21/2018	Original SOP. Effective date: 3/21/18.

TABLE OF CONTENTS

SOP Title: *Determination of Selected Organic Contaminants in Tire Crumb Rubber Subsamples for Multi-Residue Characterization by Ultra Pressure Liquid Chromatography/ Tandem Mass Spectrometry (UPLC-MS/MS)*

1. Scope and Applicability

This standard operating procedure (SOP) describes a method for analyzing Tire Crumb Research Study (TCRS) subsamples for selected organic contaminants in Dermal Wipes, Air SVOCs, Field Dust, and Field Wipe.

2. Summary of Method

TCRS samples are extracted with acetone and hexane for gas chromatograph tandem mass (GCMSMS). After the GCMSMS analysis, approximately one milliliter (mL) is allowed to evaporate until dryness and reconstituted to 1mL with water and acetonitrile. After the extract is reconstituted, the listed compounds (Table 1) are determined by ultra-performance liquid chromatograph/tandem mass spectrometer (UPLC/MS/MS, also known as ultra-high pressure or UHPLC).

3. Definitions/Acronyms

3.1. Internal Standard (IS) A fixed amount of reference compound or solution is added to each sample and standard solution prior to extraction. The ratio of the detector signal of the native analyte to the detector signal of the internal standard (IS) is compared to the ratio obtained from the calibration curves where the IS level remains fixed and the native analyte levels vary. The internal standard is used to correct for minor sample-to-sample differences in extraction, purification, injection volume, chromatographic behavior, and mass spectrometry ionization efficiency.

3.2. Quality Control (QC) Samples Blanks, spikes, and duplicates are used as quality control measures.

- 3.2.1. Blanks are prepared with deionized laboratory water.
- 3.2.2. Spikes, deionized water is spiked with known amounts of reference materials targeted by each analysis. The concentrations of analytes in these QC samples are compared to their theoretical values for the determination of the accuracy of the analysis

3.3. TCRS: Tire Crumb Research Study

4. Health and Safety Warnings

Personnel must be thoroughly acquainted with the potential hazards of the reagents, products, solvents, equipment, and procedures described in this SOP. The current Material Safety Data Sheets (MSDS) for the chemicals used in this method should be consulted. The Health and Safety Research Protocol for this laboratory activity is on file. Care should be observed with

the use of all compounds specified in this protocol as some may be hazardous if used incorrectly (e.g., Formic Acid).

Waste Management: Solid, liquid, and glass waste are disposed of in separate containers. Solvents and samples used in this procedure should be disposed according to Health and Safety regulations and mindful of appropriate labeling and record keeping

5. Cautions/Interferences

Do not wash glass bottles in detergent, with other glasses or in washing unit that may have detergent residue. Washing glassware in a common dishwashing unit can contaminate it with detergent residues, which contain polyethylene glycol (PEG) and other sticky substances with vinyl coated steel racks can be and additional source of contaminations.

Do not perform further filtration on pre-filter solvents additional filtering can introduce contaminations.

Routine instrument maintenance is critical to achieve optimum sensitivity. All laboratory materials must be determined to be free of contamination to ensure potential background interferences are minimized.

User must review Waters Manual: Controlling Contamination in Ultra Performance LC®/MS and HPLC/MS Systems 715001307, Rev. D.

6. Personnel Qualifications/Responsibilities

This SOP assumes a thorough working knowledge of basic laboratory skills, reagents, and instrumentation. This document is designed to guide a competent laboratory worker. It is assumed that the user of this method is very familiar with liquid chromatography / mass spectrometry, MassLynx software, Microsoft Excel, and computer programs in general (1-2 years of experience).

7. Equipment and Supplies

UPLC-MS/MS system Waters Acquity (UPLC) ultra- performance liquid chromatography system (Waters Corporation, Milford, MA) with binary pump, auto sampler, column heater, or other equivalent automated HPLC system suitable for the instrument parameters outlined in this method.

The characteristics of the UPLC used in this SOP:

All solvent lines are polyether ether ketone (PEEK) tubing
Mobile phase stainless-steel filters.
Sample Syringe (10 µL)
Injection syringe (250 µL)

Sample Loop ASSY EXTENSION LOOP 100uL; ACQUITY APC

- Waters Xevo TQD Tandem mass spectrometer - Ion source: positive ion electrospray ionization, ESI+. and negative ion electrospray ionization ESI
- Waters Acquity UPLC BEH C18 reverse phase UPLC column, 2.1×50 mm, 1.7 µm (P/N 186002350) or equivalent column.
- Waters Acquity UPLC BEH C18 1.7um VanGuardtm Pre-Column 2.1 X 5 mm column PN 186003975
- N-EVAP 12 nitrogen evaporator (Organomation Associates INC West Berlin, MA) or equivalent nitrogen with 10–15 psi nitrogen.
- VWR 24/16 Galaxy 16 DH Centrifuge: rotation speed ≧ 3500 ppm. Serial no. R-104098
- Sonicator- Aquasonic Model 150HT Cat# 21811-804 VWR Scientific Products.
- Vortex mixer. - Maxi Mix II Barnstead/Thermolyze Model No M371615 Dubuque Iowa

Supplies

1. Microfiber Filters, Ultra Free MC Filters Cat# UFC30LG25 Hydrophilic PTFE Membrane.
2. Sample container, high density polyethylene HDPE, 1000 mL, wide mouth, with screw top (P/N 2189-0032 24/case Nalgene Labware, Rochester, NY) or equivalent
3. Centrifuge tube and cap, polypropylene, sterile, 15 mL (BD Falcon brand, BD, Franklin Lakes, NJ, P/N 352096, or equivalent)
4. Pipettor, variable volume, positive displacement, capability of 10 to 100, and 100 to 1000 microliter ranges (Eppendorf, Westbury, NY), or equivalent
5. Disposable polypropylene pipettor tips of various sizes (10, 100, 1000, 5000 uL) (Genesee Scientific, Research Triangle Park, North Carolina USA) or equivalent

8. Reagents and Standards

8.1. TCRS Standards 100 ug/ml in dichloromethane (DCM). These compounds were received from LAB D551.
8.2. Atrazine (Ring-13C3, 99%) 100 ug/ml in nonane -Cambridge Isotope Laboratories Andover MA.

8.3. Methanol (MEOH)- A456-4 Optima LC/MS UN1230 Fisher Chemicals
8.4. Acetonitrile(_ACN) – A955-4 Lot 17132 Optima LC/MS Fisher Chemicals
8.5. Mili-Q water, resistivity ≧ 18 MΩ·cm (@25℃)
8.6. Formic Acid A117-50 Optima LC/MS Cas 54-18-6 Fisher Chemicals
8.7. Ammonium Formate (NH4COOH) 99% Cas-540-69-2 ACROS

Table 1. Analytes for LC-MS/MS analysis.

Analyte	Molecular Formula	Monisotopic Mass	CAS#
Cyclohexylamine	C6H13N	99.104797 Da	108-91-8
Dicyclohexylamine	C12H23N	181.183044 Da	101-83-7
N,N-Dicyclohexylmethylamine	C13H25N	195.198700 Da	7560-83-0
2-Hydroxybenzothiazole (benzothiazolinone)	C7H5	151.009186 Da	934-34-9
2-Mercaptobenzothiazole	C7H5NS	166.986343 Da	149-30-4
Bis(2,2,6,6-tetramethyl-4piperidyl) sebecate	C44H84N	736.632935 Da	52829-07-9

9. Procedures

9.1 Mobile Phase Solution Preparation

Two different methods were developed to analyzed different compounds.

9.1.1. <u>Mobile Phase Preparation For Method #1</u>

Compounds List 1: *Cyclohexylamine*, *2-Hydroxybenzothiazole, N,N-Dicyclohexylmethylamine,* and *Bis(2,2,6,6-tetramethyl-4piperidyl sebecate)*

9.1.1.1. Solvent A – preparation of 0.1% formic 90/10 water: acetonitrile solution: Using 1000 ml graduated cylinder measure 100 ml of acetonitrile and add to a one-liter mobile phase bottle. Add 100 µl of formic acid. Add 900 ml of water. Labeled bottle A1- 0.1% FA 90:10 H20/ACN

9.1.1.2. Solvent B - preparation of 0.1% formic acid 90/10 acetonitrile: water solution: Using 1000 ml graduated cylinder measure 100 ml of water and add to a one-liter mobile phase bottle. Add 100 µl of formic acid. Add 900 ml of acetonitrile. Labeled bottle B1 - 0.1% FA 90:10 ACN/Water

9.1.2. <u>Mobile Phase Preparation For Method #2</u>

Compounds List 2: *Dicyclohexylamine* and *2-mercaptobenzothiazole*

9.1.2.1. Solvent A – preparation of 0.4 mM Ammonium Formate 90/10 water: Acetonitrile solution:
Using 1000 ml graduated cylinder measure 100 ml of methanol and add 25.2 mg of ammonium formate to a one-liter mobile phase bottle. Add 900 ml of water. Label bottle A1 - 0.4 mM NH4COOH 90:10 H20/MEOH

9.1.2.2. Solvent B - preparation of 0.4 mM Ammonium Formate 90/10 Acetonitrile: water solution:
Using 1000 ml graduated cylinder measure 100 ml of water and add 25.2 mg of ammonium formate to a one liter mobile. Add 900 ml of methanol. Label bottle B1 – 0.4 mM NH4COOH 90:10 MEOH/Water

9.2. Standard Solution Preparation #1

Compounds to be analyzed: *Cyclohexylamine, 2-Hydroxybenzothiazole, N,N-Dicyclohexylmethylamine*, and *Bis(2,2,6,6-tetramethyl-4piperidyl sebecate)*

9.2.1. Add 1 mL of MeOH to an evaporated TCRS standard to give a concentration of 10,000 pg/µL. The TCRS standard analytes can be found in the SOP titled Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples (SOP# D-EMMD-PHCB-033-SOP-01) . From this sample, prepare a series of solutions to generate a calibration curve. Prepare dilutions in 50:50 ACN:H_2O with 0.1% formic acid. The table below shows the dilutions and constituents of each sample which are prepared to a final volume of 1 mL.

Table 2. TCR standards for calibration curve

Concentration of STD (pg/µL)	Vol of Std (µL)	Vol of IS* @ 1000 pg/µL	Vol of solvent ACN:H_2O (µL)
200	200	100	700
150	150	100	750
100	100	100	800
80	80	100	820
60	60	100	840
40	40	100	860
20	20	100	880
10	10	100	890
5	5	100	895
*Internal Standard is $^{13}C_6$Atrazine			

9.2.2. Prepare vials (1 mL) of blank solvent and of IS (100 pg/µL) for analysis.

9.3. Standard solution preparation #2

Compounds to be analyzed: *Dicyclohexylamine* and *2-mercaptobenzothiazole*

9.3.1. Add 1 mL of MeOH to an evaporated TCRS standard to give a concentration of 10,000 pg/µL. From this sample, prepare a series of solutions to generate a calibration curve. Prepare dilutions in 50:50 MeOH:H2O with 0.1% formic acid. The table below shows the dilutions and constituents of each sample which are prepared to a final volume of 1 mL. Note that there is no internal standard for this procedure.

Table 3. TCR standards for calibration curve

Concentration of STD (pg/µL)	Vol of Std (µL)	Vol of solvent MeOH:H2O (µL)
200	200	700
150	150	750
100	100	800
80	80	820
60	60	840
40	40	860
20	20	880
10	10	890
5	5	895

9.3.2 Prepare vials (1 mL) of blank solvent for analysis.

9.4. Tire Crumb Rubber Study Standard Preparation

Two sets of calibration standards were prepared from TCRS Standard mix (1 ng/µL); one set was prepared in 50:50 MeOH:H2O and the other was prepared in in 50:50 ACN:H_2O. Table 4 below displays the volumes of solvents used to prepare the standards.

Standard Concentration (pg/µL)	**Volume of TCR mix (1 ng/µL)**	**Volume of solvent / µL** MeOH:H_2O or ACN:H_2O
5	5	995
10	10	990
20	20	980
40	40	960
60	60	940
80	80	920
100	100	900
150	150	850
200	200	800
250	250	750

Table 4. Calibration Standards prepared from the Tire Crumb Rubber standard mix.

9.5. TCRS LC sample prep

9.5.1. Organize TCRS samples per batch according to earlier Gas Chromatography preparations. Prepare 0.1% Formic Acid (FA) by adding 100 µL of FA in 100 mL of H2O and 100 mL of ACN separately.

9.5.2. Prepare a stock solution of methyl paraben 13C6 by adding 50 µL of 1,000,000 pg/ µL methyl paraben 13C6 to 5 mL of MeOH for a resulting concentration of 10,000 pg/µL.

9.5.3. Prepare a stock solution of atrazine 13C3 by adding 200 µL of 100,000 pg/µL atrazine 13C3 to 20 mL of MeOH for a resulting concentration of 1,000 pg/µL.
9.5.4. Prepare 150 mL of the IS by mixing 1.5 mL of 10,000 pg/µL methyl paraben 13C6 with 15 mL of 1,000 pg/µL atrazine 13C3 and diluting to a final volume of 150 mL using 50:50 0.1 % FA in H2O:ACN, resulting in 100 pg/µL solution.
9.5.5. Add 1 mL of IS to each TCRS sample and vortex. Some samples contain particulate matter after shaking and will need to be filtered before LC analysis.
9.5.6. Pipette the samples into centrifuge tube containing a filter (microcon-10 centrifugal filter, regenerated cellulose 10,000 NMVL).
9.5.7. Centrifuge the samples for 25 min at 13000 rpg. If the full volume of the sample does not fit entirely into the filter, repeat the process until the entire sample is filtered.
9.5.8. After filtering, transfer the samples into labeled amber vials for LC analysis

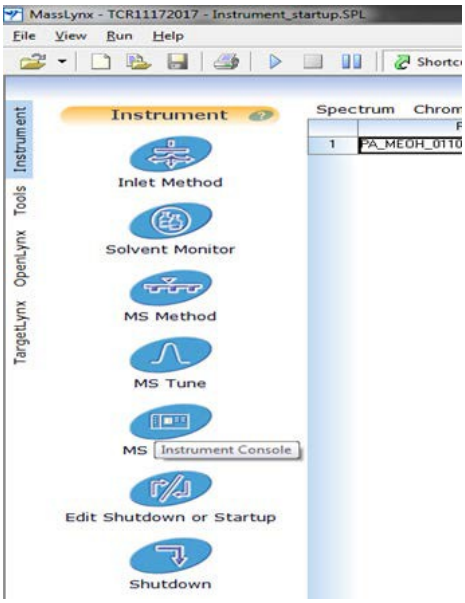
9.6. Instrument setup

General operation and maintenance of the UPLC-MSMS system are not detailed here. Users show follow Waters® Xevo® TQD ACQUITY UPLC® System Customer Familiarization Guide l for UPLC-MSMS and manufacturers recommendations. User must be familiar with MassyLnx 4.1 operational software.

Start up:

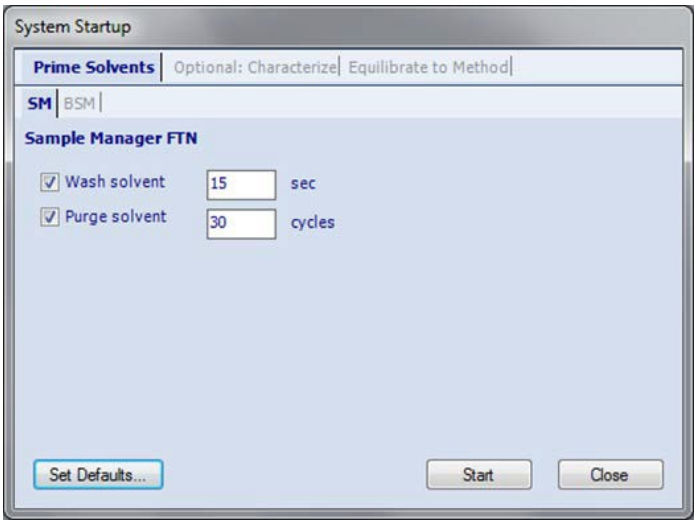
9.6.1. Double click to startup Masslynx from Icon in Desktop
9.6.2. At the default sample list go to file and open project
9.6.3. Select TCR1172017.PRO or Desired Project

9.6.4. Single Click on MS Instrument Console

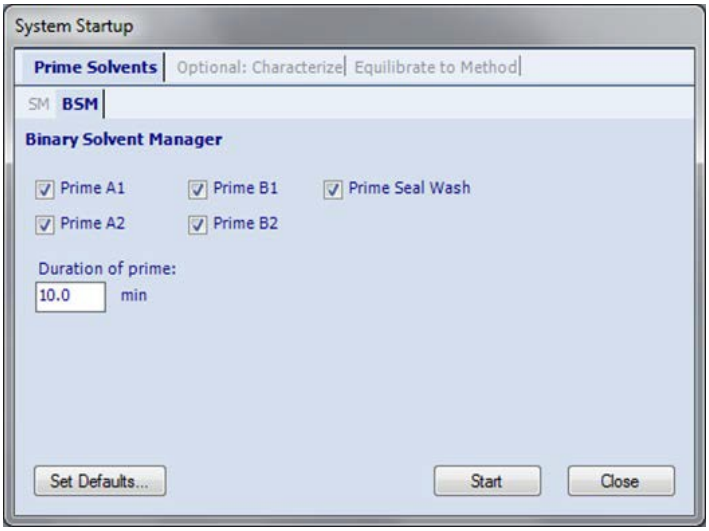


9.6.5. Select System startup for MS Console Window

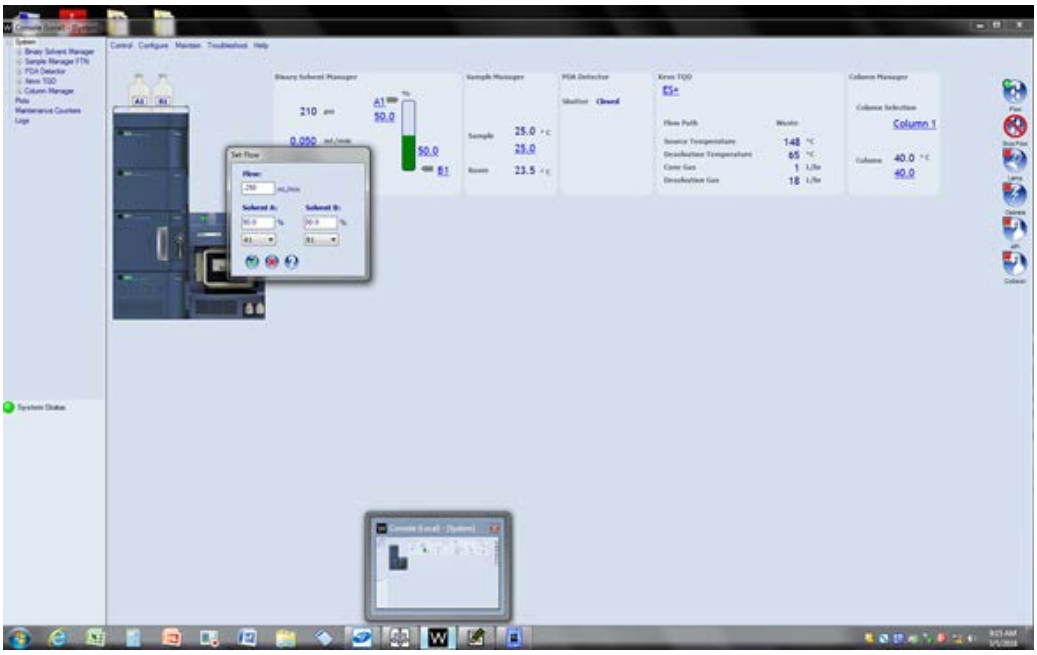
9.6.6. Check all



9.6.7. Select BSM, check all and set Duration for 10 min

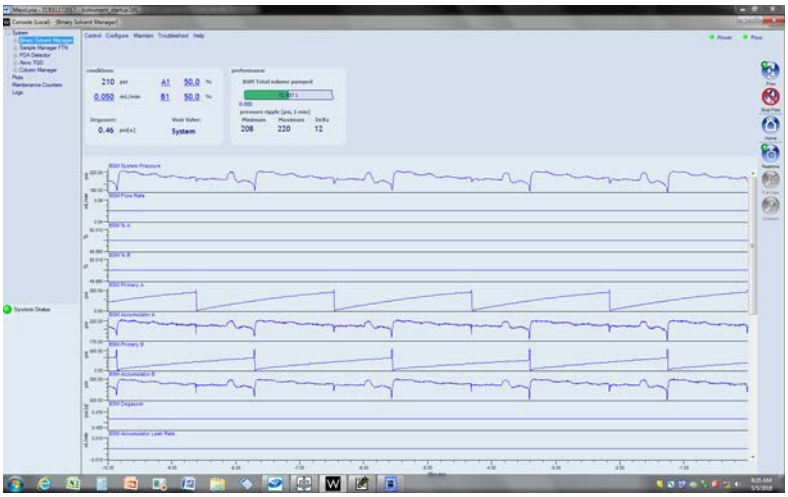


9.6.8. From MS Console Window Click on flow ml/min and set to flow =0.250, A1=50, B1 =50



9.6.9. Turn on PDA Lamp, Collison gas, API Gas and Operate by clicking the Icons on the let of MS Console Page. The lights will turn form red to green.

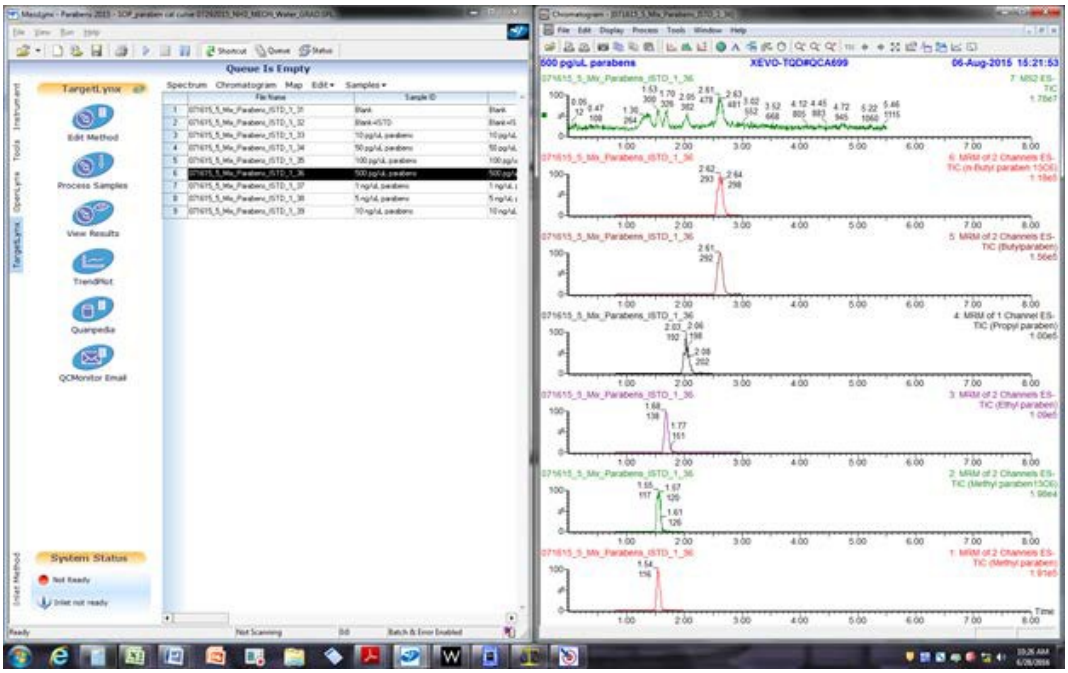
9.6.10. Select Binary Solvent Manager from MS Console Page. The Delta shown should be below 25.



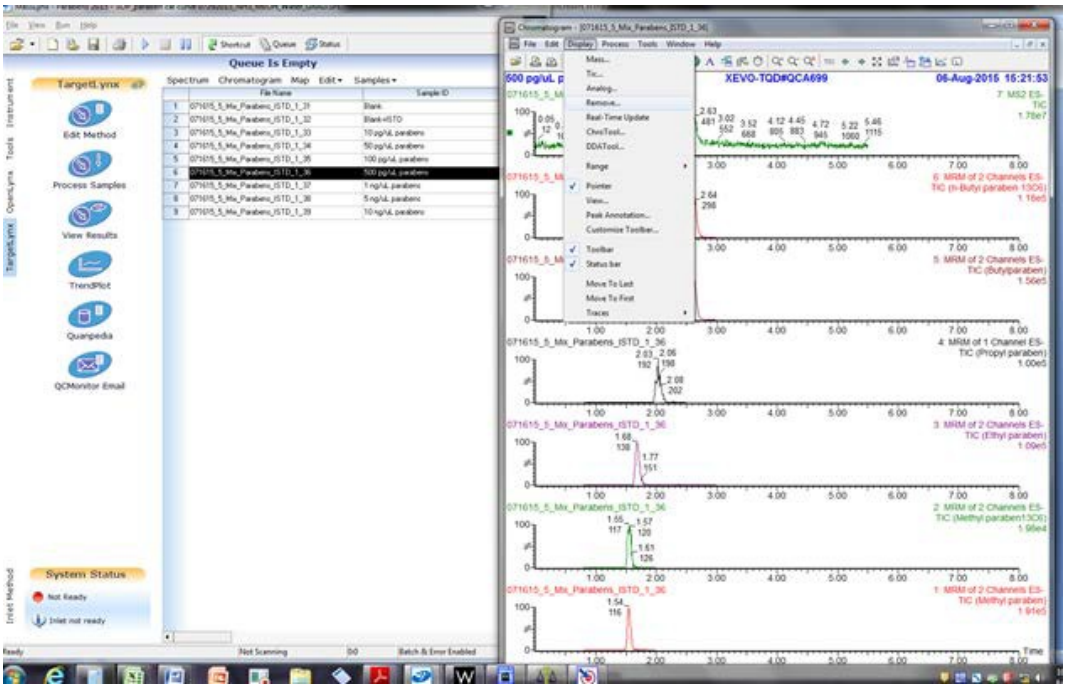
9.6.11. System is ready for use

9.7. Quantitation- Setting up Standard Curve

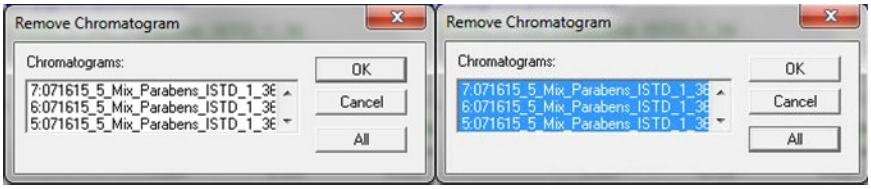
9.7.1. Choose a mid-point in the standard range i.e 500 pg/µl by clicking to highlight row 6.



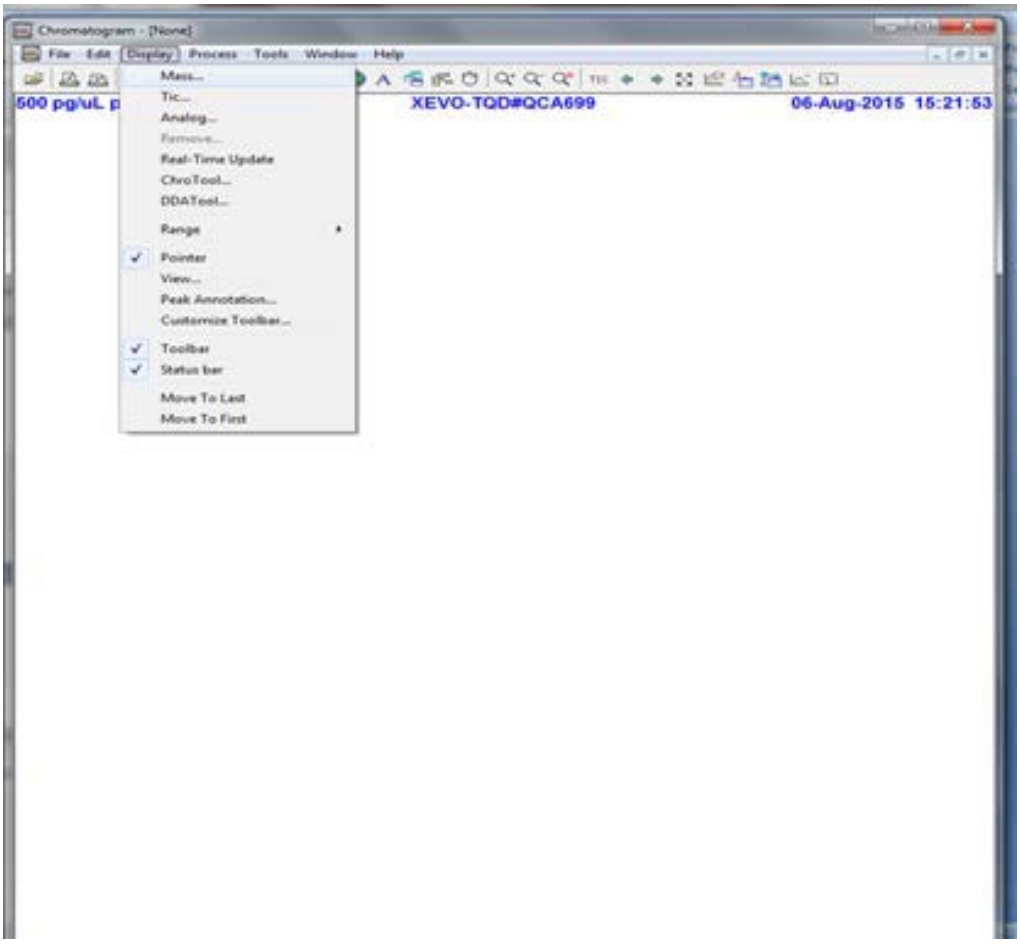
9.7.2. On the chromatogram screen select: Display Remove



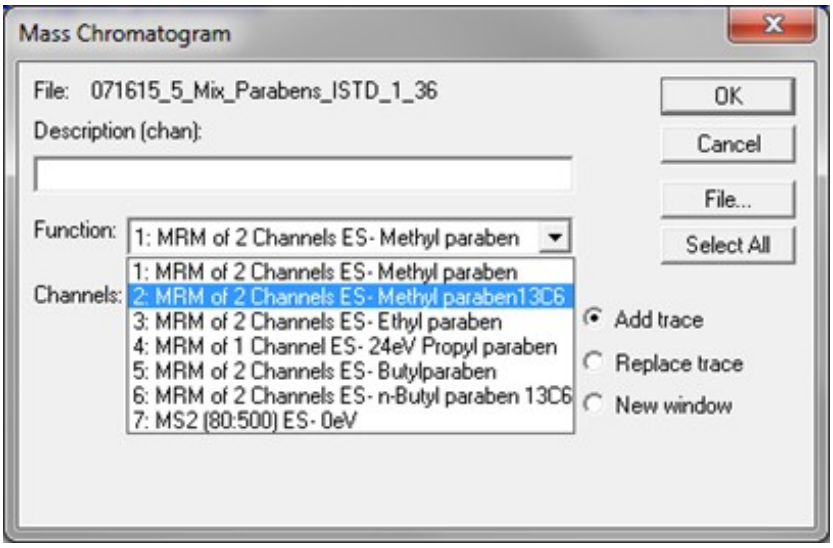
9.7.3. Select remove all and click OK



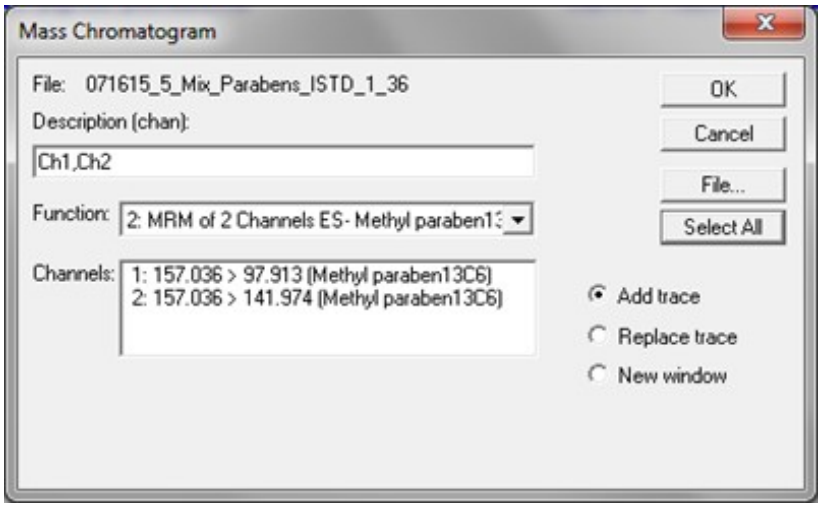
9.7.4. Go to display and select Mass

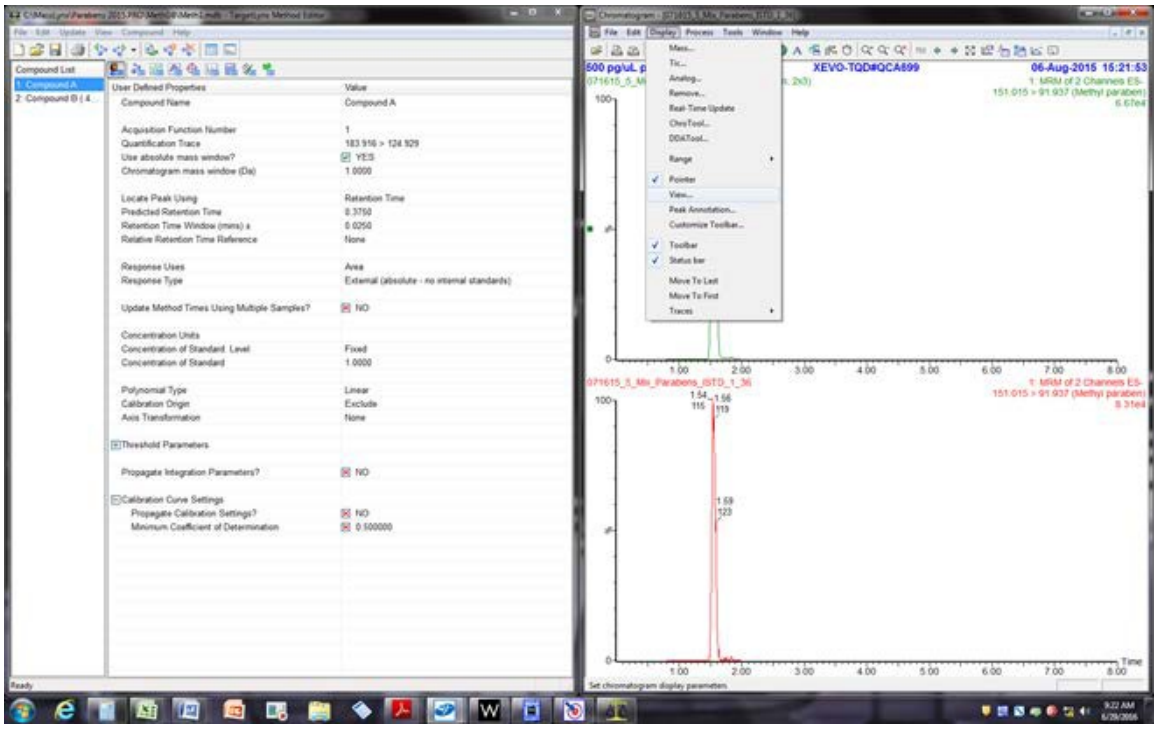


9.7.5. Select the Methyl paraben 13C6 - Note to be used as the internal standard. Double click on Channels 2.



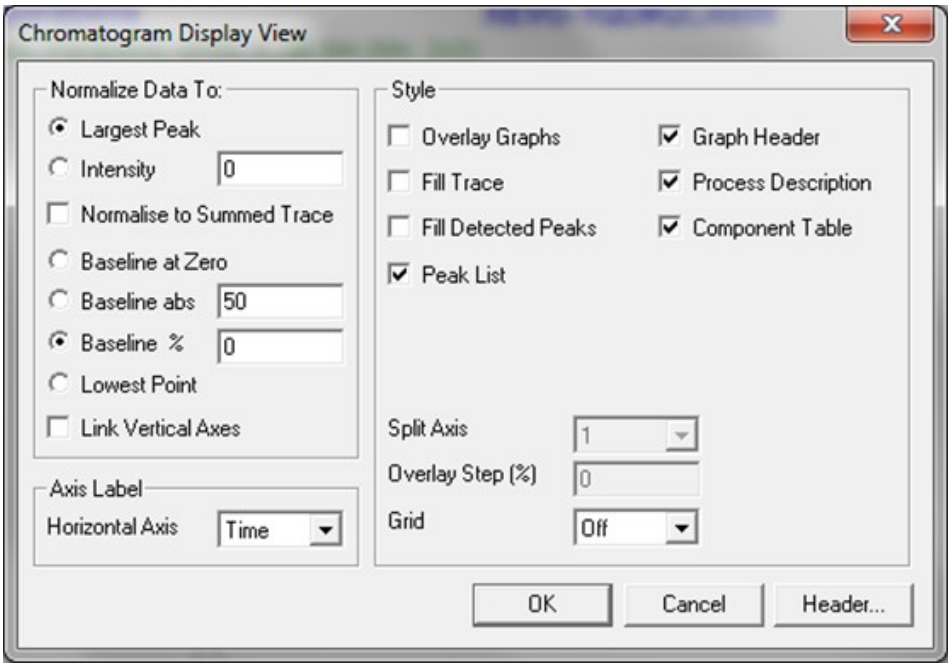
9.7.6. Click Select All. Select Add trace. Click OK.



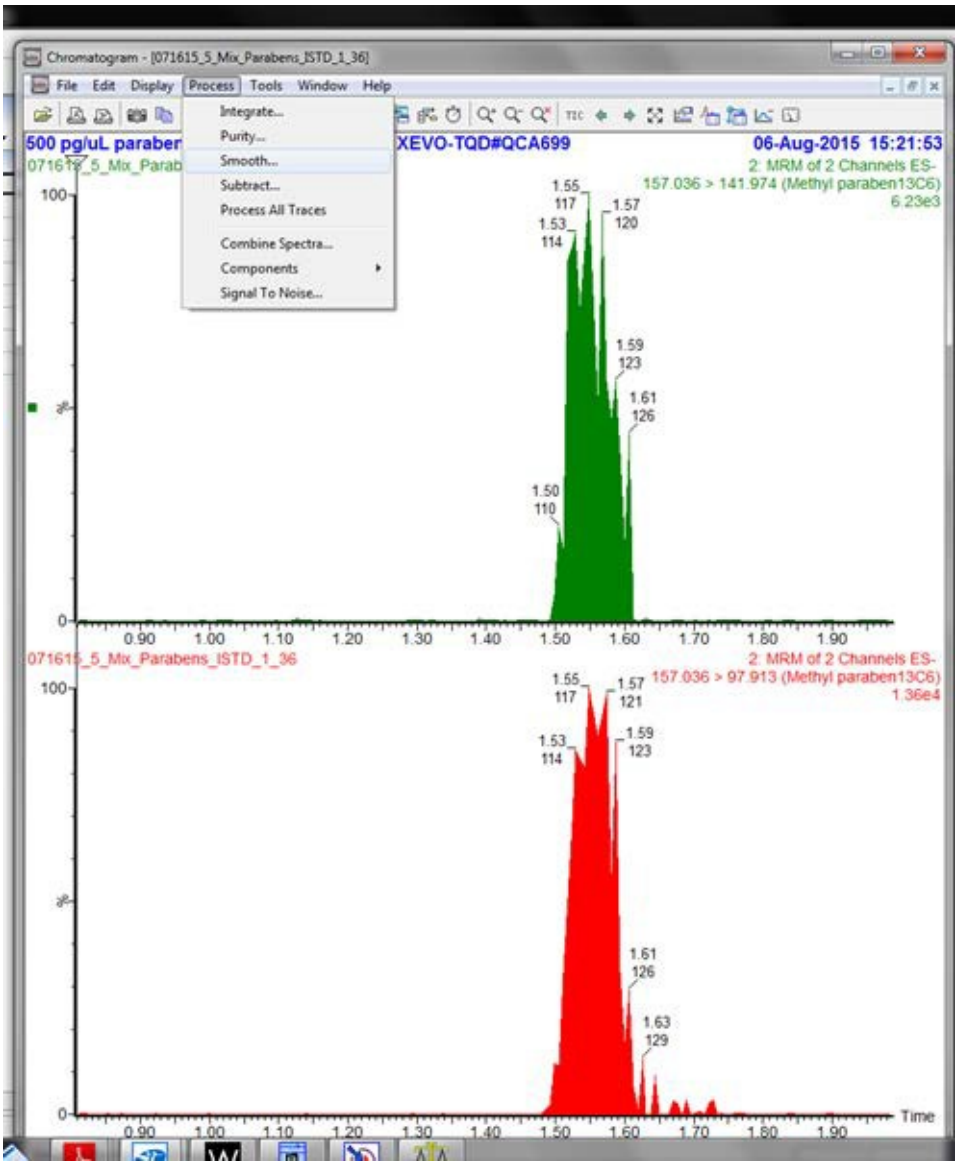


9.7.7. Click on Display and select View. Check the following:

a. Fill Trace
b. Fill Detected Peak
c. Graph Header
d. Process Description
e. Component Table



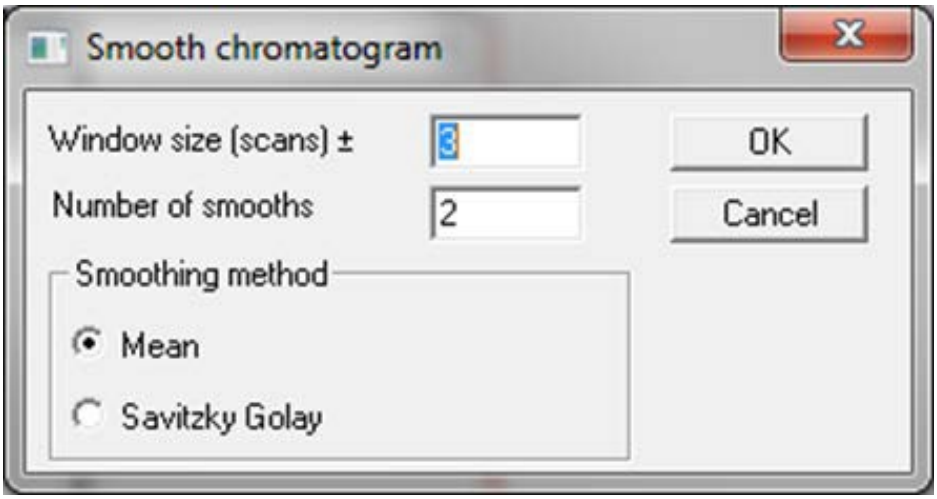
9.7.8. Click on the peak chromatogram window and color square appears across from the % sign to select. Click on process and select smooth.



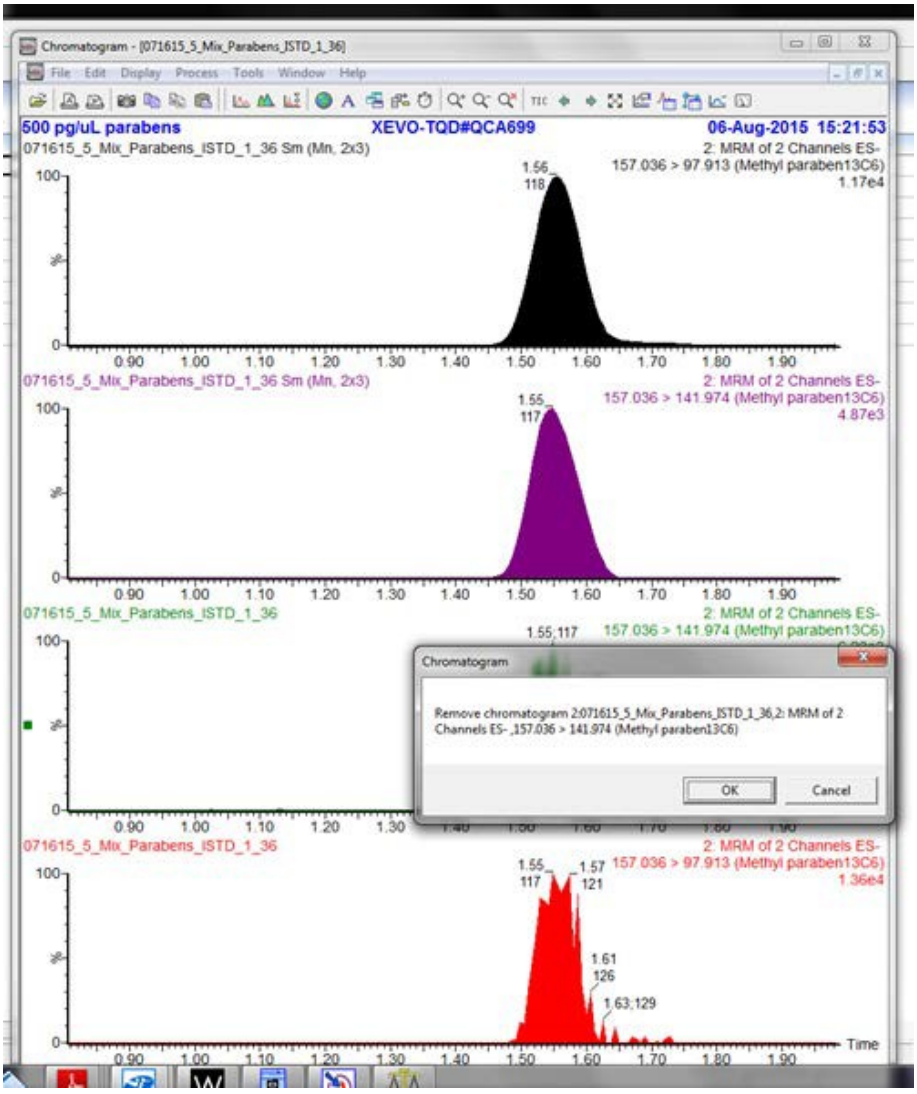
9.7.9. Enter 3 for Window size (scan)=/-

9.7.10. Enter 2 for Number of Smooths

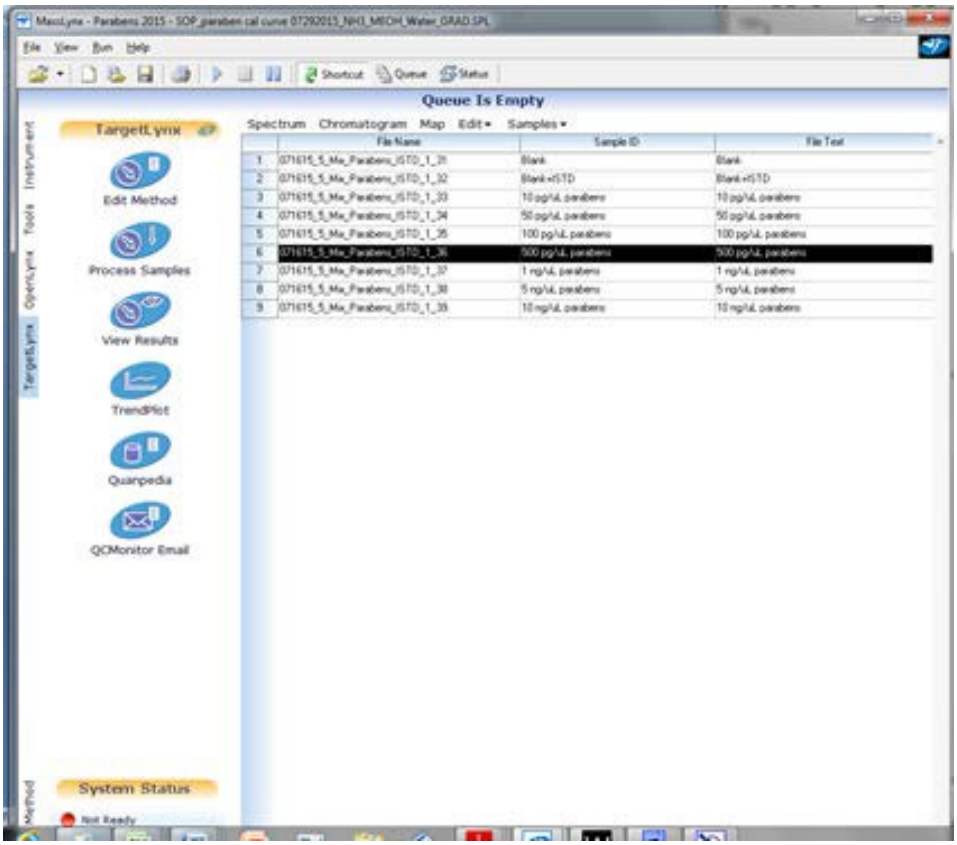
9.7.11. Select Mean for smoothing method. Click OK.



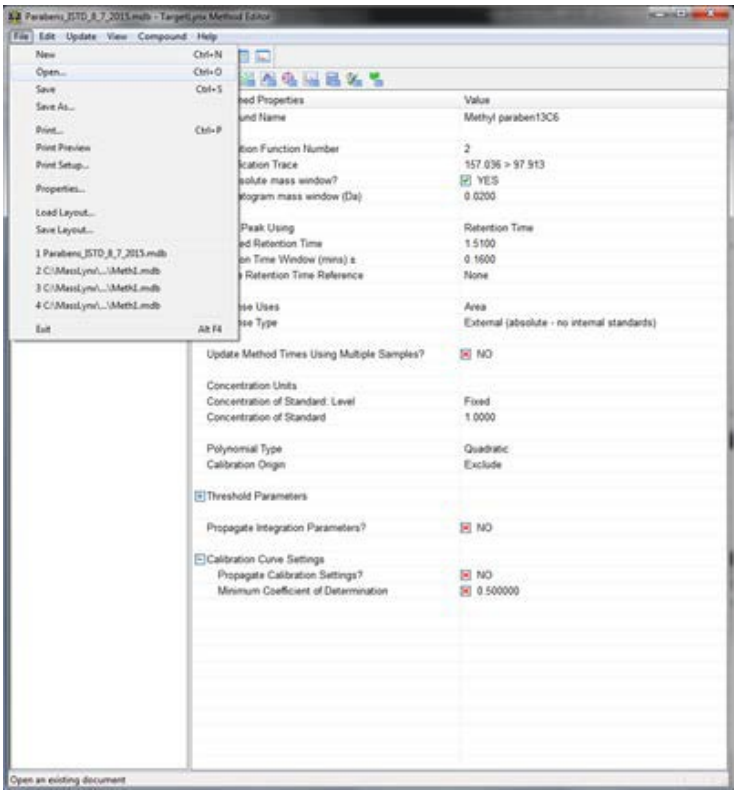
9.7.12. Click on unsmoothed peaks and press the delete key.



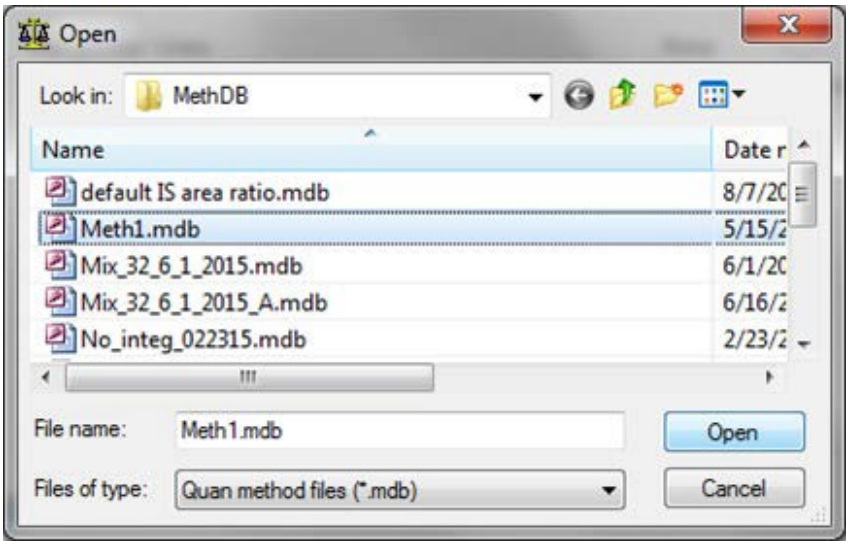
9.7.13. On the sample list page Click on TargertLynx. Click on Edit Method.



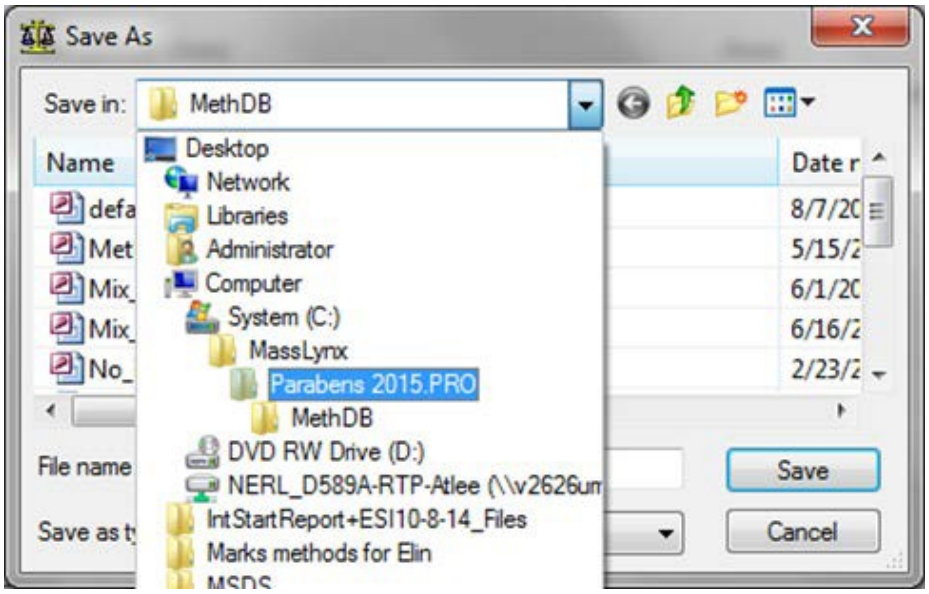
9.7.14. Go to file and select Open.



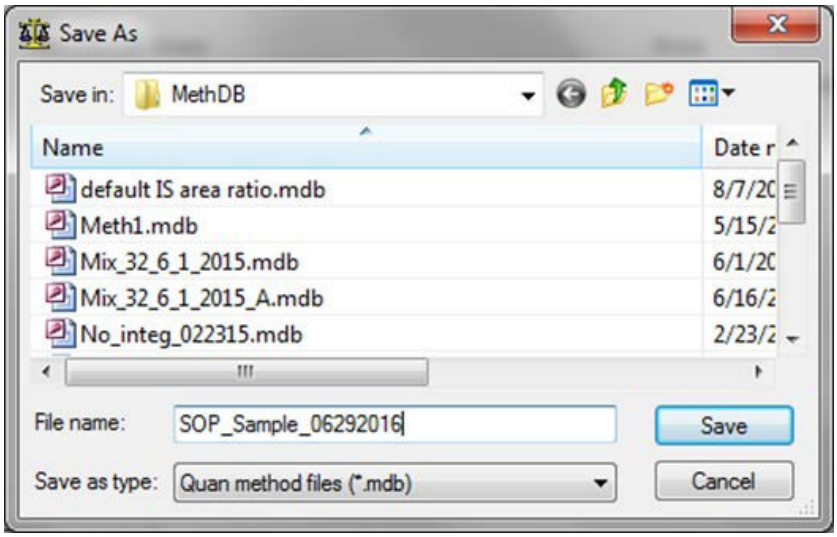
9.7.15. Click on Meth1.mdb



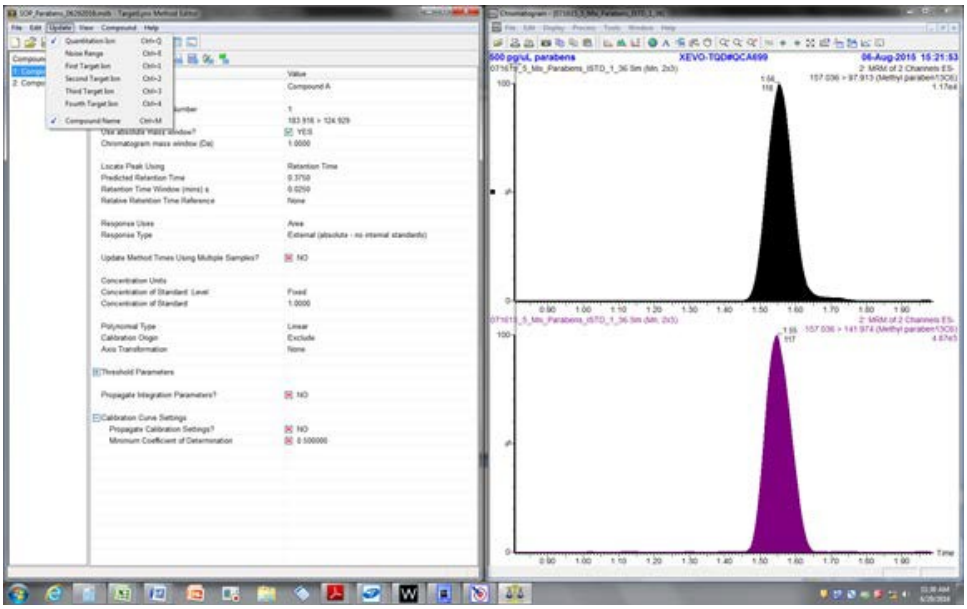
9.7.16. Go to file click on save as Select Project



9.7.17. Enter File Name and Click on Save.

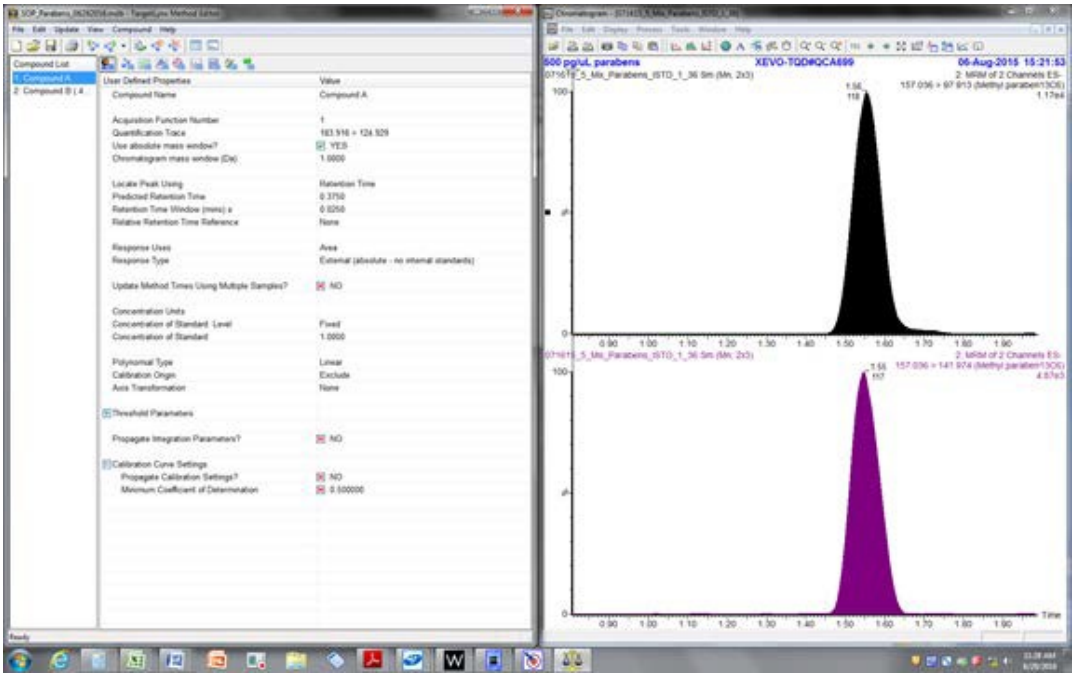


9.7.18. Click on Update. Check Quantitation Ion. Check Compound name.



9.7.19. Arrange the Method window and the Chromatogram windows side by side by holding the left mouse button on the Method page and dragging to left. Click on compound A. Note: Compound will be used as the internal standard i.e. 13C6 Methylparaben.

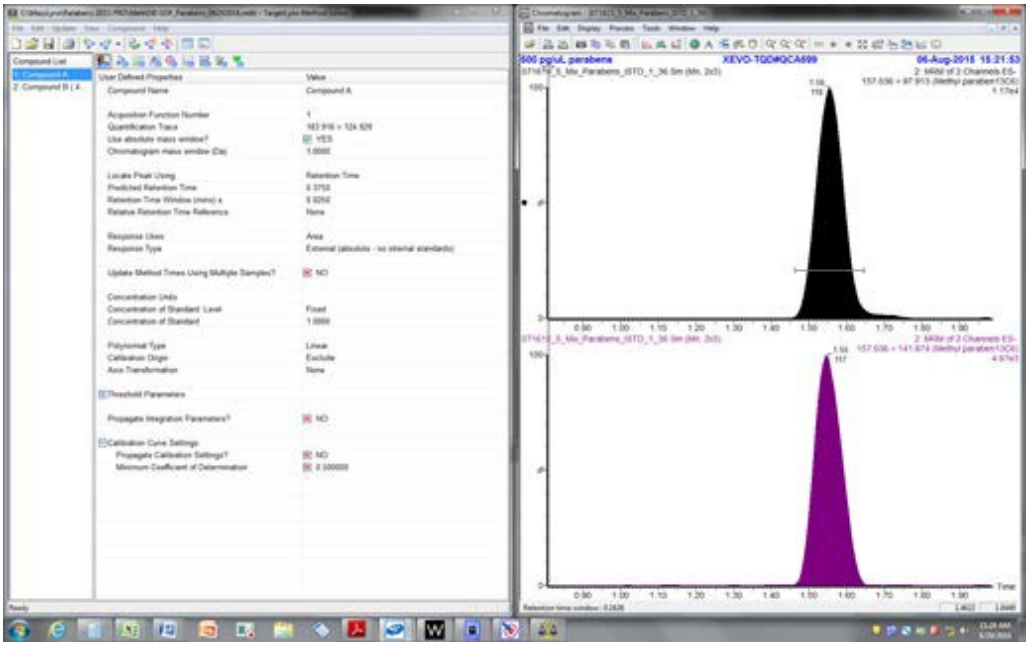
9.7.20. Click on First Icon “User Defined Properties”



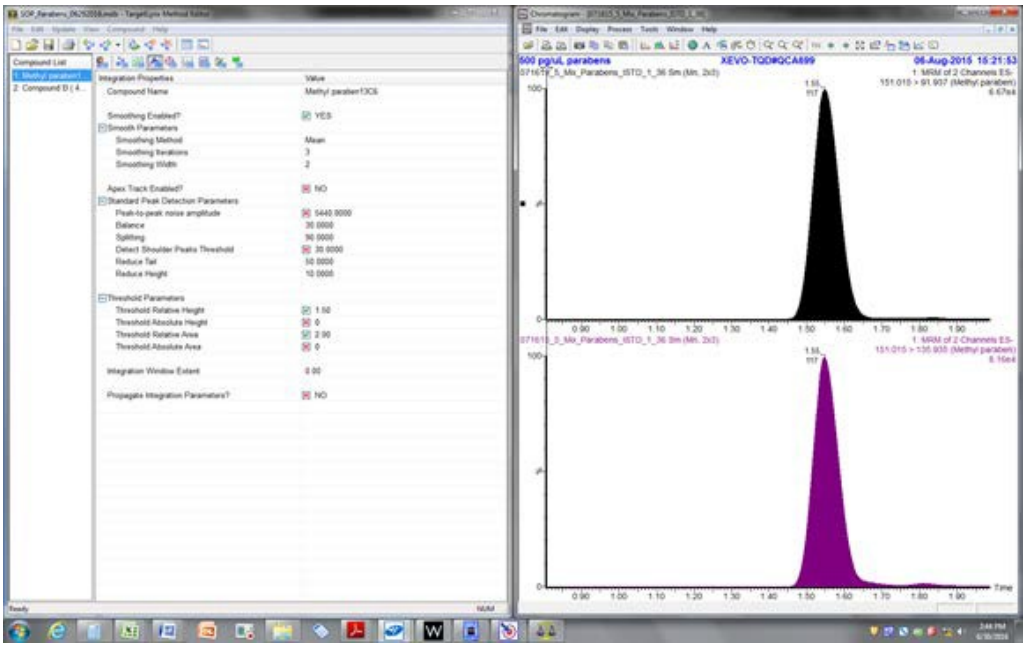
9.7.21. Click on Compound A

a. Select user define properties 
b. Right click on Peak 156.036>97 in Chromatogram window and drag a bracket across the peak.

9.7.22. Enter the following:
a. Response Uses – Area
b. Response Type – External (absolute- no internal standard)

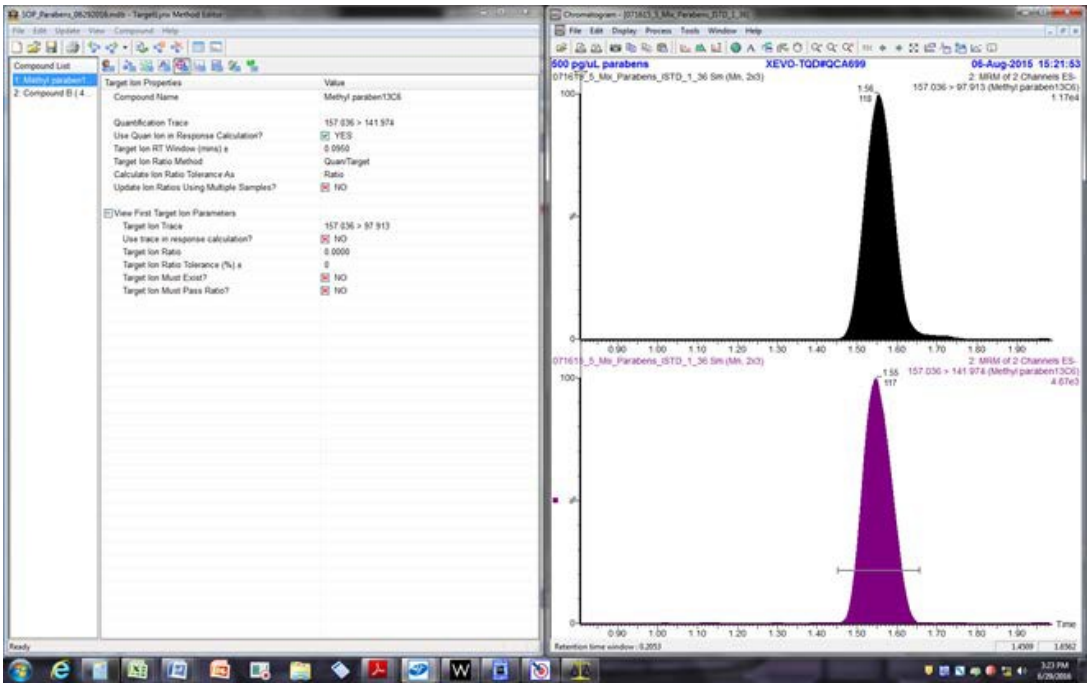


9.7.23. Click on Integration Properties 
9.7.24. Click “Yes” on Smoothing Enabled
a. Enter 3 Smoothing Iterations
b. Enter 2 for Smoothing Width



9.7.25. Click on Target Ion Properties 

9.7.26. Right click on Peak 156.036> 141.974 in Chromatogram window and drag a bracket across the peak.

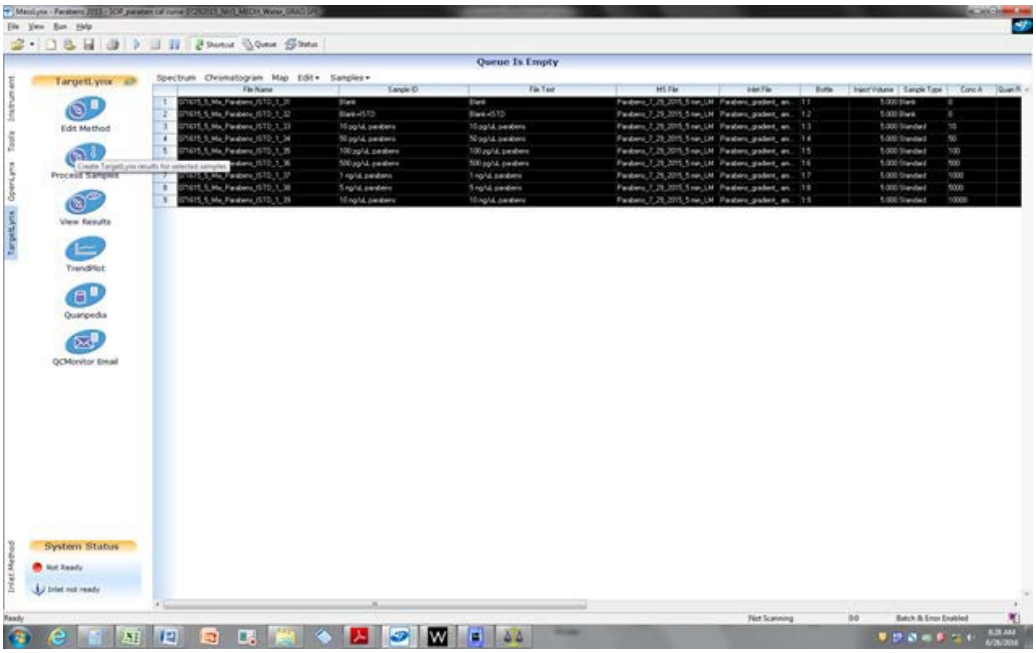


9.7.27. Click on the save button. 

9.7.28. Return to the Sample List.

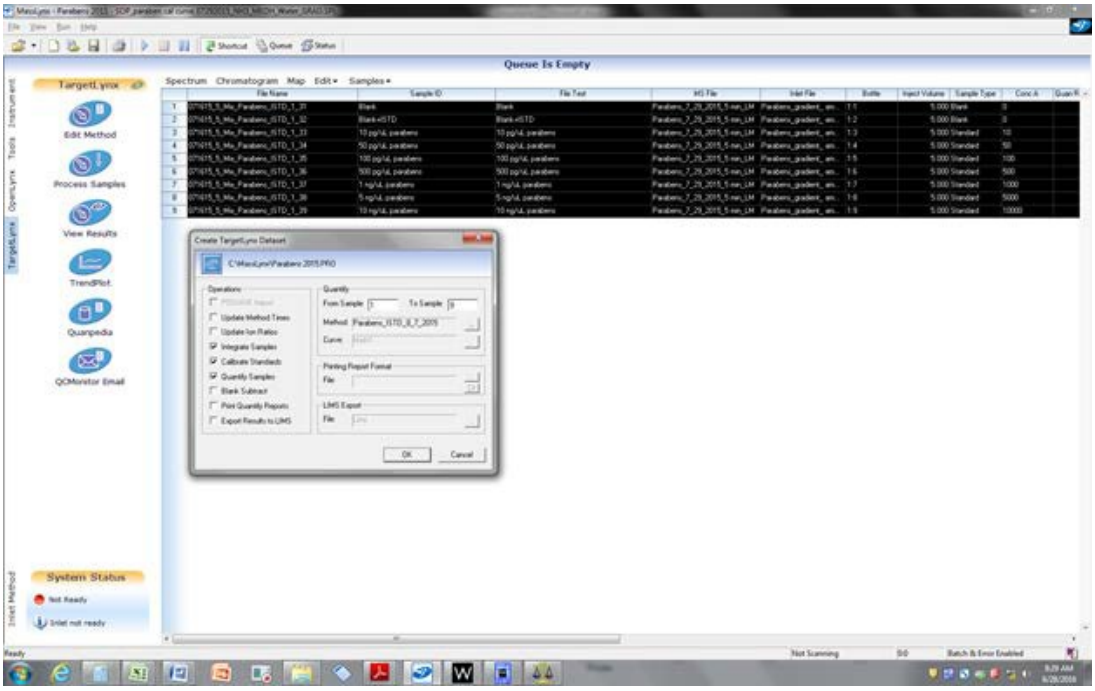
9.7.29. Highlight Row 2 -11 by dragging Mouse from row 2 -10.

9.7.30. Click on Process samples.

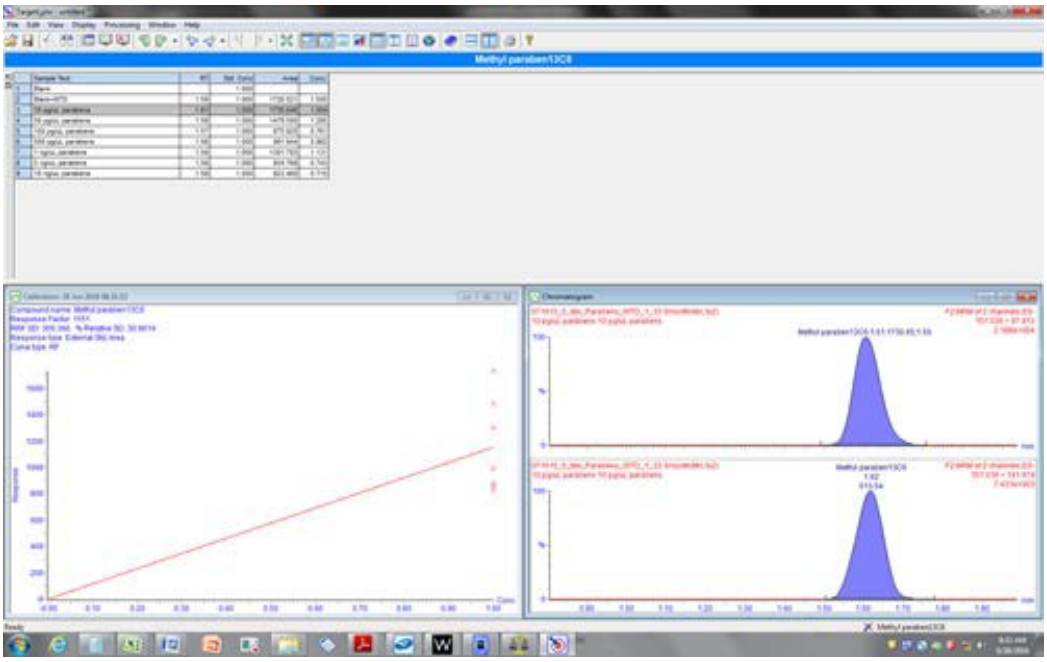


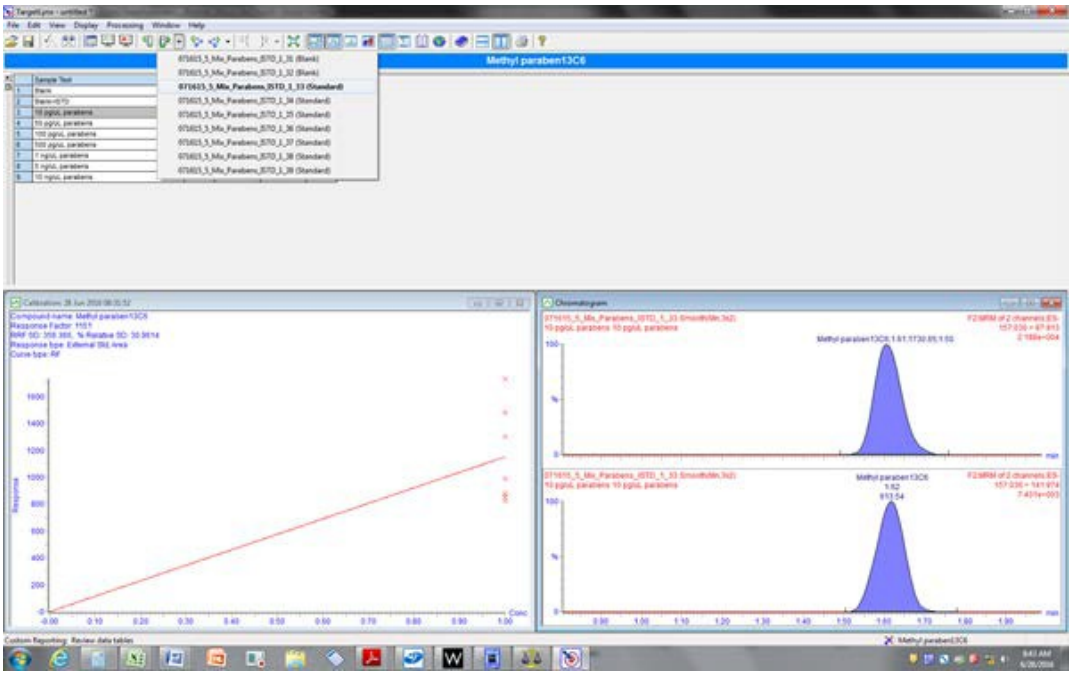
9.7.31. Check the following:

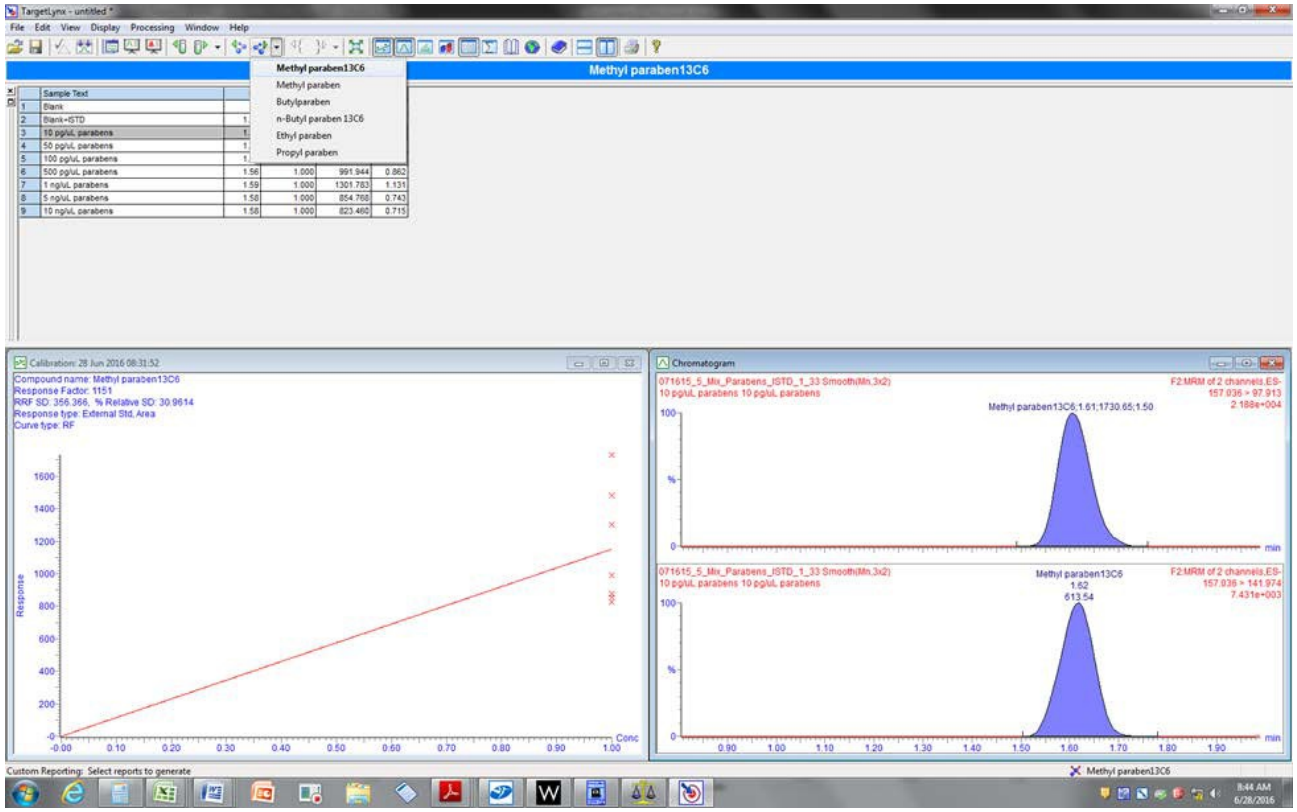
a. Intergrate Sample
b. Calibrate Standards
c. Quantify Sample
d. Click OK



9.7.32. Click on Line 3-10 pg/µl parabens. Note: All retention times show be with +/- % set in Method.






TargetLynx - untitled *
File Edit View Display Processing Window Help
Methyl paraben13C6
Methyl paraben13C6
Methyl paraben
Butylparaben
n-Butyl paraben 13C6
Ethyl paraben
Propyl paraben
Sample Text
1 Blank
2 Blank+ISTD
3 10 pg/uL parabens
4 50 pg/uL parabens
5 100 pg/uL parabens
6 500 pg/uL parabens 1.56 1.000 991.944 0.862
7 1 ng/uL parabens 1.59 1.000 1301.783 1.131
8 5 ng/uL parabens 1.58 1.000 854.766 0.743
9 10 ng/uL parabens 1.58 1.000 823.460 0.715
Calibration: 28 Jun 2016 08:31:52
Compound name: Methyl paraben13C6
Response Factor: 1151
RRF SD: 356.366, % Relative SD: 30.9614
Response type: External Std, Area
Curve type: RF
Response
Conc
Chromatogram
071615_5_Mix_Parabens_ISTD_1_33 Smooth(Mn,3x2)
10 pg/uL parabens 10 pg/uL parabens
F2:MRM of 2 channels,ES-
157.036 > 97.913
2.188e+004
Methyl paraben13C6;1.61;1730.65;1.50
071615_5_Mix_Parabens_ISTD_1_33 Smooth(Mn,3x2)
10 pg/uL parabens 10 pg/uL parabens
Methyl paraben13C6
1.62
613.54
F2:MRM of 2 channels,ES-
157.036 > 141.974
7.431e+003
min
Custom Reporting: Select reports to generate
Methyl paraben13C6
8:44 AM
6/28/2016

10. Data and Records Management

10.1. Raw data (including electronic data on individual PC hard drives and group shared drives) should be backed up, maintained and made available for review.

10.2. A consistent file naming convention should be documented and used for each specific project and data type generated.

10.3. Upon completion, data should be stored in accordance with EPA's record management policy.

10.4. All instrument data should be backed up to network drives on a regular basis and should be archived along with other supporting data and relative correspondence at the completion of the study.

10.5. Printed data should be referenced, signed and dated in accordance with the Office of Research & Development's Policies and Procedures Manual.

11. Quality Assurance/Quality Control

11.1. Analysis of standard solutions should result in a best fit regression coefficient of determination (r2) of 0.99 or greater, using a minimum of six independent concentrations that bracket the sample concentrations. Sample concentrations resulting in peak area ratios that are lower or greater than the range of standards should be reanalyzed with appropriate re- extraction or dilution of original water sample, respectively.

11.2. A blank sample must be analyzed every 10 samples. The blank must be prepared for 50:50 Water: Acetonitrile (H2O:ACN). When contaminations is noted analysts should stop the analysis and investigate further to identify the source of contamination before continuing.

11.3. A method blank must be analyzed prepared from mobile phase solvents A and B between every 10 samples. If significant analyte levels (S/N > 5) are found in the laboratory method blank, the source of contamination should be identified, corrected, and verified as being eliminated before additional analyses of unknown samples proceed.

11.4. For quality control check samples of deionized water fortified at one concentrations that bracket the six-point curve. A QC standard should be analyzed between every 10 samples. Data is considered acceptable if the calculated recoveries are within 70% of the expected values.

11.5. Recovery % = (Actual/Known) * 100 where actual is result received from analysis and known is the prepared results.

12. References

Determination of Dicyclohexylamine and Fumagillin in Honey by LC-MS/MSJohan P. van den Heever & Thomas S. Thompson & Jonathan M. Curtis & Stephen F. Pernal Springer Science+Business Media New York 2014

Simultaneous determination of benzotriazole and benzothiazole derivatives in aqueous matrices by mixed-mode solid-phase extraction followed by liquid chromatography–tandem mass spectrometry; I. Carpinteiro & B. Abuin & M. Ramil & I. Rodríguez & R. Cela # Springer-Verlag 2012

Rapid and sensitive LC–MS–MS determination of 2-mercaptobenzothiazole, a rubber additive, in human urine; Wolfgang Gries & Katja Küpper & Gabriele Leng # Springer-Verlag Berlin Heidelberg 2015

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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: [redacted] Date: 9/12/16

Approval

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

Signature: [redacted] Date: 09/12/2016

Concurrence*

Name:
Title:

Signature: Date:

For Use by QA Staff Only:

SOP Entered into QATS: _____ _____
Initials Date

Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples

Section

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 -20º C 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 content from the bottom to the top of the jar. Two separate 1g 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. 10 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 4000 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, QAQC samples will consist of a duplicate preparations, 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 ten 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 has 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 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 0.1ng/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 under freezer (-20º C) 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 -20º C) 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 1g 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 QAQC samples.

1 Reagent Spike
1 Reagent Blank
1 Reference TCR Sample

10.1.1 Reagent Spike Preparation

1. Label a clean 50mL 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 anutosampler 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 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).

12.0 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.

13.0 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 3 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 splitless mode Pulsed splitless 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	50º C for 2 min to 325º C at 10º C/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

U.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurements Division
Public Health Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: Standard Operating Procedure for Preparation of Synthetic Field Dust Samples for SVOC Analysis

SOP ID: D-EMMD-PHCB-068-SOP-01	Effective Date: 07/07/2017

SOP was Developed: ☒ In-house ☐ Extramural: enter organization

SOP Discipline*: Organic Chemistry

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

Standard Operating Procedure for Preparation of Synthetic Field Dust Samples for SVOC Analysis

Table of Contents

1.0 Scope and Application

This SOP details the extraction and work-up procedures for dust samples that are collected from synthetic turf fields. This SOP is written for the extraction and preparation of samples for analysis of semi-volatile organic chemicals (SVOCs).

2.0 Summary of Method

Dust samples are stored in a freezer at -20º C after receipt at the EPA lab. The samples are allowed to warm to room temperature before weighing 100 mg of dust into a 50 mL polypropylene centrifuge tube. Internal Standard solution is added to each tube along with a ceramic homogenizer. 10 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 4000 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. Quality assurance/quality control (QA/QC) samples will consist of a duplicate preparation (if enough sample is available), a reagent spike, reagent blank, method spike, method blank and a recovery spike.

3.0 Definitions

3.1. **Synthetic Field Dust Samples** – Samples of dust collected from a synthetic turf field and processed to 150 µm during collection.

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 method 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 ten 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 has been through the extraction procedure to determine losses or interferences from the method.

3.7. **Method Spike** – An extract prepared from fortifying diatomaceous earth with spiking solution prior to extraction to evaluate recovery from matrix.

3.8. **Method Blank** – An extract prepared using unfortified diatomaceous earth to evaluate analyte contribution from the matrix.

3.9. **Recovery Spike** - An extract prepared using diatomaceous earth that is extracted and then fortified to evaluate matrix effects and solution errors assuming 100% recovery.

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.

5.3 Diatomaceous earth, pre-cleaned, muffled

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

5.15 Stainless steel spatula

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 QA 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 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 2 and 3 prior to sample analysis. Calibrate the GC/MS/MS system in a range from 0.1ng/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 Analytical Procedure

9.1 Sample and QA/QC Sample Preparation and Extraction

9.1.1 Sample Preparation

1. Remove the synthetic field dust samples from the freezer and allow to warm to room temperature (approximately 30 minutes). Mix the dust inside the collection vial well using a clean stainless steel spatula. For each sample, weigh a 100 mg aliquot into a 50 mL polypropylene tube. Record the weight.
2. One sample batch will consist of up to 24 tire synthetic field dust samples along with the following QA/QC samples.
    - 1 Reagent Spike
    - 1 Reagent Blank
    - 1 Method Spike
    - 1 Method Blank
    - 1 Recovery Spike

9.1.2 Reagent Spike Preparation

1. Label a clean 50mL 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 by adding 100 µL of spiking solution and 100 µL of Internal Standard solution.
4. Cap and process with dust samples as outlined in 10.1.7.

9.1.3 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 dust samples as outlined in 10.1.7.

9.1.4 Method Spike Preparation

1. Label a clean 50mL polypropylene tube as the Method Spike.
2. Weigh 100 mg of diatomaceous earth into the tube.
3. Fortify the diatomaceous earth with 100 µL of spiking solution and 100 µL of Internal Standard solution.

4. Add a ceramic homogenizer and 10 mL of 1:1 Acetone:Hexane to the tube.
5. Cap and process with dust samples as outlined in 10.1.7.

9.1.5 Method Blank Preparation

1. Label a clean 50mL polypropylene tube as the Method Blank.
2. Weigh 100 mg of diatomaceous earth into the tube.
3. Fortify the diatomaceous earth with 100 µL of Internal Standard solution.
4. Add a ceramic homogenizer and 10 mL of 1:1 Acetone:Hexane to the tube.
5. Cap and process with dust samples as outlined in 10.1.7.

9.1.6 Recovery Spike Preparation

1. Label a clean 50mL polypropylene tube as the Method Blank.
2. Weigh 100 mg of diatomaceous earth into the tube.
3. Add a ceramic homogenizer and 10 mL of 1:1 Acetone:Hexane to the tube.
4. Cap and process with dust samples as outlined in 10.1.7.
5. After sample extraction, fortify the extract with 100 µL of spiking solution and 100 µL of Internal Standard Solution.

9.1.7 Internal Standard Addition to the Samples

1. Transfer a 100 µL aliquot Internal Standard solution to the surface of the dust for each sample.
2. Add a ceramic homogenizer and 10 mL or 1:1 acetone:hexane.
3. Cap and process with QA/QC samples as outlined in 10.1.7.

9.1.8 Dust and QA/QC Sample Extraction

1. Vortex for 1 minute.
2. Allow to sit for 2 minutes.
3. Vortex for an additional minute.
4. Centrifuge at 4000 RPM for 5 minutes.
5. Transfer the solvent layer to a 15 mL amber tube with PTFE-lined cap.
6. Transfer a 1 mL aliquot to an autosampler vial for GC/MS/MS analysis.

7. Transfer a second 1 mL aliquot from each sample extract to a clean anutosampler vial for solvent exchange to methanol for LC/TOF analysis.
8. Store remaining extracts in a freezer at -20º C.

9.2 Sample Analysis by GC/MS/MS

Analyze samples by GC/MS/MS using the conditions specified in Tables 2 and 3.

9.3 Sample Analysis by LC/TOF

Analyze samples by LC/TOF using the conditions specified in 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).

10.0 Records

Chain of custody records will be maintained to document the removal and extraction of each dust 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 NERL laboratory notebook.

11.0 Quality Control and Quality Assurance

Data will be reviewed by the analyst. 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, Method Spike, Method Blank, and Recovery Spike will serve to measure method performance for each sample. Target recovery for the reagent spike, method spike, and recovery 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.

Table 1. Spiking and Internal Standard 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 splitless mode Pulsed splitless 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	50º C for 2 min to 325º C at 10º C/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[a]	Pre1[b]	Prod 1[c]	CE[d]	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			

Time Segment	Compound	Class	RT[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE

Time Segment	Compound	Class	RT[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
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			
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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			

Time Segment	Compound	Class	RT[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
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			
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

U.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
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Public Health Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: Standard Operating Procedure for Preparation of Dermal and Surface Wipe Samples for SVOC Analysis

SOP ID: D-EMMD-PHCB-067-SOP-01	Effective Date: 07/07/2017

SOP was Developed: ☒ In-house ☐ Extramural: enter organization

SOP Discipline*: Organic Chemistry

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Standard Operating Procedure for Preparation of Dermal and Surface Wipe Samples for SVOC Analysis

Table of Contents

1.0 Scope and Application

This SOP details the extraction and work-up procedures for wipe samples that are collected for both dermal and surface measurements for measurement of semi-volatile organic chemicals (SVOCs). This SOP is written to address specific conditions used for samples collected for tire crumb rubber research, but the general procedure can be used and modified as appropriate providing quality assurance/quality control (QA/QC) criteria are met and any modifications are recorded in a laboratory notebook.

2.0 Summary of Method

Samples are first removed from freezer storage and are allowed to warm to room temperature. The jars containing the samples are carefully opened and internal standard solution is added to the surface of each wipe sample. Each jar containing a dermal or 4"x4" surface wipe is filled with 50 mL of 1:1 acetone:hexane and is then sealed with a PTFE-lined cap. Jars containing the larger drag sled wipes are filled with 250 mL of 1:1 acetone:hexane. 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 containing ~ 10g of anhydrous sodium sulfate 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 through a 0.2 μm PTFE syringe filter into autosampler vials for analysis.

3.0 Definitions

3.1 **Dermal Wipe** – Wipe sample collected from a participant's skin.

3.2 **Surface wipe** – Wipe collected from a field surface.

3.3 **Drag Sled Wipe** – Large wipe sample collected using a weighted drag sled.

3.4 **Internal Standard (IS)** – Internal standard solution which is used in quantification to establish response ratios.

3.5 **Method Blank** – Unfortified media that is extracted to evaluateinterferences and possible contamination in the media or lab.

3.6 **Method Spike** – Media that is fortified and extracted to evaluate analyte recovery from the extraction process.

3.7 **Recovery Spike** – Unfortified media that is extracted and processed like the Method Blank. The resulting 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 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 Clean wipe media, (Twillwipes MG Chemicals 4”x4” or Texwipe TX 100 12”x12”)

5.2 Stainless steel forceps

5.3 Spiking Solution, applicable to analytes being measured

5.4 Internal Standard Solution (IS), applicable to analytes being measured

5.5 Pipette or syringe capable of accurately delivering 100 µL of solution

5.6 Hexane, pesticide grade or equivalent

5.7 Acetone, pesticide grade or equivalent

5.8 Amber glass jars (60 or 500 mL, I-Chem 300 series or equivalent)

5.9 Glass analytical funnels

5.10 Glass wool

5.11 Anhydrous Sodium Sulfate, 10-60 mesh

5.12 250 mL glass bottles, narrow mouth

5.13 Parallel evaporator, Buchi Multivapor P6 or equivaleR-200 or equivalent

5.14 Boiling flasks, 500 mL

5.15 Rotary evaporator, Buchi

5.16 Glass serological or volumetric pipette capable of 5 mL

5.17 Pasteur pipettes – 9”

5.18 Graduated tubes, glass, 15 mL

5.19 Nitrogen evaporator with heated water bath (N-Evap or equivalent) or dry block

5.20 Glass Lauer-tip syringes

5.21 Syringe filters, PTFE, 13 mm 0.2 µm

5.22 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 QA 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 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 Method Calibration

The GC/MS/MS instrument should be calibrated for the analytes using the parameters listed in Tables 2 and 3 prior to sample analysis. Calibrate the GC/MS/MS system in a range from 0.1ng/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 Extraction Procedure for 4"x4" Wipe Samples

9.1 Wipe Preparation

9.1.1 Remove 4"x4" wipe samples from the freezer and let warm to room temperature.

One sample batch will consist of the following:

- Up to 24 wipe samples (from freezer)
- 1 Method spike
- 1 Method blank
- 1 Recovery spike

9.1.2 While the samples from the freezer are warming to room temperature, the Method spike, Method blank and recovery spike can be prepared for extraction.

9.2 Method Spike Preparation

9.2.1 Place one clean 4" x 4" wipe into a 60 mL wide mouth glass jar.
9.2.2 Transfer a 50 µL aliquot of Spiking Solution to the wipe.
9.2.3 Transfer a 100 µL aliquot of Internal Standard solution to the wipe.
9.2.4 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

9.3 Method blank Preparation

9.3.1 Place a clean 4" x 4" wipe into a 60 mL wide mouth glass jar.
9.3.2 Transfer a 100 µL aliquot of Internal Standard solution to the wipe.
9.3.3 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

9.4 Recovery Spike Preparation

9.4.1 Place a clean 4" x 4" wipe into a 60 mL wide mouth glass jar.
9.4.2 Add 50 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

9.4.3 After extraction and concentration, add 50 µL of spiking solution to the extract.

9.4.4 Add 100 µL of internal standard solution, cap and vortex along with the other samples processed in the sample batch.

9.5 Internal Standard Addition

9.5.1 After the sample has reached room temperature, write the sample ID on the top of the cap and carefully remove the cap.

9.5.2 Fortify the wipe sample with 100 µL of Internal Standard solution.

9.5.3 Add 50 mL of 1:1 acetone:hexane to the jar and seal with the sample ID labeled PTFE-lined cap.

9.6 Sample Extraction (Spikes, Blanks and Wipe Samples)

9.6.1 Place samples into an Ultrasonic cleaner with the water level well below the level of the jar's cap and start the cleaner.

9.6.2 Allow the cleaner to run for 15 minutes.

9.6.3 Carefully remove the samples from the ultrasonic cleaner, drying the outside of each jar with a paper towel as it is removed.

9.6.4 Assemble an analytical funnel on a 250 mL glass Boston round bottle and label one bottle for each sample extract.

9.6.5 Place a glass wool plug into each funnel and add ~ 10g of anhydrous sodium sulfate into each funnel.

9.6.6 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. Carefully remove the jars.

9.6.7 Add another 50 mL of 1:1 acetone:hexane to each jar. Recap the jars.

9.6.8 Rinse the inside of each funnel with hexane from a wash bottle and collect the rinse into its bottle.

9.6.9 Repeat steps 9.6.1 to 9.6.3 and 9.6.6 to 9.6.8 two more times, omitting step 9.6.7 after the third extraction.

9.7 Extract Processing

9.7.1 Concentrate the sample extracts inside of the bottles to a volume of ~2-5 mL on a parallel evaporator.

9.7.2 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 corresponding graduated tube.

9.7.3 Concentrate to a volume of 1 mL under nitrogen using the nitrogen evaporator.

9.7.4 Transfer the sample solution to a syringe fitted with a 13mm 0.2 µm PTFE syringe filter and filter into a labeled autosampler vial.

9.7.5 Cap the autosampler vial and analyze by GC/MS.
9.7.6 If the sample cannot be analyzed immediately, store in a freezer at -20 ºC until they can be analyzed.

10.0 Extraction Procedure for 12"x 12" Wipe Samples

10.1 Wipe Preparation

10.1.1 Remove 12"x12" wipe samples from the freezer and let warm to room temperature.

One sample batch will consist of the following:

- Up to 12 wipe samples (from freezer)
- 1 Method spike
- 1 Method blank
- 1 Recovery spike

10.1.2 While the samples from the freezer are warming to room temperature, the method spike, method blank and recovery spike can be prepared for extraction.

10.2 Method Spike Preparation

10.2.1 Place one clean 12" x 12" wipe into a 500 mL wide mouth glass jar.
10.2.2 Transfer a 50 µL aliquot of Spiking Solution to the wipe.
10.2.3 Transfer a 100 µL aliquot of Internal Standard solution to the wipe.
10.2.4 Add 250 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.3 Method blank Preparation

10.3.1 Place a clean 12" x 12" wipe into a 500 mL wide mouth glass jar.
10.3.2 Transfer a 100 µL aliquot of Internal Standard solution to the wipe.
10.3.3 Add 250 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.4 Recovery Spike Preparation

10.4.1 Place a clean 12" x 12" wipe into a 500 mL wide mouth glass jar.
10.4.2 Add 250 mL of 1:1 acetone:hexane to the jar and seal with a PTFE-lined cap.

10.4.3 After extraction and concentration, add 50 µL of spiking solution to the extract.

10.4.4 Add 100 µL of internal standard solution, cap and vortex along with the other samples processed in the sample batch.

10.5 Internal Standard Addition

10.5.1 After the sample has reached room temperature, write the sample ID on the top of the cap and carefully remove the cap.

10.5.2 Fortify the wipe sample with 100 µL of Internal Standard solution.

10.5.3 Add 250 mL of 1:1 acetone:hexane to the jar and seal with the sample ID labeled PTFE-lined cap.

10.6 Sample Extraction (Spikes, Blanks and Wipe Samples)

10.6.1 Place samples into an Ultrasonic cleaner with the water level well below the level of the jar's cap and start the cleaner.

10.6.2 Allow the cleaner to run for 15 minutes.

10.6.3 Carefully remove the samples from the ultrasonic cleaner, drying the outside of each jar with a paper towel as it is removed.

10.6.4 Assemble an analytical funnel on a 500 mL boiling flask and label one flask for each sample extract.

10.6.5 Place a glass wool plug into each funnel and add ~ 10g of anhydrous sodium sulfate into each funnel.

10.6.6 Carefully pour the solvent content of each sample jar into the corresponding flask through the funnel. Allow the jar to sit in the funnel until solvent stops dripping. Carefully remove the jars.

10.6.7 Evaporate the content of each flask to a volume < 10 mL using a rotary evaporator.

10.6.8 Return each flask to its position under the corresponding funnel.

10.6.9 Add another 250 mL of 1:1 acetone:hexane. Recap the jars.

10.6.10 Rinse the inside of each funnel with hexane from a wash bottle and collect the rinse into its flask.

10.6.11 Repeat steps 10.6.1 to 10.6.3, then 10.6.6 to 10.6.7, and finally step 10.6.10.

10.7 Extract Processing

10.7.1 Concentrate the sample extracts inside of the boiling flasks to a volume of ~2-5 mL on a rotary evaporator.

10.7.2 Transfer the concentrated extract to a 15 mL graduated tube. Rinse the flask twice with 2 mL aliquots of hexane. Transfer the rinsate to the corresponding graduated tube.

10.7.3 Concentrate to a volume of 1 mL under nitrogen using the nitrogen evaporator.

10.7.4 Transfer the sample solution to a syringe fitted with a 13mm 0.2 µm PTFE syringe filter and filter into a labeled autosampler vial.

10.7.5 Cap the autosampler vial and analyze by GC/MS.

10.7.6 If the sample cannot be analyzed immediately, store in a freezer at -20 ºC until they can be analyzed.

11.0 Records

Chain of custody records will be maintained to document the removal and extraction of each wipe 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 research 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 analyst. 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. Target recovery for the method spike, and recovery spike should be ± 30% of the nominal concentration for each analyte. Any deviations will be recorded and investigated.

Table 1. Spiking and Internal Standard 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 splitless mode Pulsed splitless 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	50º C for 2 min to 325º C at 10º C/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[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
	2,6-Di-tert-butyl-p-cresol											
	4-tert-Octylphenol											
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			
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			

Time Segment	Compound	Class	RT[a]	Pre1[b]	Prod 1[c]	CE[d]	Pre2	Prod 2	Prod2 CE	Pre3	Prod 3	Prod3 CE
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			
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

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WU.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurement Division
Public Health Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: Extraction of Filter Media for Ion Chromatography and High Resolution Inductively Coupled Plasma Mass Spectrometry

SOP ID: D-EMMD-PHCB-071-SOP-01	Effective Date: November 1, 2017

SOP was Developed: ☒ In-house ☐ Extramural:

SOP Discipline*: Inorganic Chemistry

Alternative Identification:

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

Version History

HR-ICPMS Analysis

Version	Date	Revisions/Changes
ECAB-114.0	1/1/11	Original Effective Date. ECAB-114.0 Three-Stage Extraction of Filter Media for Ion Chromatography and High Resolution Inductively Coupled Plasma Mass Spectrometry
PHCB-071-01	11/01/17	Add **Appendix 2** for Tire Crumb Rubber Study PM filter and reference material single-stage hot acid extraction.

Extraction of Filter Media for Ion Chromatography and High Resolution Inductively Coupled Plasma Mass Spectrometry

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1. Scope and Applications

Ion chromatography (IC) and high resolution inductively coupled plasma mass spectrometry (HR-ICPMS) are solution phase analytical techniques for the analysis of stable inorganic ions. This standard operating procedure details the extraction of aqueous and acid soluble particulate matter from filter media for quantitative analysis to determine water soluble, weak acid leachable, and total mass extracted from particulate matter (PM) collected on teflon filters. For a particular inorganic species, by combining the resulting concentration results with the volume(s) of a specific extraction step, mass per filter can be calculated for a host of uses.

2. Summary of Method

Particulate matter from any sampling device collected on a variety of filter media is extracted to estimate source solubility and total mass through various-stage extraction procedures. This procedure can involve a 24-hour aqueous filter leach to determine water-soluble inorganic species. Aliquots of this stage are analyzed for pH, for major cations and anions with IC, as well as 51 elements with HR-ICPMS.

Following the 24-hour aqueous leach, the remaining filter and leachate are spiked with hydrochloric and nitric acids to make an acid solution for determination of dilute acid-soluble inorganic species. After 30 days under ambient conditions, an aliquot is collected for HR-ICPMS analysis. The filter and remaining dilute acid solution is then spiked to 2% nitric and 1% hydrochloric acids and heated for 3 hours at 70 ºC, for the last stage aliquot.

Any stage of this procedure can be performed independently, or as a series, dependent on the study design.

3. Definitions/Acronyms

mL = milliliter
µL = microliter
L = liter
cm = centimeter
NERL = National Exposure Research Laboratory
D456 = NERL ISO 5 Class 100 Clean Laboratory (Research Triangle Park, EPA)
RO = Reverse Osmosis water (“tap” or “faucet” water in D456)
MilliQ = Type I ultra clean water from the Millipore A10 Element (> 18.2 MΩ-cm)
SHEM = Safety, Health, and Environmental Management
MSDS = Material Safety Data Sheets
HDPE = High Density Polyethylene

4. Health and Safety Warnings

4.1. All acid-cleaning procedures should be performed with the safety of the scientist as the utmost importance. Lab coat, protective eye wear and particle-free clean room vinyl gloves should be worn according to basic SHEM training guidelines.

4.2. Be extremely cautious with concentrated acids. MSDS' are located labeled 3-ring binders in the clean room gowning area and in the D461A lab.

4.3. Perform all acid spiking and cleaning procedures in the exhausting laminar flow hood in D456 Clean Room.

5. Cautions/Interferences

5.1 Cautions

5.1.1. Unless otherwise noted, all work is carried out in the NERL Class 100 Clean Laboratory located in D456 of the Research Triangle Park, NC EPA campus.

5.1.2. A Class 100 Clean lab follows general Clean Lab Guidelines. You are required to remove footwear (shoes) in the outmost room and wear a clean lab coat and clean lab footwear (Crocs, or equivalent) in the middle room (gowning room). The innermost room is the actual laboratory where HEPA-filtered air is continuously circulated and the temperature and humidity are closely monitored (20 ± 2 ˚C and 55 ± 5 % Relative Humidity). A clean lab is considered "clean" because of the filtered air and because it should be free of visible contamination. If there are any questions or doubts concerning Clean Lab Practices, please see Kasey Kovalcik.

5.1.3. All filter handling procedures should focus on minimizing contamination. Perform all filter work in the laminar flow hood. While handling filters in the laminar hood, it is advisable to turn the HEPA filter fan off. This fan is forceful enough to make filter handling with forceps difficult.

5.1.4. Wear vinyl clean room gloves at all times in the clean room.

5.1.5. Let the MilliQ water run for 30-60 seconds to flush the system, possibly longer after extended shutdowns (i.e., Monday morning), to achieve 18.2 MΩ-cm water.

5.1.6. Do not move analytical balances. The balances are certified for accuracy (Precision Weighing, Cary, NC) at their present location and are placed to minimize air flow, stability, and to stay level.

5.2. Interferences

5.2.1. In trace inorganic analyses, interferences (contamination) are caused by non-clean lab practices. Refer back to Section 5.1, Cautions.

6. Personnel Qualifications and Responsibilities

Personnel are not allowed in the lab without proper SHEM training and appropriate badge-access. After this status is achieved, Clean Lab Guidelines will be covered with Kasey Kovalcik.

7. Equipment and Supplies

7.1. Clean Laboratory (see Section 5.1)
7.2. Eppendorf Research adjustable micro-pipette set and corresponding tips (D456)
7.3. If needed for large volume of samples:
  7.3.1. Sartorius Analytical Balance connected to PC Laptop (D456)
  7.3.2. WASP USB Barcode Scanner
7.4. Crest Liquid Sonicating Bath (D461B)
7.5. Fisher Isotemp laboratory convection oven capable of 70 ˚C (D461A)
7.6. Eutech Instruments Hand-Held pH Probe (D456)
7.7. Ceramic Scissors (Kyocera Tycom Corp, or equivalent)
7.8. Dionex IC Vial and Cap Tool (Dionex 068925)
7.9. NIST-traceable thermometer/hygrometer for lab monitoring
7.10. 60 position large racks, Elemental Scientific, (LR-60-16)
7.11. 72 position racks, Thermo Scientific (14-809-22)
7.12. Barcode Labeler Software and PlazStyx Waterproof Labels (1/2" x 1 ¾", 15026)
7.13. Dionex 5.0mL Vials with Filter Caps (Dionex 038141)
7.14. 20 mil ziplock Bags (18" x 12"; 8" x 5")
7.15. Acid Cleaned HDPE Forceps
7.16. Nalgene NUNC 15 mL Conical Centrifuge Tube PP w/HDPE Screw Cap (366036)
7.17. Fisher Scientific Easy Reader Centrifuge Tube, FisherBrand 50mL (06-443-20)

8. Reagents and Standards

8.1. TraceMetal Grade Nitric Acid, Fisher Scientific (A509SK 212)
8.2. Trace Grade Hydrochloric Acid, JT Baker (9530-33)
8.3. Ethyl Alcohol, 200 proof, ACS/USP Grade, Pharmco-AAPER (EPA store stock – all plastic)
8.4. Optima Nitric Acid, Fisher Scientific (A46751)
8.5. Optima Hydrochloric Acid, Fisher Scientific (A466-1)
8.6. NIST Standard Reference Material 1648a , Urban Particulate Matter
8.7. pH 4.0 and 7.01 Buffer Solutions for pH meter calibration

9. Procedures

9.1. Eutech Instruments pH Probe

9.1.1. Calibration

9.1.1.1. Remove probe from electrode storage solution and rinse/shake a few times with MilliQ water. Extra electrode storage solution is located under the exhaust hood.

9.1.1.2. Turn meter on.

9.1.1.2.1. Rinse out 1 buffer container with pH 4.01 buffer solution and then fill it with the same solution. Repeat this procedure with a second buffer container for the pH 7.0 buffer solution.

9.1.1.2.2. Ensure calibration temperature matches room temperature using NIST traceable thermometer, within ± 1ºC.

9.1.1.2.3. Dip the electrode ~2-3 cm into the pH 4.01 standard buffer solution.

9.1.1.2.4. Press the CAL button to enter calibration mode. The "CAL" indicator will be shown.

9.1.1.2.5. Allow sufficient time (can be up to 2 minutes) for the reading to stabilize. Check by moving the probe in the solution.

9.1.1.2.6. Once stabilized, press the HOLD/ENT button to confirm the first calibration point.

9.1.1.2.7. Rinse with MilliQ water and repeat with pH 7.0 buffer solution starting with step 9.1.1.2.2.

9.1.1.2.8. After calibration is complete, press the "CAL" button to exit calibration mode. The calibration data is now stored.

9.1.2. Measurement

9.1.2.1. Pre-rinse the sample cup with 0.5 mL of the first sample. Shake off excess sample.

9.1.2.2. In "MEAS" mode, dip the electrode ~2-3cm into the sample.

9.1.2.3. Let the signal stabilize, read to two decimal places and record the reading in the dedicated laboratory notebook.

9.1.2.4. Rinse the electrode tip with MilliQ water, shake. Empty the sample cup into an appropriate waste beaker.

9.1.2.5. After every 20 samples (with MilliQ water rinses in between each sample), submerse the probe in pH 4.01 solution. If the reading is more than 10% off (3.96-4.05), recalibrate using the steps in Section 9.1.1.

9.1.3. Helpful Hints

- Meter will go to standby mode after a few minutes of inactivity or simply after a fixed amount of time. Just turn the unit back on and proceed with use.
- Sometimes it takes a few moments to stabilize the pH reading. Try moving the meter and then let it settle.
- Rinse/shake pH probe glass tip with MilliQ water between measurements.
- Collected rinses can be disposed in the sink.

9.2. Sample Tube Acid-Cleaning Procedure for ICPMS Analyses

9.2.1. Obtain a desired amount of 15 mL and 50 mL tubes to be cleaned (for every 50 mL Fisherbrand tube used, three 15 mL Nalgene tubes are required). Remove the plastic covers in the first entry-way of the D456 Clean Lab, ensuring that the tubes and their Styrofoam trays are never placed on the floor. Be sure to wear personal protective equipment (e.g. lab coat, gloves, safety glasses).

9.2.2. In the laminar exhausting hood located in the D456 Clean Lab, remove the tube (15 mL or 50 mL) caps and place the caps in a plastic tub with reverse osmosis (RO) water.

9.2.3. Leave the capless tubes in the Styrofoam tray, fill them with RO water and let them sit in the hood for 1 hour.

9.2.4. After 1 hour, pour the contents of the tubes into the hood sink and empty the tub from 9.2.2.

9.2.5. Loosen the cap slightly of the 20 L carboy of 4.0% nitric and 2.0% hydrochloric acids (v/v)* to provide airflow. Carefully fill all tubes from the carboy spigot with the acid solution and securely cap them.
- *This acid solution is made by mixing 800 mL Trace Grade nitric acid and 400 mL Trace Grade hydrochloric acid to a total volume of 20 L with MilliQ water.

9.2.6. Once all desired tubes are filled with the acid cleaning solution, capped, and placed back in their Styrofoam tray, carefully take them to D461 and place them in the oven at 70 ˚C for 3 hours.

9.2.7. After 3 hours, carefully remove the tubes and let them cool in a hood before doing any further work.
- CAUTION: mixed acids are considerably more aggressive when hot.
- With the oven turned off, these tubes can be left to sit overnight, or they can be removed and let to sit in a hood to cool. Do not try to work with the tubes while hot.

9.2.8. When cool, transport the tubes back to D456.

9.2.9. Within the laminar exhausting hood, remove the caps from the tubes and place them in a plastic tub with MilliQ water and pour the acid back into the 20 L carboy.

9.2.10. After a tray of tubes is empty, overfill them twice with MilliQ water and discard rinses. Refill the tubes with MilliQ water and let the tubes sit in the laminar hood for 1 hour.

9.2.11. After 1 hour, MilliQ rinse the clean tubes twice again and rinse the caps well.

9.2.12. Cap the MilliQ-full tubes, seal in ziplock bag (12” x 18”), and store them out of the way in the D456 Clean Lab until needed.

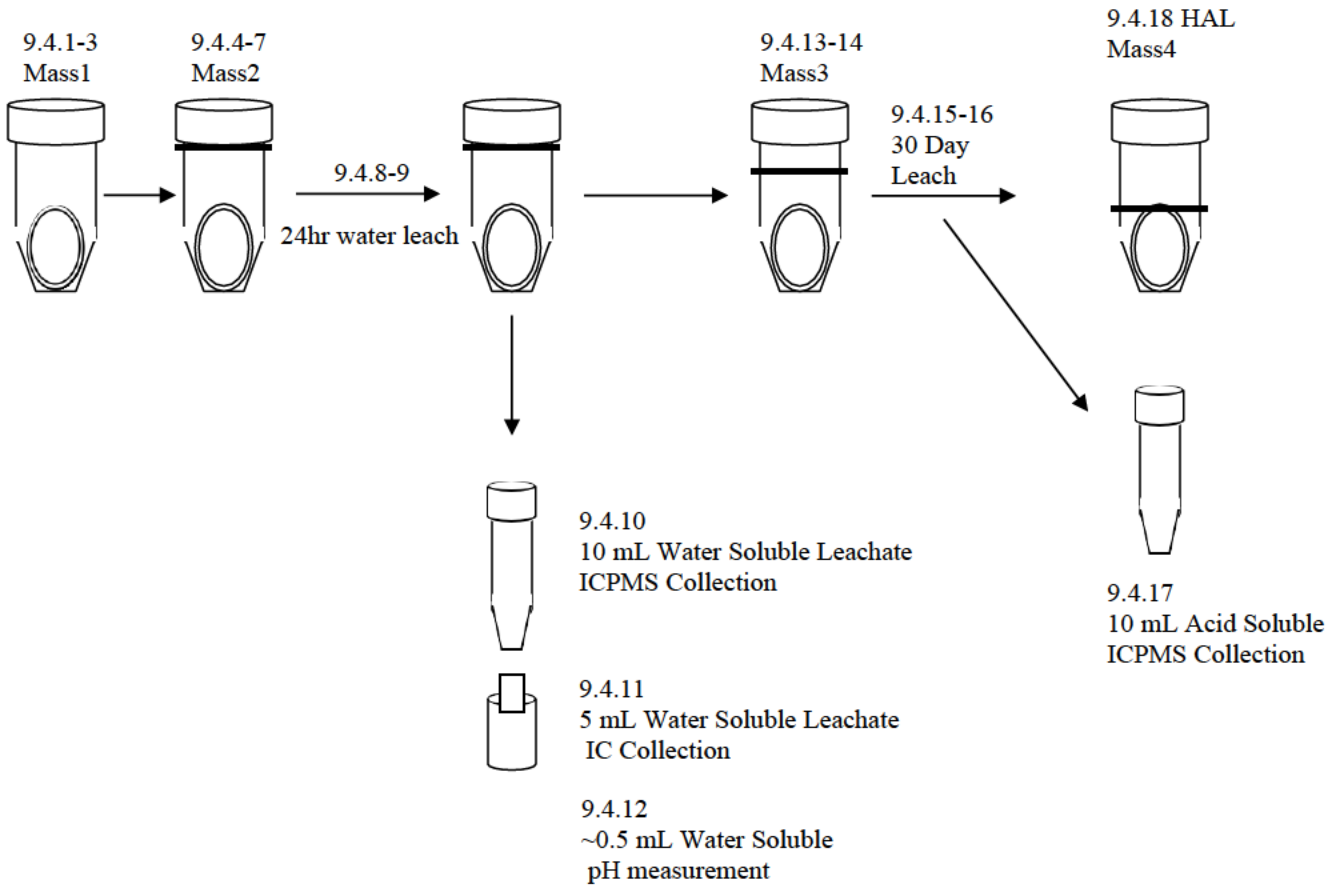
9.3. Cleaning Procedure for Ion Chromatography (Dionex) Vials and Caps

9.3.1. Fill plastic 72-position racks with 5.0 mL Dionex vials.
9.3.2. Fill and empty twice with MilliQ water. Then fill and let them sit overnight in the laminar flow hood.
9.3.3. Rinse two more times, shake any remaining droplets, and let dry in the laminar flow hood.
9.3.4. Place the dried tubes in a ziplock bag for storage.
9.3.5. The caps can be soaked in MilliQ water overnight ziplock bag. Try to remove as much air as possible from the bag to keep the caps submersed in the water.
9.3.6. The next day, discard the water and shake the caps to remove large droplets. Place them on a lint-free task wiper and air dry overnight in the laminar hood.
9.3.7. Place the dry caps in a sealed ziplock bag (12” x 18”).

9.4. Three-Stage Filter Extraction Procedure

The following section describes the three-stage, one-container extraction procedure for filters. Figure 1 shows this process, with accompanying section references. Filters are subjected to a 24-hour aqueous leach, and 30-day dilute acid leach, and an increased acid concentration under heated conditions. During each leaching step, it is imperative to know the volume of the extracting solution so that post analysis processing can calculate the mass of a desired analyte from the concentration.

Figure 1. Visual Representation of Extraction Procedure and Section Reference



If the sample filter has a stamped ID number on the polypropylene ring, carefully cut it off with acid cleaned ceramic scissors and discard.

9.4.1. Label an empty acid-cleaned 50 mL tube and cap (it does not need to be completely dry) with appropriate bar code sample ID.

9.4.2. Using acid-cleaned HDPE forceps, carefully place a Teflon filter in the bottom of the tube.

9.4.3. Weigh tube, cap, and filter (**Mass1**) using the Sartorius Analytical Balance (D456).

9.4.4. Carefully and evenly wet the filter with 200 µL ethanol using an Eppendorf micro-pipette. If the filter is polycarbonate, do <u>not</u> use ethanol. No additional solvent is needed.

9.4.5. Prepare at least 3 water/tube blanks per full carboy with _WB#_ label.

- You must still add the 200 µL ethanol per tube!

9.4.6. Use the 20 L carboy and the tube graduations to deliver approximately 50 mL of MilliQ water into the tube.
9.4.7. Weigh tube, cap, filter, and liquid contents (**Mass2**).

Example Calculation 1 – 1 DW (1 day water) Extraction Mass (see Table 1)

Mass (g) of 24 Hour Extraction Solution (1DW) = Mass2 – Mass1

Assuming a density of 1 g/mL, mass (g) = volume (mL)

9.4.8. Sonicate tubes for 30 minutes at lab temperature D461B. Be careful not to contaminate the underside of the caps with bath water.
9.4.9. Move the tubes back to the D45 Clean Lab and let the tubes sit for 24 hours.

Table 1: Sample Spreadsheet for Extraction Mass Records and Calculations

Sample ID	**MASS 1** Tube, Cap, (filter) (g)	**MASS 2** Tube, Cap, 200uL EtOH, 50mL MilliQ, filter (g)	1DW Extraction Mass (g)
PMC_WB1	13.61354	64.64846	51.03492
PMC_WB2	13.48995	64.74524	51.25529
PMC_WB3	13.76135	64.20034	50.43899
PMC_69B	13.84836	65.15102	51.30266
PMC_70B	13.65657	64.87368	51.21711
PMC_75A	13.79744	64.79582	50.99838

Water Soluble Extract Collection for ICPMS

9.4.10. Carefully pour 10 mL of the water-soluble leachate from the previous steps into a labeled, acid cleaned 15 mL tube.
- Label is SampleName**_1DW** (one day water).
- This tube will get spiked with Ultra Pure nitric (HNO_3) and Optima hydrochloric acids (HCl) to make 0.2% and 0.1% (v/v), respectively.

Example Calculation 2 – Acid Spiking for Water Soluble 1DW Extract
If poured off solution from 9.4.10 is approximately 10 mL:

$$10\,\text{mL Sol'n} \times \left(\frac{0.1\,\text{mL HCl}}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\ \mu\text{L}}{1\,\text{mL}}\right) = 10\ \mu\text{L HCl}$$

$$10\,\text{mL Sol'n} \times \left(\frac{0.2\,\text{mL HNO}_3}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\ \mu\text{L}}{1\,\text{mL}}\right) = 20\ \mu\text{L HNO}_3$$

This is an essential step for ICPMS analysis for stability and calibration standard matrix matching. It is also a very easy step to introduce contamination.

Water Soluble Extract Collection for IC and pH

9.4.11. Carefully pour 5 mL of the water-soluble leachate into a MilliQ cleaned IC vial labeled with an appropriate bar code sample ID.
- Use the appropriate MilliQ rinsed vial tool to correctly position the cap.
- Samples can be stored at 4 ˚C until analysis.

9.4.12. Pour ~ 0.5 mL water soluble leachate for pH measurement (see section 9.1.2).

Dilute Acid Soluble Extract Collection for ICPMS

9.4.13. Obtain the mass (g) of the tube, cap, filter, and remaining water soluble leachate after pour off from steps 9.4.11 and 9.4.12 (**Mass3**).

Table 2: Sample Spreadsheet after Mass 3 and Acid Spiking

	4/23/09	4/23/09	4/23/09	4/24/09		
Sample ID	**MASS 1** Tube, Cap, (filter) (g)	**MASS 2** Tube, Cap, 200uL EtOH, 50mL MilliQ, filter (g)	1DW Extraction Mass (g)	**MASS 3** after 1DW pour off (g)	uL HNO3 for 0.2% (v/v)	uL HCl for 0.1% (v/v)
PMC_WB1	13.61354	64.64846	51.03492	35.53492	71	36
PMC_WB2	13.48995	64.74524	51.25529	35.75529	72	36
PMC_WB3	13.76135	64.20034	50.43899	34.93899	70	35
PMC_69B	13.84836	65.15102	51.30266	35.80266	72	36
PMC_70B	13.65657	64.87368	51.21711	35.71711	71	36
PMC_75A	13.79744	64.79582	50.99838	35.49838	71	36

Example Calculation 3 – Mass Lost to IC and pH Pour Offs

Remaining Solution Mass (g) after 1DW pour off for 30 Day Acid Spiking =

[(Mass2 – Mass1) – Mass3]

9.4.14. To the remaining ~ 34.5 mL of leachate and filter, spike with ultra high purity nitric and hydrochloric acids to make 0.2% and 0.1% (v/v), respectively.
- See Table 2 and Example Calculation 4 below.

Example Calculation 4 – Acid Spiking for **Acid Soluble Extract**

If remaining mass from SPREADSHEET = ~ 34.5 g,

$$34.5\,\text{mL Sol'n} \times \left(\frac{0.1\,\text{mL HCl}}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\ \mu\text{L}}{1\,\text{mL}}\right) = 34.5\ \mu\text{L HCl}$$

$$34.5\,\text{mL Sol'n} \times \left(\frac{0.2\,\text{mL HNO}_3}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\ \mu\text{L}}{1\,\text{mL}}\right) = 69\ \mu\text{L HNO}_3$$

9.4.15. Sonicate 30 minutes at 80 ºC.

9.4.16. Let sit in D456 (20 ºC) for 30 days.

9.4.17. After 30 days, pour approximately 10 mL of the acid soluble leachate into a labeled, acid cleaned 15 mL tube.
- Label is SampleName_**30DA** (30-day acid)
- This sample now has the matrix-matched acid concentrations needed for multi element ICPMS analysis for D-EMMD-PHCB-042-SOP-03 Analysis, Standard Operating Procedure for Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument.

Concentrated Hot Acid Leach (HAL) for ICPMS Analysis

9.4.18. Record the mass of the remaining ~25 mL of leachate and filter (**Mass4**), spike with ultra high purity nitric and hydrochloric acids to make 2% and 1% (v/v), respectively.

Table 3: Sample Spreadsheet After Mass 4 and Acid Spiking

	4/23/09	4/23/09	4/23/09	4/24/09			5/24/2009		
Sample ID	**MASS 1** Tube, Cap, (filter) (g)	**MASS 2** Tube, Cap, 200uL EtOH, 50mL MilliQ, filter (g)	1DW Extraction Mass (g)	**MASS 3** after 1DW pour off (g)	uL HNO3 for 0.2% (v/v)	uL HCl for 0.1% (v/v)	**Mass 4** after 30 DA pour off (g)	uL HNO3 for 2 % (v/v) HAL	uL HCl for 1% (v/v) HAL
PMC_WB1	13.61354	64.64846	51.03492	35.53492	71	36	25.53492	511	255
PMC_WB2	13.48995	64.74524	51.25529	35.75529	72	36	25.75529	515	258
PMC_WB3	13.76135	64.20034	50.43899	34.93899	70	35	24.93899	499	249
PMC_69B	13.84836	65.15102	51.30266	35.80266	72	36	25.80266	516	258
PMC_70B	13.65657	64.87368	51.21711	35.71711	71	36	25.71711	514	257
PMC_75A	13.79744	64.79582	50.99838	35.49838	71	36	25.49838	510	255

Example Calculation 5 – HAL Acid Spiking

If poured off solution from 9.4.18 is approximately 25 mL:

$$25\,\text{mL Sol'n} \times \left(\frac{1\,\text{mL HCl}}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\,\mu\text{L}}{1\,\text{mL}}\right) = 250\,\mu\text{L HCl}$$

$$25\,\text{mL Sol'n} \times \left(\frac{2\,\text{mL HNO}_3}{100\,\text{mL total Sol'n}}\right) \times \left(\frac{1000\,\mu\text{L}}{1\,\text{mL}}\right) = 500\,\mu\text{L HNO}_3$$

9.4.19. Place the concentrated samples inside a convection lab oven at 70 ºC for 3 hours.
9.4.20. After 3 hours, carefully remove from the oven and let to cool at lab temperature.
9.4.21. Once cooled, pour a ~10 mL aliquot into a labeled acid-cleaned 15 mL tube for ICP-MS analysis, using _HAL as the label suffix.
9.4.21.1. ICPMS calibration standards should be matrix-matched to 2% and 1% nitric and hydrochloric acids, respectively.

ONCE ACIDIFIED, ALL TUBES FOR ICPMS CAN BE STORED AT ROOM TEMPERATURE.

10. Data and Records Management

Tubes with filters and any other samples for long-term storage are locked in the Filter Storage Room (D458). Label the sample trays appropriately with Study Name, Analysis

Date, and Analyst Initials. Bag the samples for storage before leaving the clean lab environment (D456). See also section 11.2.

10.1. Sample Calculations for Final Results of Extractions

- All sample calculations below assume data has been flagged valid. Error propagation has been omitted for simplicity.

1DW Extraction Mass (Mass2 - Mass1 (see 9.4.7 and Table 1)) = 51.30266 g
30DA Extraction Mass (Mass3 (see 9.4.13)) = 35.80266 g
Mass lost to IC and pH pour offs (1DW – 30DA) = 15.50000 g

ICPMS Sample Results for Fe57

PMC_69B_1DW	1.12 µg/L
PMC_69B_30DA	4.20 µg/L

10.1.1. Single-stage extraction procedure. Also works for water soluble mass per filter for ICPMS (same calculation for IC) or soluble mass after single-stage extraction procedure

- First, calculate the single stage extraction mass (Appendix 2, Step 5) or the 1DW extraction mass (see 9.4.7), assuming density of solution is 1.0 g/mL.

Example Calculation 5 – Water Soluble Mass Per Filter

$$\frac{1.12\ \mu g\ Fe}{L\ soln} * \frac{1\ L\ soln}{1000\ mL\ soln} * \frac{1.0\ mL\ soln}{1\ g\ soln} * 51.30266\ g\ soln = 0.0575\ \mu g\ Fe\ per\ filter$$

10.1.2. Total mass per filter for ICPMS, combination of two extraction steps.

Example Calculation 6 – Total Mass Per Filter

$$\frac{1.12\ \mu g\ Fe}{L\ soln} * \frac{1L\ soln}{1000\ mL\ soln} * \frac{1.0\ mL\ soln}{1\ g\ soln} * 15.50000 g\ soln = 0.0176 \mu g\ Fe lost\ to pour\ offs$$

$$\frac{4.20\ \mu g\ Fe}{L\ soln} * \frac{1L\ soln}{1000\ mL\ soln} * \frac{1.0\ mL\ soln}{1\ g\ soln} * 35.80266 g\ soln = 0.150 \mu g\ Fe after\ 30DA$$

Total Mass = 0.0176 µg + 0.150 µg = 0.168 µg Fe per filter

11. QA/QC

11.1 Water or Tube Blanks collected during the filter extraction procedure (9.4.5) serve as quality control samples to determine any water and tube contaminations. These samples will be analyzed alongside filter samples and filter blanks and will be used in the final interpretation of quantitative data.

11.2 Excel spreadsheets used for capturing pour-off mass data should be saved and archived on the ICPMS computer (D456) that automatically archives to a removable hard drive. These files can also be emailed to Kasey Kovalcik (kovalcik.kasey@epa.gov) for duplicate archiving.

12. References

12.1. Graney, J.R.; Landis, M.S.; Norris, G.A; Concentrations and solubility of metals from indoor and personal exposure $PM_{2.5}$ samples. *Atmospheric Environment*, 2004, 237-247.

12.2. Analytical Balance and Weight Certification performed annually by Precision Weighing, Cary, NC.

12.3. D-EMMD-PHCB-042-SOP-03, Standard Operating Procedure for Operation and Maintenance of the Element 2 High-Resolution Inductively Coupled Plasma Mass Spectrometry Instrument, November 2017.

12.4. Oakes, M.M.; Burke, J.M.; Norris, G.A.; Kovalcik, K.D.; Pancras, J.P.; Landis, M.S: Near-road enhancement and solubility of fine and coarse particulate matter trace elements near a major interstate in Detroit, Michigan. *Atmospheric Environment*, **2016**, 213-224.

Appendix 1: Two Stage Extraction Procedure for NEXUS and CMAPS Filter Samples.

1) Rinse the already acid-cleaned 50 mL sample tubes three times with MilliQ water, and air dry the tubes in the Laminar Flow hood in D456 for 60 minutes.
2) Insert a filter sample into the dry 50 mL tube, add 100 µL of ethanol to cover the filter surface, cap the tube and label it appropriately. Weigh the tube (W1).
3) Add approximately 40 mL of MilliQ water from the carboy and weigh again (W2).
    a) Extraction Volume (mL) = W2 – W1
4) Sonicate the samples at room temperature for 3 hours and let the filter leach in the aqueous phase for 24 hours.
5) After 24 hours, pour 4 mL of the aqueous leachate into a 15-mL acid-cleaned tube that has been rinsed three times with MilliQ water, and labeled. Then add 120 µL of freshly prepared (2+1) concentrated HNO_3 and HCl acid mix. Store for analysis.
    a) Final concentrations are 2% HNO_3 and 1% HCl (v/v).
6) Weigh the remaining filter, leachate, and tube after the above pour-off (W3).
    a) Volume Poured Off for Aqueous Extraction (mL) = W2 – W3
7) To the approximately remaining 36 mL of leachate and filter, add 1000 µL of the (2+1) concentrated acid mix to the tube and cap tightly.
    a) Final concentrations are 2% HNO_3 and 1% HCl (v/v).
8) Set the ultrasonic water temperature to 70 °C and place the 50 mL sample tubes (24 tubes per rack) in the bath. Once the set temperature is reached, Sonicate the samples for 3 hours.
    a) To minimize potential contamination, ensure the ultrasonic bath water does not reach the cap.
9) Set the 50 mL sample tubes aside for 9 days. Then pour off approximately 4 mL for ICP-MS analysis.

Appendix 2: Single Stage Extraction Procedure for Tire Crumb Rubber Study Samples

1) Insert media to be extracted into a dry 50 mL acid-cleaned centrifuge tube.
2) Add 100 µL of ethanol to cover the filter surface or to completely wet the reference material cap the tube and label it appropriately. Weigh the tube (W1).
    i) Filter media is a teflon filter and teflon support ring with collected PM or
    ii) NIST SRM 1648a. Refer to NIST insert for handling care. Typically, 100 mg of the well-mixed material is used.
3) Add approximately 35 mL of a 2% HNO_3 and 1% HCl (v/v) solution using MilliQ water and Optima acids.
    a) The total volume added can vary depending on the size of the filter.
4) Weigh the filter, leachate, and tube after the above pour-off (W2).
    a) Extraction Mass (g) = W2 – W1
5) Set the ultrasonic water temperature to 70 °C and place the 50 mL sample tubes (24 tubes per rack) in the bath. Once the set temperature is reached, sonicate the samples for 30 minutes and leave the tubes in the water bath for 3 hours.
    a) To minimize potential contamination, ensure the ultrasonic bath-water does not reach the cap.
6) Set the 50 mL sample tubes aside for 9 days.
7) Into a cleaned 15 mL sample tube, pour approximately 10 mL for ICP-MS analysis, leaving the original filter/sample submerged.
8) Calculations to determine the elemental mass per filter are shown in Section 10.1.1

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:

Approval

Name: Tammy Jones-Lepp

Title: ECB Chief

Signature:

Concurrence*

Name: Margie Vazquez

Title: EMMD QA mgr

Signature:

* Optional field

STANDARD OPERATING PROCEDURE FOR TOTAL NITRIC ACID EXTRACTABLE METALS FROM SOLID SAMPLES BY MICROWAVE DIGESTION

Prepared by

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For

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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	Reversed 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 of the Environmental Measurement and Monitoring Division (EMMD) of the U.S. Environmental Protection Agency 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 METHOD**. 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/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 encourage 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™ 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 either 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

8.0 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.

8.1 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.

8.2 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.

8.3 The analyst should be familiar with the location and proper use of the fume hoods, eye washes, safety showers, and fire extinguishers.

8.4 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.

8.5 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.

8.6 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.

8.7 Never heat liquids in a sealed vessel that is not equipped with a pressure relief device.

8.8 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.

8.9 Fumes from the microwave unit should be exhausted to a hood.

8.10 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.

8.11 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 procedure 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.

9.1.0 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.

9.1.1 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.

9.1.2 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.

9.1.3 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.

9.1.4 **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.

9.1.5 Use program ECBTRC-1 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 pre-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.

9.1.6 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.

9.1.7 Vent the vessels in a fume hood and weigh to verify that no significant amount of solution was lost.

9.1.8 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.

9.1.9 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.

9.1.10 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 / Quality Control (QA/QC)

10.0 **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, than a blank will also need to be filtered to assess if the filter is contributing any contamination.

10.1 **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.

10.2 **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.

10.3 **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.

10.4 **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.

10.5 **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.

10.6 **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

12.0 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.

12.1 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.

12.2 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 U.S.E.P.A. 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 = 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 = 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 200psi. The temperature should be 200°C +/- 10C at 200psi. 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 (200psi = 200°C). Look for volume loss instead.
6. If the QC test fails (i.e., you do not get 200°C +/-10C at 200psi), 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 read “0000-0000-0000-0000-0000-0000” refer to operation manual pg 60-66 or contact manufacturer for proper calibration constant for your model. The calibration number used for our 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 pg 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 reversed 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 HNO_3 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.
   - Use the Optima grade acid to prepare 1 liter of 10% Nitric acid.
   - 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.
   - 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.
7. 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.

8. All sample bottles and microwave vessels should be capped and stored in sealable plastic bags or plastic containers to prevent contamination.

9. Label bag or container with the cleanup date and batch number.

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.

- Remove the caps and let the tubes stand in the Styrofoam base.

- 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.

- Fill each tube with 10% nitric acid and let sit overnight or at least twelve hours at room temperature in the Styrofoam container with their lids on.
- 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.
- Pour the acid content into an acid cleaning bottle.
- Rinse tubes and lids several times with deionized water and allowed to air dry.
- Cap the tubes, place them back in their Styrofoam base and keep them seal 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 = 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 = 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 = 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 Digesion Sheet S

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 Digesion 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**: Hydrogen Peroxide

▪ Weight of Nitric acid needed: _____ g ▪ Weight of Hydrochloric acid needed: _____ g [wt(g) = V(mL) **X** density (g/mL)]

***** Volume of spike standard solution: _____ µL ▪ 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 ≤ 24

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**: Hydrogen Peroxide

▪ Weight of Nitric acid needed: _____ g ▪ Weight of Hydrochloric acid needed: _____ g [wt(g) = V(mL) **X** density (g/mL)]

***** Volume of spike standard solution: _____ µL ▪ 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 ≤ 24

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U.S Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurement Division
Environmental Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: **TOTAL NITRIC ACID EXTRACTABLE METALS FROM WIPE SAMPLES BY MICROWAVE DIGESTION**

SOP ID: D-EMMD-ECB-013-SOP-01	Effective Date: 11/13/2017

SOP was Developed: ☒ In-house ☐ Extramural:

SOP Discipline*: Inorganic Chemistry

Alternative Identification: QA Track ID

SOP Contact Signature

Name: Georges-Marie Momplaisir, author

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Signature/Date:

QA Signature

Name: Margie Vazquez

Title: EMMD QA manager

Signature/Date:

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

Version History

Version No.	Name	Date of Revision	Description of Change(s)
1	Georges-Marie Momplaisir	11/13/2017	Modification of ECB-003- SOP-01 for wipe samples.

TABLE OF CONTENTS

SOP Title: TOTAL NITRIC ACID EXTRACTABLE METALS FROM WIPE SAMPLES BY MICROWAVE DIGESTION

1. *Scope and Applicability*

This document describes a procedure for the preparation of wipe samples, for elemental 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.

This standard operating procedure has been prepared for use of the Environmental Measurement and Monitoring Division (EMMD) of the U.S. Environmental Protection Agency and may not be specifically applicable to the activities of other organizations. It is a modified version of ECB-003- SOP-01 [Ref. 13.1]. It uses the multi-element nitric acid/ hydrochloric acid-leach microwave-assisted digestion procedure described in EPA Method 3051a [Ref. 13.2] 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 document has not been through the Agency's peer review process nor the EMMD clearance/publication process. **THIS IS NOT AN OFFICIAL EPA APPROVED Method.**

2. *Summary of Method*

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 15 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.

3. *Definitions/Acronyms*

ECB	Environmental Chemistry Branch
EMMD	Environmental Measurement and Monitoring Division
ICPMS	Inductively Coupled Plasma Mass Spectrometry
DI	Deionized
RO	Reversed Osmosis
HNO3	Nitric acid
HCl	Hydrochloric acid
H2O2	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
FCS	Field Control Sample
LCS	Laboratory Control Sample
LBLK	Laboratory Blank
FBLK	Field Blank
RBLK	Reagent Blank

4. *Health and Safety Warnings*

4.0 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.

4.1 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.

4.2 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.

4.3 The analyst should be familiar with the location and proper use of the fume hoods, eye washes, safety showers, and fire extinguishers.

4.4 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.

4.5 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 5.0.

4.6 When working with samples of unknown composition, always perform a pre-digestion step in an unsealed, open vessel, allowing a minimum of 15 minutes of time for reaction of volatile or easily oxidized compounds to subside before sealing the vessel and microwave heating.

4.7 Never heat liquids in a sealed vessel that is not equipped with a pressure relief device.

4.8 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.

4.9 Fumes from the microwave unit should be exhausted to a hood.

4.10 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.

4.11 All unused acids should be properly disposed in the acid waste collection container.

5. *Cautions/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 either 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.

6. *Personnel Qualifications/Responsibilities*

The procedures detailed in this SOP are to be conducted only by staff trained by the SOP contact listed on the cover page or the contact's designee. Training will be documented and the trainee will be allowed to conduct the procedures after demonstrating proficiency at the discretion of the trainer.

7. *Equipment and Supplies*

7.1 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 13.3] for more information on routine maintenance and cleaning.

7.2 MARS Digestion Vessels: CEM XP-1500 plus Control Vessel with a TFM liner

7.3 Centrifuge: Model IEC Centra MP4R International Equipment Company

7.4 Water Purification System: Water Pro Plus LABCONCO

7.5 Assorted micropipettes and appropriate tips Rainin E4^{TM} XLS, Mettler Toledo

7.6 0.45 µm PTFE filter membranes

7.7 Analytical balance: Mettler Toledo XP504, 4-decimal analytical balance, 520-gram maximum capacity, and minimum sensitivity of 0.1 mg

7.8 Calibrated Class 1 NIST Certified Reference Weights

7.9 Sample bottles and tubes of varying sizes

8. *Reagents and Standards*

8.1 Deionized (DI) water: in house 18.2 MΩ DI water.

8.2 Concentrated Nitric Acid Optima: high purity concentrated HNO_3 (65%-70% Fisher Scientific, 1-liter bottle, catalog # A467-2).

8.3 Concentrated Hydrochloric Acid Optima: high purity concentrated HCl (Fisher Scientific, 500 mL bottle, A466-500) use if needed only.

8.4 Hydrogen Peroxide Optima: 30% H_2O_2 Trace-pure (Fisher Scientific, 500 mL bottle, P170).

8.5 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).

8.6 Concentrated HNO_3: Trace-metal clean concentrated HNO_3 (65%, Fisher Scientific, catalog # A509212) for washing labware.

8.7 10% Nitric Acid: Add 100 mL of concentrated nitric acid to 500 mL deionized water and dilute to 1 L.

9. *Procedures (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 procedure for cleaning vessels.

9.1 Microwave Extraction of Wipe 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 wipe material and can withstand a maximum pressure of 800 psi. Whole wipe samples will be used.

9.1.0 Place the microwave vessel and lid on the analytical balance and record the weight in the digestion Table. Using acid cleaned forceps (made of teflon or acid resistant plastic), transfer 1 wipe sample (approximately 4.5 - 5.0 g) into the tared vessel. Record the weight of the wipe. Tare the sample vessel. Place it in the fume hood and add 9 mL of concentrated nitric acid and 3 mL of hydrochloric acid. Make sure that the entire wipe is covered with reagent. Wait approximately 20 minutes before closing the vessel. **Caution:** The reaction between wipe and acid is rapid. It produces brown fumes and is quite exothermic (the experimentalist should exert extreme caution when performing this task).

9.1.1 Place a safety membrane in the vessel cap and seal firmly by hand. Weigh the vessel and record the weight before digestion.

9.1.2 Repeat the above procedure for a batch of up to 24 samples. 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.

9.1.3 **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.

9.1.4 Use program ECBTRCW-1 1 – 4 vessels, ECBTRCW-2 for 4 – 8 vessels and ECBTRWC-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.

9.1.5 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.

9.1.6 Vent the vessels in a fume hood and weigh to verify that no significant amount of solution was lost.

9.1.7 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.

9.1.8 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 0.45 µm membrane filter.

9.1.9 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.

9.2 Troubleshooting

9.2.1 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.

9.2.2 Follow instructions in Appendix B or 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.

10. *Data and Records Management*

Calculations

10.1 **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

10.2 **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.

10.3 **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.

10.4 **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.

Records Management

10.5 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 bench sheets will be placed in the Tire Crumb digestion binder located in CHL-43. Sample preparation and digestion notes not included on the digestion bench sheets will be taken in a research notebook maintained as per ORD requirements in ORD PPM 13.2

11. *Quality Assurance/Quality Control*

11.0 **Reagent Blank (RBLK):** A reagent blank will accompany each digestion batch of 24 or fewer samples. The mixture of acid reagents (Section 9.1.0) will be used. The reagent blank is used to track potential contamination during sample preparation and extraction. The reagent 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.

11.1 **Laboratory and Field Blank (LBLK and FBLK):** Two matrix blanks will be prepared prior to field-sampling, but the Laboratory Blank will remain in the lab and will not travel to the sampling site, while the Field Blank will travel to the field sampling site. In an ISO 5 Class 100 Clean Lab, acid-cleaned plastic forceps will be used to place a wipe sample into an acid-cleaned 50mL flat-bottom polypropylene sample tube.

11.2 **Laboratory Control Sample (LCS):** Another matrix blank will be prepared and to this, will be added 250 µl of the custom spike standard solution (Section 8.5) before digestion. The LCS results will be used to determine if the laboratory can perform the analysis in a clean matrix. Acceptable recovery range of this sample is 65-125%.

11.3 **Field Control Sample (FCS):** This sample is similar to the LCS sample in 11.2, but it travels to the field. A 250 µl aliquot of the custom spike standard is added to the ghost wipe.

11.4 **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 wipes.

11.5 **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.

11.6 **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).

12. *References*

12.0 U.S.E.P.A. SW-846 Method 3051A. Microwave Assisted Acid Digestion of sediments, sludges, soils and oils.

12.1 SOP # D-EMMD-ECB-003-SOP-01. Microwave Assisted Digestion of Solid Matrices Rev #0.0

12.2 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: ECBTRCW-1**
   Max Power = 400W
   % Power = 100%
   Ramp = 15 minutes
   Pressure = 0 psi
   Temperature = 200 °C
   Hold Time = 15 minutes
   Number of Vessels = 1 to 4

2. **Program Name: ECBTRC-2**
   Max Power = 800W
   % Power = 100%
   Ramp = 15 minutes
   Pressure = 0 psi
   Temperature = 200 °C
   Hold = 15 minutes
   Number of Vessels = 5 to 8

3. **Program Name: ECBTRC-3**
   Max Power = 1600W
   % Power = 100%
   Ramp = 15 minutes
   Pressure = 0 psi
   Temperature = 200 °C
   Hold = 15 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 200psi. The temperature should be 200°C +/- 10C at 200psi. 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 (200psi = 200°C). Look for volume loss instead.
6. If the QC test fails (i.e., you do not get 200°C +/-10C at 200psi), 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 read “0000-0000-0000-0000-0000-0000” refer to operation manual pg 60-66 or contact manufacturer for proper calibration constant for your model. The calibration number used for our model is: **4315-4316-0621-0623-6637-2241**

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 pg 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 reversed 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 HNO_3 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.
   - Use the Optima grade acid to prepare 1 liter of 10% Nitric acid.
   - 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.
   - 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.
7. 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.

8. All sample bottles and microwave vessels should be capped and stored in sealable plastic bags or plastic containers to prevent contamination.

9. Label bag or container with the cleanup date and batch number.

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.

- Remove the caps and let the tubes stand in the Styrofoam base.

- 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.

- Fill each tube with 10% nitric acid and let sit overnight or at least twelve hours at room temperature in the Styrofoam container with their lids on.
- 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.
- Pour the acid content into an acid cleaning bottle.
- Rinse tubes and lids several times with deionized water and allowed to air dry.
- Cap the tubes, place them back in their Styrofoam base and keep them seal 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 = 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 = 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 = 30 minutes
   Number of Vessels = 9 to 12
   Average Sample Volume = 50 mL

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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

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 (Δm/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 4000, the MR setting is used for data acquisition. If R is in the 4000 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 90 ºC 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 Mulit-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 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	10	1 mL from every PSS	100 ppb each element	
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.

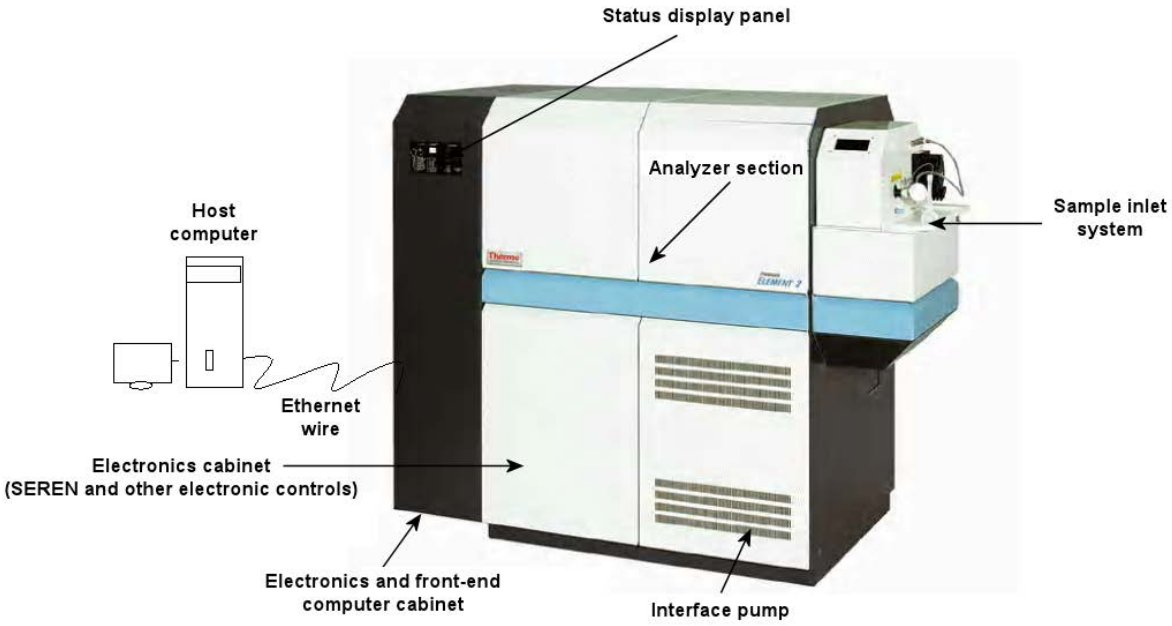


Figure 1. Components of the Element 2 instrument.

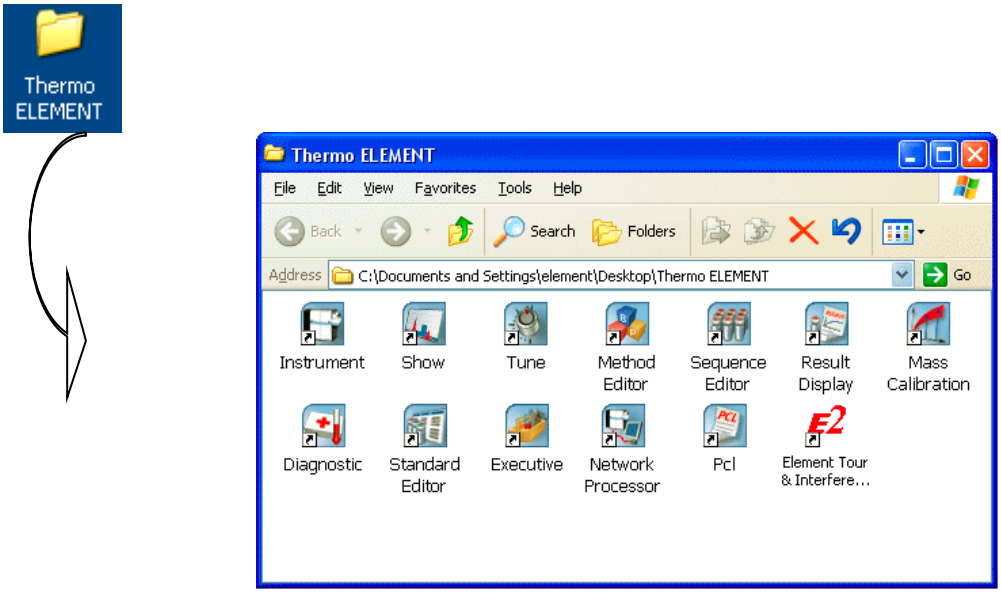


Figure 2. Task window folder.

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.

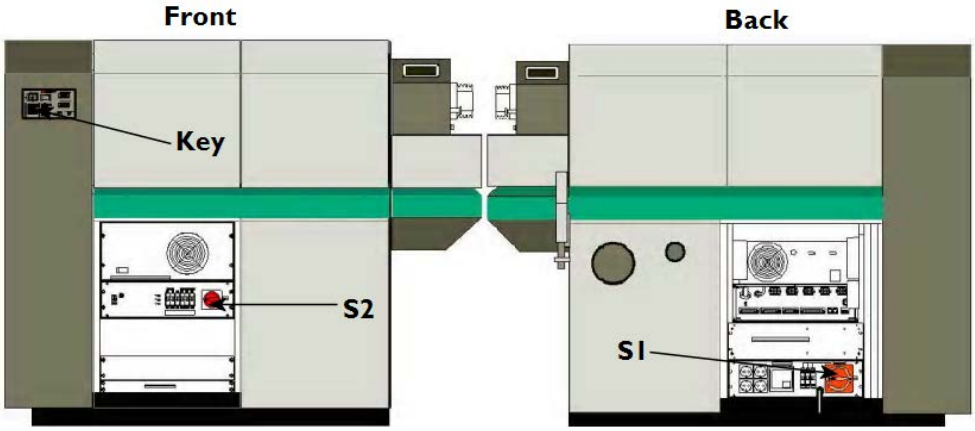


Figure 3. Front and back views of E2.

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 min 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 50-mL polypropylene sample vials and four removable trays (Racks 01–04) that are each capable of holding up to sixty 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.

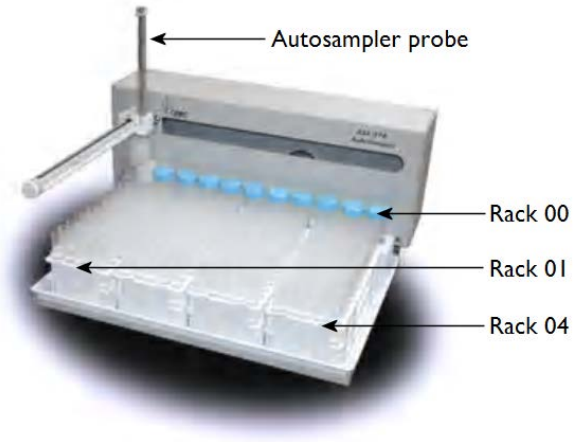


Figure 4. ASX-510 autosampler (source: Cetac web site).

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 the GO TO button at the top of the GUI is pressed, 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.

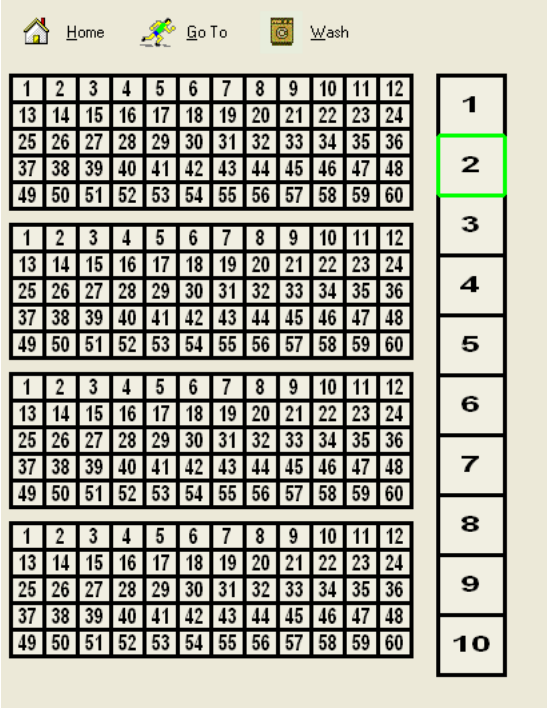


Figure 5. GUI of ASX-510 sample trays with position numbers.

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 ~ 18 ºC) 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.

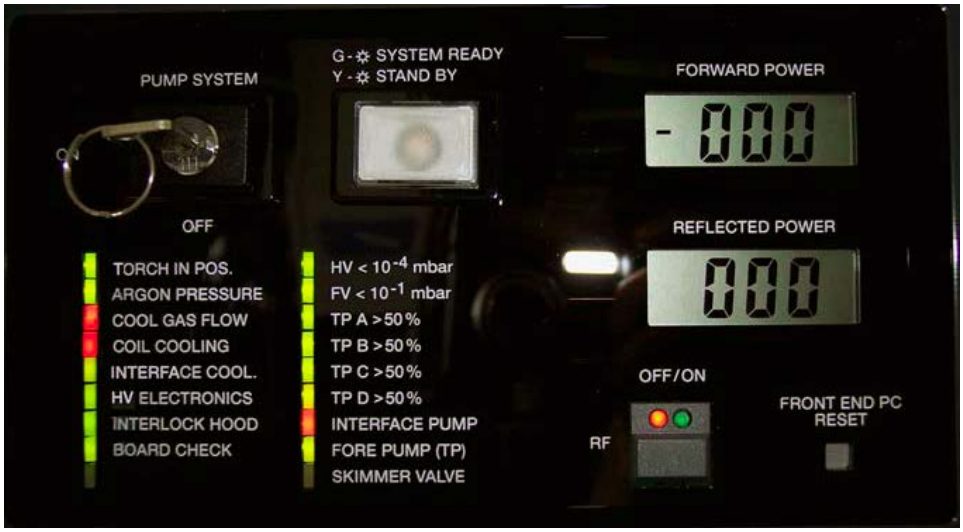


Figure 6. Status display panel when E2 is in standby.

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).

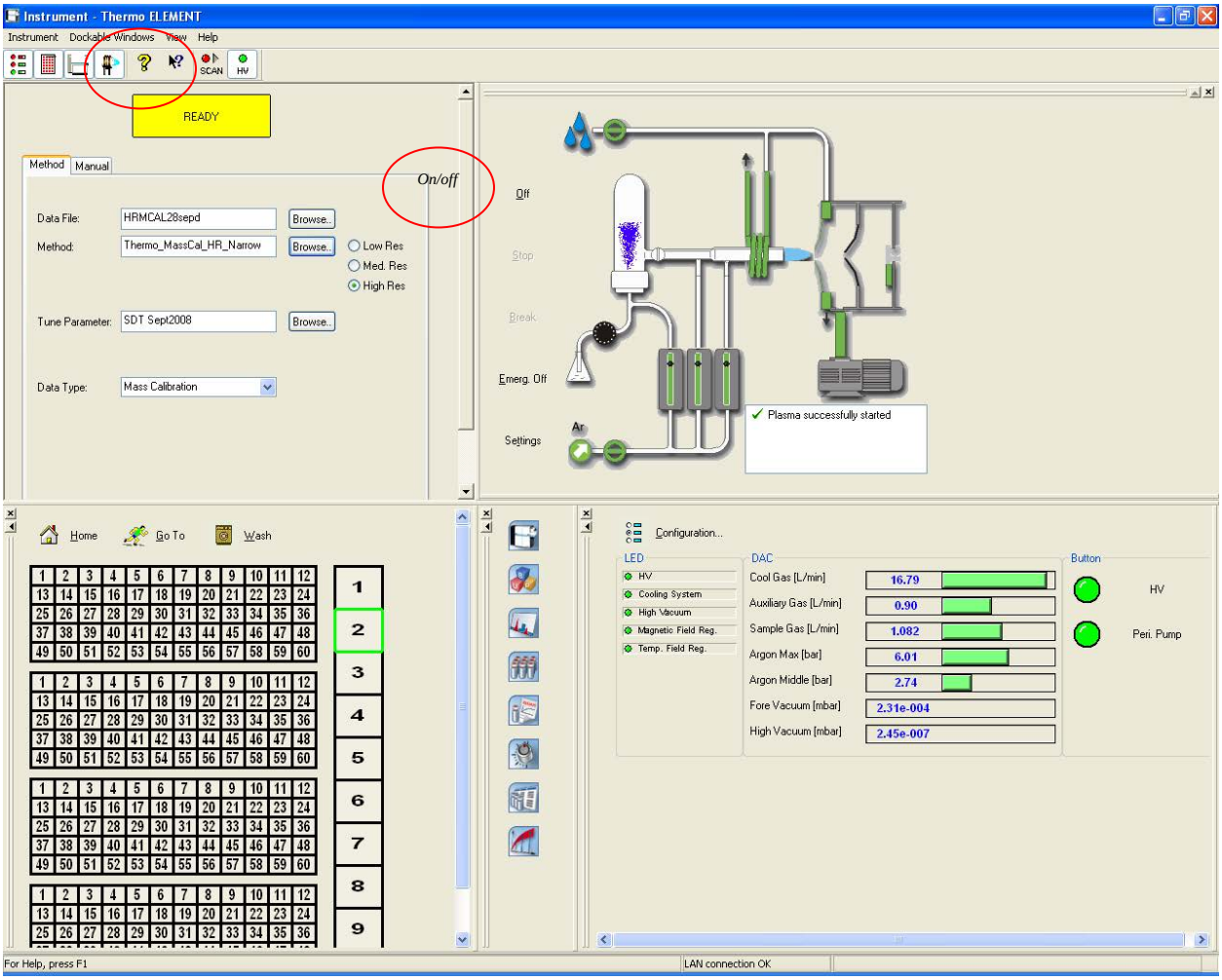


Figure 7. Instrument task window.

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.

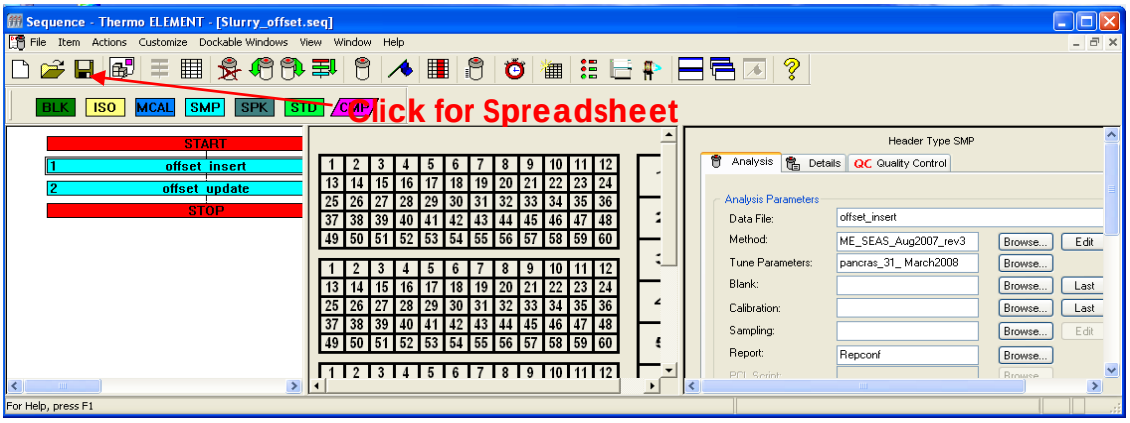


Figure 8. Spreadsheet view.

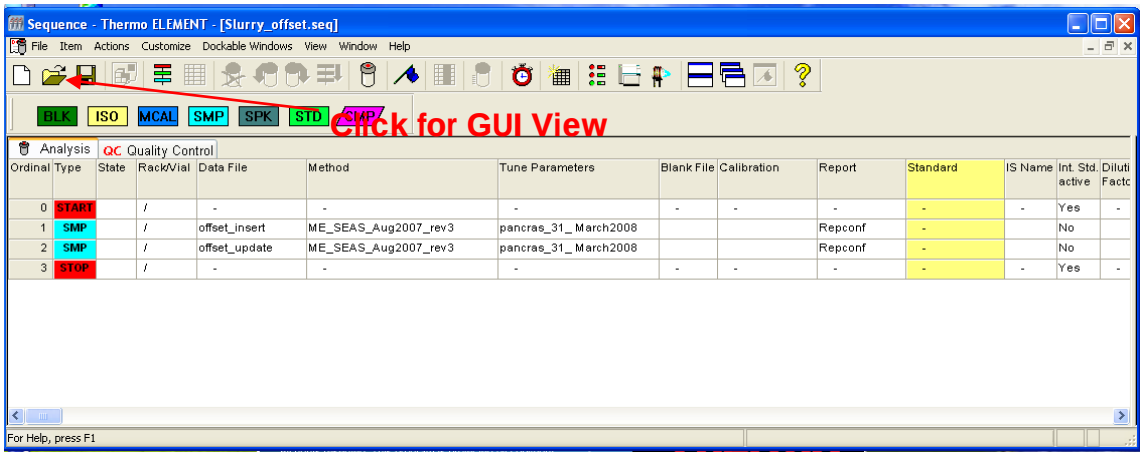


Figure 9. GUI view.

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.

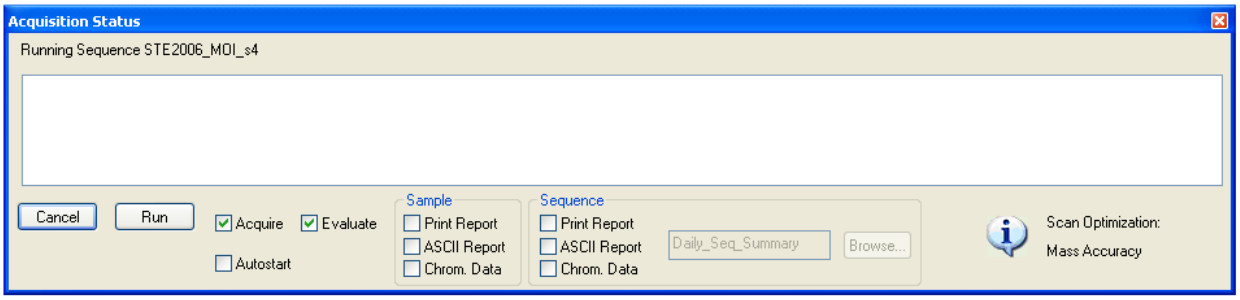


Figure 10. Run task window.

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 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.

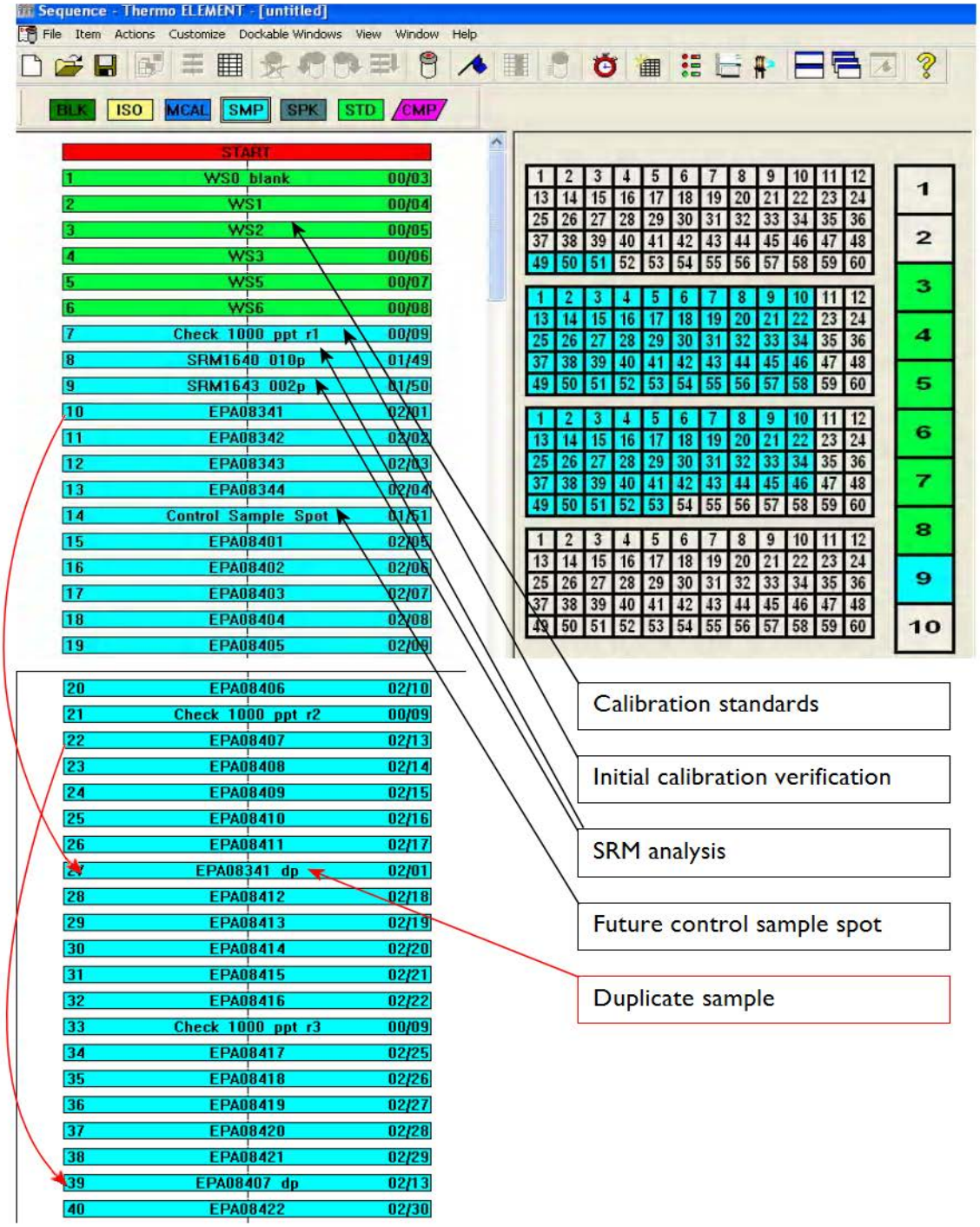


Figure 11. Sequence Editor with QC samples.

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.

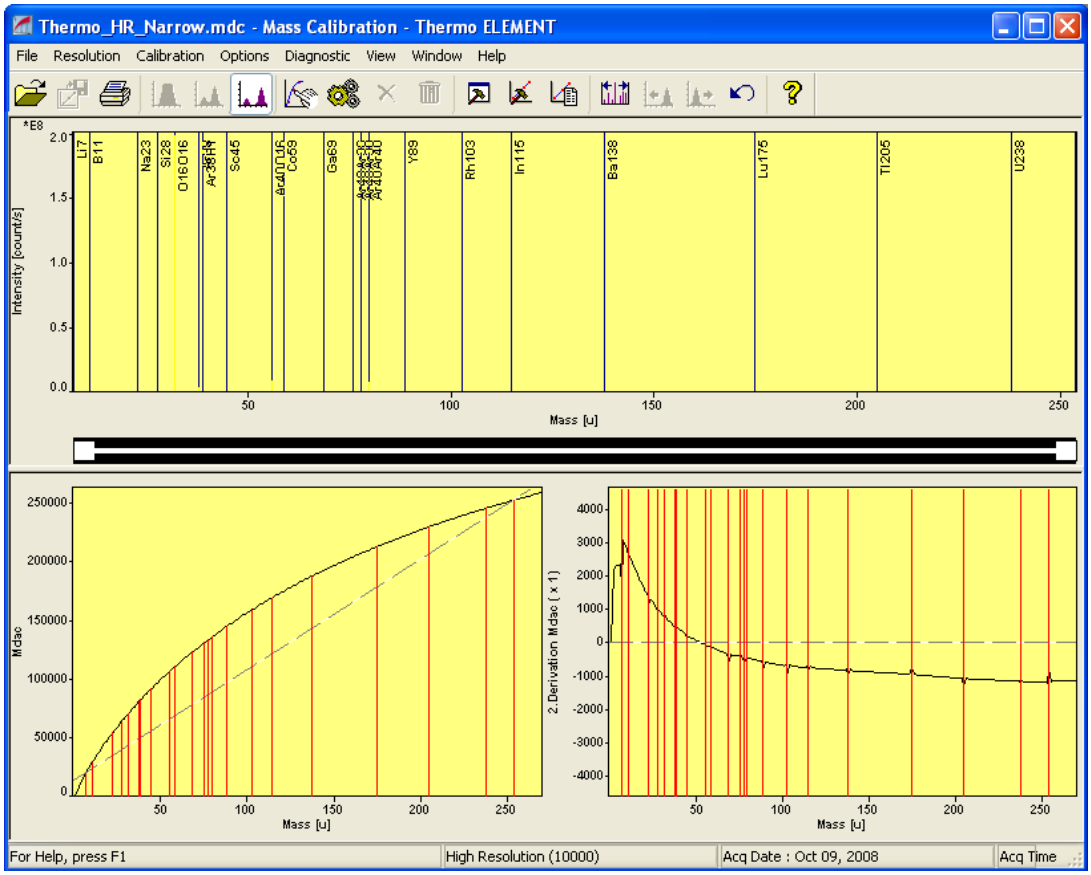
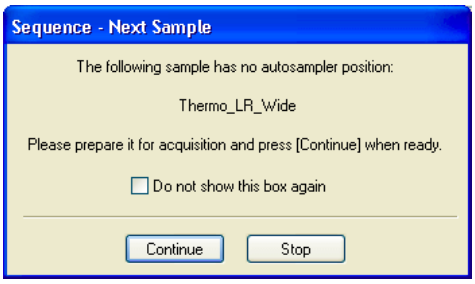


Figure 12. Mass Calibration task window.

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.

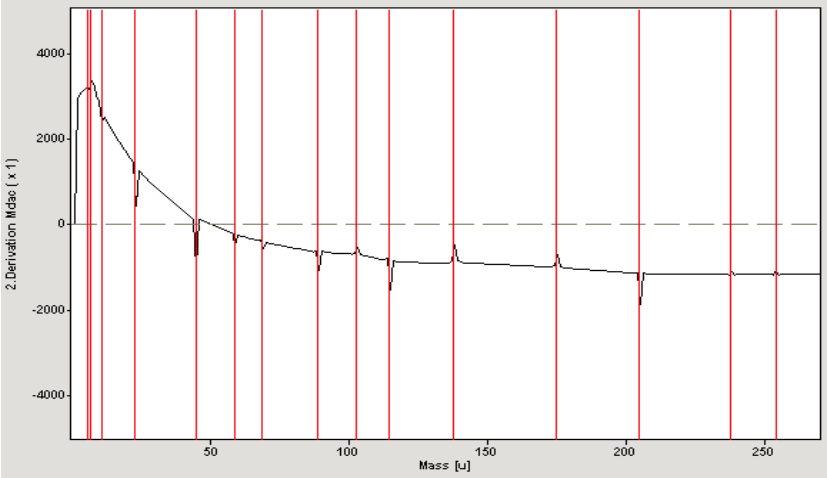


Figure 13. Typical LR 2nd deriv diagnostic curve.

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.

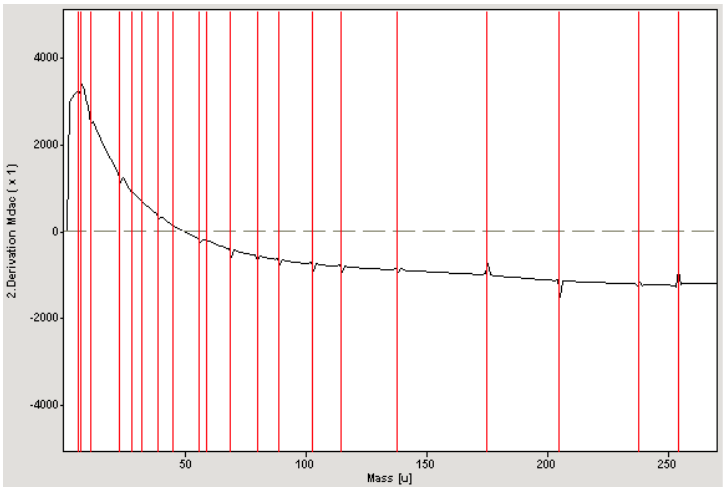


Figure 14. Diagnostic curve for MR.

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.

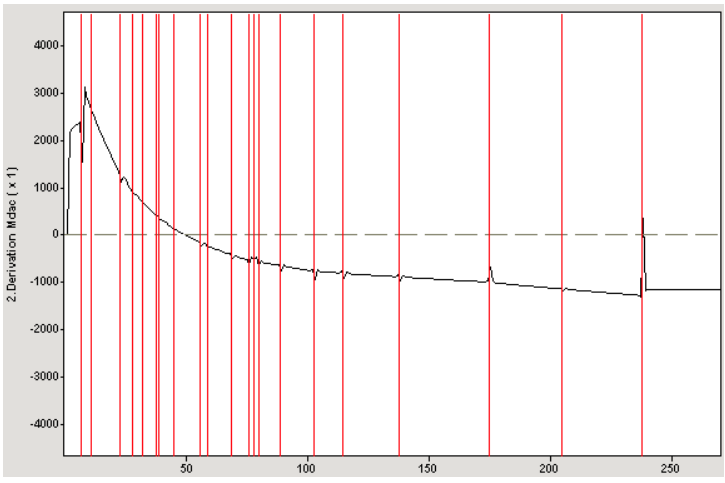


Figure 15. Diagnostic curve for HR.

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.

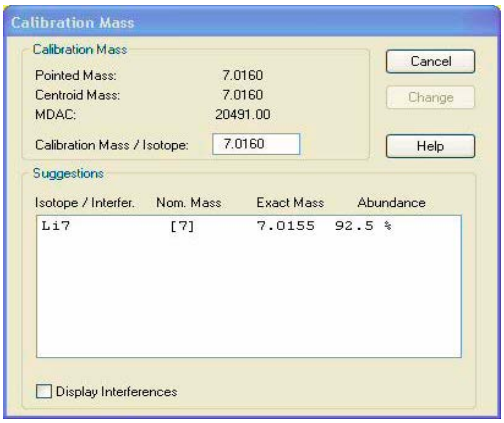


Figure 16. Calibration Mass dialog box.

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.

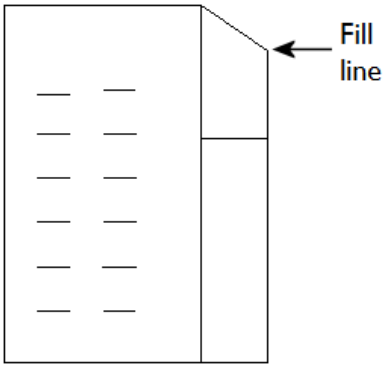
Perform the following steps to reestablish mass calibration:

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, US EPA, 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

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

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								

3.3 Usage Dependent Log

Date	Item Replaced	Cause of Change	Operator Initials

3.7 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.

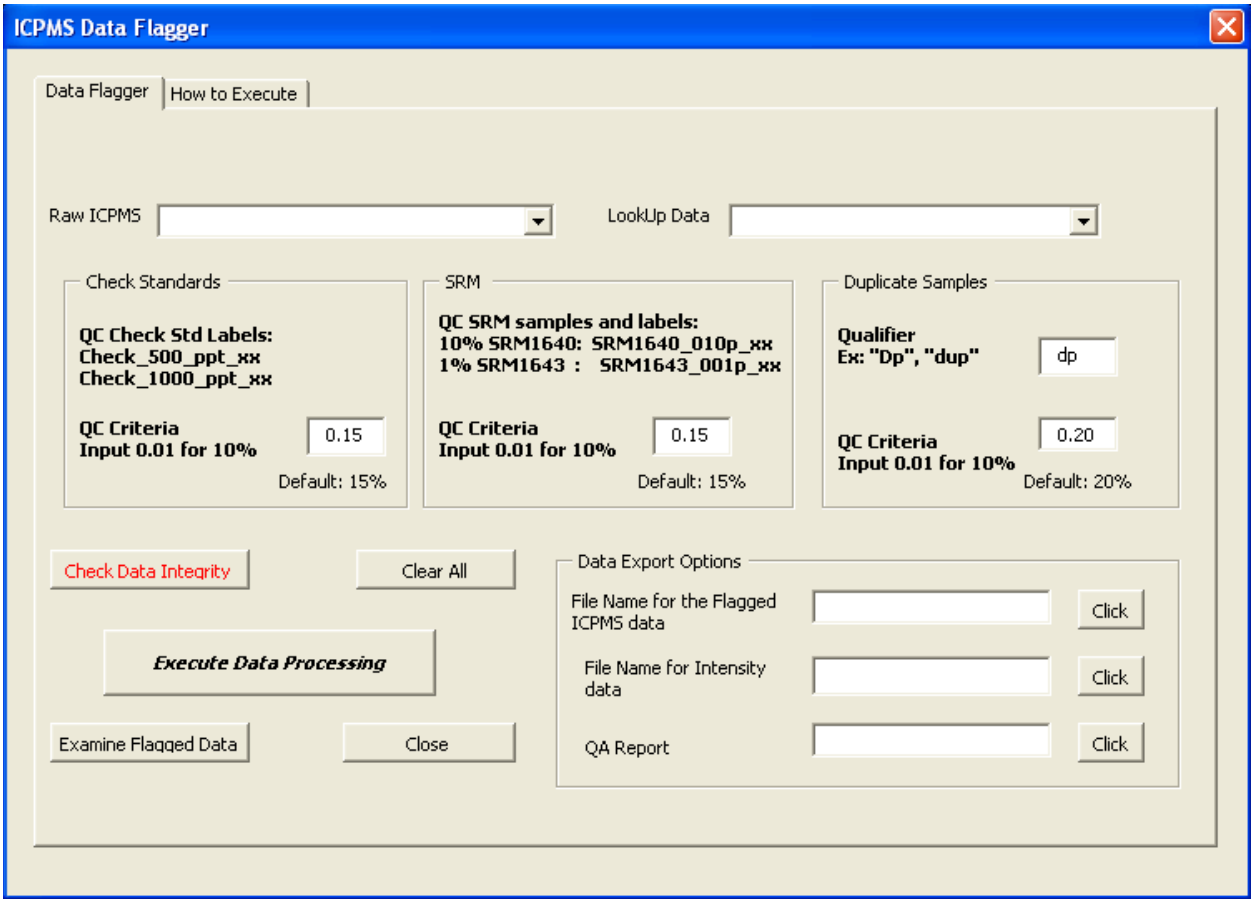


Figure 1. ICPMS Data Flagger application screen.

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 Ca, Mg, K, Si, Na	300 ppb 1000 ppb	2% Nitric Acid
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 Ca, Mg, K, Si, Na	300 ppb 1000 ppb	2% Nitric Acid
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.

Appendix D

Synthetic Turf Field Facility User Questionnaires – Adult/Adolescent and Youth/Child Versions

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

Adult/Adolescent Field User Questionnaire

PID		Site ID Number	
Facility Name		Facility Location	
Interview Date		Interviewer ID	

Interviewer: I would like to ask you some questions about activities that may affect your exposures to, and contact with synthetic turf fields that contain crumb rubber materials.

Field Contact Frequency and Duration Questions

Interviewer: I have several questions about the time you spend on synthetic turf fields at this facility.

B1. How long have you been coming to this facility?

	(years)
	(months)

B2. Specifically on the synthetic fields at this facility, what sports, physical education classes, or other activities have you actively participated in by season (specify) over the past year?

Season	Sport	Specify Other

B3. Over the past year, how many days per week by season have you typically spent ***on the synthetic fields at this facility***?

Spring		(days per week)
Summer		(days per week)
Fall		(days per week)
Winter		(days per week)

B4. Over the past year, how many hours per day by season have you typically spent ***on the synthetic fields at this facility***?

Spring		(hours per day)
Summer		(hours per day)
Fall		(hours per day)
Winter		(hours per day)

B5. Over the past year, what was the longest period of time that you spent ***on the synthetic fields at this facility*** during a single day?

	(number of hours)

Contact Types and Scenarios per Each Type of Field Use

Interviewer: I have several questions about the kinds of activities that you take part in specifically **on synthetic turf fields installed at this facility**.

For the following question, please use one of the three responses (often, sometimes, and rarely/never). "Often" means > 50% of the time and "sometimes" means < 50%.

B6. How frequently do you do the following activities while **on synthetic fields** at this facility each season?

	Dive on ground	Fall on ground	Sit on turf	Eat snacks	Drink
Spring					
Summer					
Fall					
Winter					

Inhalation Exposure-Related Questions

B7. When using ***synthetic fields at this facility***:

What % of your time are you highly active, for example, running?	
What % of your time are you moderately active, for example, jogging?	
What % of the time do you have low activity, for example, walking?	
What % of the time are you resting, for example, sitting or standing?	

Dermal and Non-dietary Ingestion Exposure-related Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B8. When ***using synthetic turf fields at this facility***:

	Every Time	Often	Some times	Rarely /Never
How often do you chew gum?	3	2	1	0
How often do you use a mouth guard?	3	2	1	0
How often do you eat?	3	2	1	0
How often do you drink?	3	2	1	0
How often do you play in the rain?	3	2	1	0
How often do you wipe your hands with a hand wipe before eating?	3	2	1	0
How often do you sweat heavily?	3	2	1	0
How often do you touch the turf with your hand?	3	2	1	0
How often do you touch the turf with your other body parts excluding hands?	3	2	1	0
How often do you sit on the turf with bare skin wearing shorts?	3	2	1	0
How often are you barefooted on the turf?	3	2	1	0
How often do you play with the turf materials or rubber granules?	3	2	1	0
How often do you touch your mouth with your hands or fingers?	3	2	1	0
How often do you place non-food objects in your mouth like toothpicks, or pens or use your mouth to hold an object?	3	2	1	0

If rarely/never, skip next.

What type of object do you most often place in your mouth while at this facility?	

How often to you get cuts or abrasions from contact with the turf?	3	2	1	0

If rarely/never, skip next.

What is the body part that usually has the most cuts or abrasions: knee, elbow, hand, thigh, shin, or other?	

B9. What clothing do you typically wear in this facility during each season (check all that apply)?

	Spring	Summer	Fall	Winter
Shorts	☐	☐	☐	☐
Short-sleeve shirt	☐	☐	☐	☐
Long pants	☐	☐	☐	☐
Long-sleeve shirt	☐	☐	☐	☐
Gloves	☐	☐	☐	☐
Socks	☐	☐	☐	☐
Helmet	☐	☐	☐	☐
Hat	☐	☐	☐	☐
Pads	☐	☐	☐	☐

Tire Crumb Take-Home Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B10. After using this facility:

How often do you notice tire crumbs, dirt, or debris

	Every Time	Often	Sometimes	Rarely/Never
on your body?	3	2	1	0
in your car?	3	2	1	0
in your home?	3	2	1	0
In your laundry room/mudroom?	3	2	1	0
In your living room?	3	2	1	0
In your bedroom?	3	2	1	0
In your bathroom(s)?	3	2	1	0

Post-Use Hygiene Practices Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B11. After using this facility:

	Every Time	Often	Sometimes	Rarely/Never
How often do you take shower and change clothes immediately after engaging in activities on the synthetic turf at this facility?	3	2	1	0
How often do you take actions to prevent tire crumbs from getting into your car?	3	2	1	0
How often do you wipe or remove shoes/equipment before entering your home?	3	2	1	0

For the following questions, please use one of the six responses (never, once a month, 2 to 3 times a month, once a week, 2-3 times a week, or four or more times a week).

B12. At other locations:

	Never	*Once a month*	2 to 3 times a month	Once a week	2 to 3 times a week	4 or more times a week
How often have you played on any other synthetic turf fields during the past year?	0	1	2	3	4	5
How often have you played on any synthetic turf fields in the last five years?	0	1	2	3	4	5
How often have you played on any natural grass fields during the past year?	0	1	2	3	4	5
How often have you played on any natural grass turf fields in the last five years?	0	1	2	3	4	5
How often have you played on playgrounds with rubber mulch, mats or synthetic turf during the past year?	0	1	2	3	4	5
How often have you played on playgrounds with rubber mulch, mats or synthetic turf during in the last five years?	0	1	2	3	4	5

General Hygiene Questions

B13. How many times in general do you wash hands per day? _____

B14. How many times in general do you bathe or shower per week _____

General Demographic Questions

D1. How old are you? ☐

D2. Are you male or female? ◉ Male ◉ Female ◉ Refused

D3. Do you consider yourself to be Hispanic or Latino? ◉ Yes ◉ No ◉ Refused

D4. Which of the following categories best describes your race? (select one or more)

◉	Native American Indian or Alaska Native	◉	Black or African American	◉	White	◉	Don't know
◉	Asian	◉	Native Hawaiian or Other Pacific Islander	◉	Refused		

D5. How tall are you? ☐ (ft) ☐ (in)

D6. How much do you weigh? ☐ (lbs)

D7. Are you still in school? ☐ yes ☐ no

If so, what is your current grade in school?

◉ 7th ◉ 8th ◉ 9th

◉ 10th ◉ 11th ◉ 12th

◉ Technical School ◉ College ◉ Graduate School

◉ Other ◉ Refused

Specify Other Grade ☐

D8. If No, what is your highest education level?

- ◉ 11th or less
- ◉ High School Graduate/ GED
- ◉ Post High School Training
- ◉ Some College
- ◉ College Graduate School
- ◉ Post-graduate
- ◉ Other
- ◉ Refused

D9. What is your occupation?

This concludes the survey. Thank you 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.

Form Approved
OMB No. 0923-xxxx
Exp. Date xx/xx/201x

Youth/Child Field User Questionnaire

PID		Site ID Number	
Facility Name		Facility Location	
Interview Date		Interviewer ID	

Interviewer: I would like to ask you some questions about activities that may affect your child's exposures to, and contact with synthetic turf fields that contain crumb rubber materials.

Field Contact Frequency and Duration Questions

Interviewer: I have several questions about the time your child spends on synthetic turf fields at this facility

B1. How long has your child been coming to this facility?

	(years)
	(months)

B2. Specifically on the synthetic fields at this facility, what sports, physical education classes, or other activities has your child actively participated in by season (specify) over the past year?

Season	Sport	Specify Other

B3. Over the past year, how many days per week by season has your child typically spent **on synthetic fields at this facility**?

Spring		(days per week)
Summer		(days per week)
Fall		(days per week)
Winter		(days per week)

B4. Over the past year, how many hours per day by season has your child typically spent **on the synthetic fields at this facility**?

Spring		(hours per day)
Summer		(hours per day)
Fall		(hours per day)
Winter		(hours per day)

B5. Over the past year, what was the longest period of time that your child has spent **on the synthetic fields** at this facility during a single day?

	(number of hours)

Contact Types and Scenarios per Each Type of Field Use

Interviewer: I have several questions about the kinds of activities that your child takes part in specifically ***on synthetic turf fields installed at this facility****.*

For the following question, please use one of the three responses (often, sometimes, and rarely/never). "Often" means > 50% of the time and "sometimes" means < 50%.

B6. How frequently does your child do the following activities **on synthetic fields** at this facility each season?

	Dive on ground	Fall on ground	Sit on turf	Eat snacks	Drink
Spring					
Summer					
Fall					
Winter					

Inhalation Exposure-Related Questions

B7. When using **synthetic fields at this facility**:

What % of the time is your child highly active, for example, running?

What % of the time is your child moderately active, for example, jogging?

What % of the time does your child have low activity, for example, walking?

What % of the time is your child resting, for example, sitting or standing?

Dermal and Non-Dietary Ingestion Exposure-Related Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B8. When **using synthetic turf fields at this facility**:

	Every Time	Often	Some times	Rarely / Never
How often does your child chew gum?	3	2	1	0
How often does your child use a mouth guard?	3	2	1	0
How often does your child eat?	3	2	1	0
How often does your child drink?	3	2	1	0
How often does your child play in the rain?	3	2	1	0
How often does your child wipe their hands with a hand wipe before eating?	3	2	1	0
How often does your child sweat heavily?	3	2	1	0
How often does your child touch the turf (with their hand)?	3	2	1	0
How often does your child touch the turf with their body excluding hands?	3	2	1	0
How often does your child sit on turf with bare skin wearing shorts?	3	2	1	0
How often is your child barefooted on the turf?	3	2	1	0
How often does your child play with the turf materials or rubber granules?	3	2	1	0
How often does your child touch their mouth with their hands or fingers?	3	2	1	0
How often does your child place non-food objects in their mouth every time like toothpicks, or pens or use their mouth to hold an object? If rarely/never, skip next.	3	2	1	0
What type of object does your child most often places in their mouth while at this facility?				
How often does your child get cuts or abrasions from contact with the turf?	3	2	1	0
If rarely/never, skip next.				
What is the body part that usually has the most cuts or abrasions: knee, elbow, hand, thigh, shin, or other?				

B9. What clothing does your child typically wear in this facility during each season (check all that apply)?

	Spring	Summer	Fall	Winter
Shorts	☐	☐	☐	☐
Short-sleeve shirt	☐	☐	☐	☐
Long pants	☐	☐	☐	☐
Long-sleeve shirt	☐	☐	☐	☐
Gloves	☐	☐	☐	☐
Socks	☐	☐	☐	☐
Helmet	☐	☐	☐	☐
Hat	☐	☐	☐	☐
Pads	☐	☐	☐	☐

Tire Crumb Take-Home Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B10. After using this facility:

How often do you notice tire crumbs, dirt, or debris

	Every Time	Often	Sometimes	Rarely/Never
on your child's body?	3	2	1	0
in your car?	3	2	1	0
in your home?	3	2	1	0
In your laundry room/mudroom?	3	2	1	0
in living room?	3	2	1	0
in your child's bedroom?	3	2	1	0
in your bathroom(s) your child uses?	3	2	1	0

Post-Use Hygiene Practices Questions

For the following questions, please use one of the four responses (every time, often, sometimes, or rarely/never):

B11. After using this facility:

	Every Time	Often	Sometimes	Rarely/Never
How often does your child shower and change clothes immediately after engaging in activities on the synthetic turf at this facility?	3	2	1	0

How often does your child's shoes/equipment get wiped or removed before entering your home?	3	2	1	0

For the following questions, please use one of the six responses (never, once a month, 2 to 3 times a month, once a week, 2-3 times a week, or four or more times a week).

B12. At other locations:

	Never	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 or more times a week
How often has your child played on any other synthetic turf fields during the past year?	0	1	2	3	4	5
How often has your child played on any synthetic turf fields in the last five years?	0	1	2	3	4	5
How often has your child played on any natural grass fields during the past year?	0	1	2	3	4	5
How often has your child played on any natural grass turf fields in the last five years?	0	1	2	3	4	5
How often has your child played on playgrounds with rubber mulch, mats or synthetic turf during the past year?	0	1	2	3	4	5
How often has your child played on playgrounds with rubber mulch, mats or synthetic turf during in the last five years?	0	1	2	3	4	5

General Hygiene Questions

B13. How many times in general does your child wash their hands per day? ☐

B14. How many times in general does your child bathe or shower per week? ☐

General Demographic Questions

D1. How old is your child? ☐

D2. Is your child male or female? ◉ Male ◉ Female ◉ Refused

D3. Do you consider your child to be Hispanic or Latino? ◉ Yes ◉ No ◉ Refused

D4. Which of the following categories best describes your child's race? (select one or more)

- ◉ Native American Indian or Alaska Native
- ◉ Black or African American
- ◉ White
- ◉ Don't know
- ◉ Asian
- ◉ Native Hawaiian or Other Pacific Islander
- ◉ Refused

D5. How tall is your child? [ ] (ft) [ ] (in)

D6. How much does your child weigh? [ ] (lbs)

D7. What is your child's current grade in school?

- ◉ 2nd
- ◉ 3rd
- ◉ 4th
- ◉ 5th
- ◉ 6th
- ◉ 7th
- ◉ 8th
- ◉ 9th
- ◉ Other
- ◉ Refused

Specify other grade [ ]

That concludes the survey. Thank you 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 E

Exposure Characterization Meta-Data Collection Forms

E.1 Meteorological Conditions

TCRS Exposure Characterization Study		Meteorological Conditions	
Note: Complete this form for each day of monitored participant activities at each field.			
		Field ID Number	
		Date	
		Outdoor or Indoor Field	
Metric/Information	Near Start of Participant Activities	Near Middle of Participant Activities	Near End of Participant Activities
Time of Day (Military time format)			
Field Air Temperature at 1 m (°C)			
Field Surface Temperature (°C)			
Wind 1-minute average speed (km/h)			
Wind 1-minute maximum speed (km/h)			
Wind direction (compass degrees, where wind is coming from)			
Dew on field (yes/no)			
Field wet from rain or watering (yes/no)			
Conditions (sunny, partly cloudy, cloudy, drizzle, rain)			

E.2 General Activity Information

TCRS Exposure Characterization Study		General Activity Information	
Note: Complete this form for each day of monitored participant activities at each field.			
		Field ID Number	
		Date	

Metric/Information	At Start of Participant Activities	Near Middle of Participant Activities	At End of Participant Activities
Approx. Number People in Active Play on Study Field			
Approx. Number People as Bystanders on Study Field			
Sport Name on Study Field (soccer, football, etc.)			
Type of Active Play on Study Field (1)			
Type of Active Play on Study Field (2)			
Type of Active Play on Study Field (3)			
Type of Active Play on Study Field (4)			
Type of Active Play on Study Field (5)			
Adjacent Synthetic Fields in Use (yes/no)			
Approx. Number People at Adjacent Synthetic Fields			
Adjacent Grass Fields in Use (yes/no)			
Approx. Number People at Adjacent Grass Fields			

E.3 Participant Activity Information

TCRS Exposure Characterization Study	**Participant Information**					
Note: Complete this form for each participant at each field.						
	Field ID Number					
	Date					
	Participant ID (from 1 to 8)					

Metric/Information	At Start of Participant Activities	Approx. 30 minutes Into Participant Activities	Approx. 60 minutes Into Participant Activities	Approx. 90 minutes Into Participant Activities	Approx 120 minutes Into Participant Activities	At End of Participant Activities
Time of Day (military time format)						
Activities						
Sport Name (soccer, football, etc.)						
Sport Position (goal keeper, soccer field player, football receiver, etc)						
Type of Activities on Study Field						
Physical Activity Level (low, medium, high)						
Contacting Turf (yes/no)						
Types of Turf Contact (hands, arms, legs, face, body)						
Frequency of Turf Contact (≥ 1/min; ≥ 1/5min; < 1/5min)						
Clothing/Equipment Types (record only once - not at each time period)						
Shirt (yes/no; long/short)						
Pants (long/short)						
Socks (yes/no; high/mid/low)						
Gloves (yes/no and type)						
Head Gear (yes/no and type; hat, helmet, other)						
Mouth Guard (yes/no)						
Pads (yes/no and type; shoulder, hip, leg, other)						
Wearing sunscreen						
Wearing bug repellent						
Other Information						

E.4 General Field Information

TCRS Exposure Characterization Study		General Field Information/Observations		
Use this form to record observations that may be relevant to the research study.				
Examples include, but are not limited to, condition of field, field maintenance, construction on/near field,				
adjacent high traffic on roads or parking areas, ventilation information for indoor fields, other relevant information.				
Use a different row for each type of observation.				
			Field ID Number	
			Date	

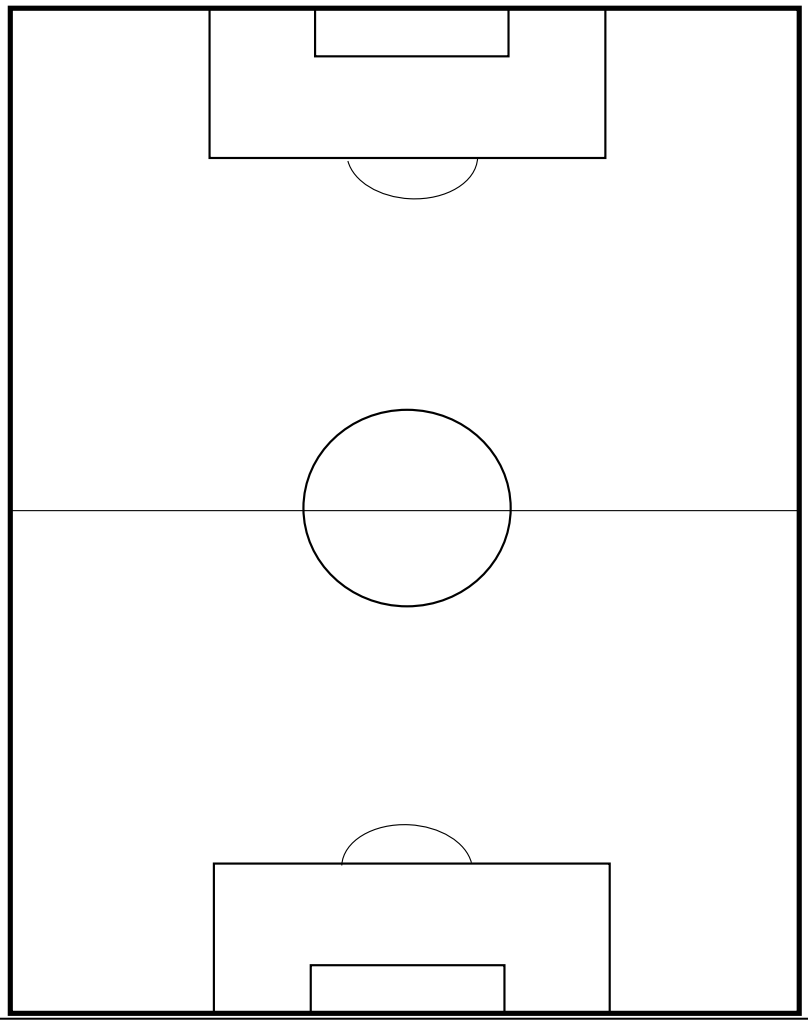
E.5 Field Sampling Locations

Field Sampling Location Record Form

Field ID Number

Field SVOC wipe locations S
Field metals wipe locations M
Field SVOC drag sled locations D
Air sample station locations A
(For off-field upwind air sample station estimate and record distance from field center; record type of ground surface)

Draw Magnetic North Direction Arrow Here



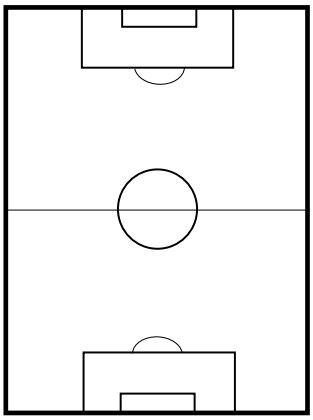
E.6 Field Environment Information

Field Environment Record Form

Field ID Number

Sketch and label features within approx.. 100 m of field
Include roads, parking areas, other fields, buffers, buildings or other natural and built features
Record approximate off-field sampling station location

Draw Magnetic North Direction Arrow Here



Appendix F

Blood Metals and Serum Metals Analysis Protocols

Division of Laboratory Sciences

Laboratory Protocol

Analytes: Cadmium, Lead, Manganese, Mercury, and Selenium

Matrix: whole blood

Method: blood multi-element analysis by ICP-DRC-MS

Method code: DLS 3016.8-05

Branch: Inorganic and Radiation Analytical Toxicology

Prepared By: Deanna M. Jones, PhD _____ _____
author's name signature date

_____ _____ _____
author's name signature date

Supervisor: Jeffery M Jarrett, MS _____ _____
supervisor's name signature date

Branch Chief: Robert L Jones, PhD _____ _____
Branch Chief signature date

Date current version of method first used in lab: _____
Date

Director's Signature Block:

Reviewed: _____ _____
Signature Date

Procedure Change Log

Procedure: Blood multi-element analysis by ICP-DRC-MS

DLS Method Code: 3016.8-05

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
4/1/2011	1UB and 2UB for Mn changed from 15 to 25 ug/L and from 30 to 50 ug/L, respectively.	JHJ8	JHJ8	4/1/2011
4/1/2011	Limit Rep Delta for Mn changed from 1.0 to 2.0.	JHJ8	JHJ8	4/1/2011
7/28/2011	Clarified matrix of internal standard intermediate from "dilute HNO3" to "1% v/v HNO_3".	JHJ8	JHJ8	7/28/2011
8/9/2011	Changed BMN 1UB (25 ug/L to 20 ug/L) and 2UB (50ug/L to 35 ug/L). Supporting references added.	JHJ8	JHJ8	8/9/2011
10/7/2011	Added comment to CV standard tables regarding use of gravimetric preparation.	JHJ8	JHJ8	10/7/2011
3/20/2012	Sample Diluent Preparation: Triton X-100 percentage correction (typo)	nap1	JHJ8	3/20/2012
3/20/2012	DRC Stability Test Preparation: alternate preparation procedure using the intermediate working calibrators	nap1	JHJ8	3/20/2012
3/20/2012	Preparation of Samples for Analysis: changed the Blood Blank name from "BldBlkChk" to "WB Blank" and WB Blank2"	nap1	JHJ8	3/20/2012
3/20/2012	Contaminated Blanks: added clarification on procedure to follow in the event of contaminated blanks	nap1	JHJ8	3/20/2012
3/20/2012	Linear Calibration Curves: clarification on dropping points	nap1	JHJ8	3/20/2012
3/20/2012	Appendix B, Table 1: Added description for method file names Method Parameters: updated sample flush times and sample wash times Autosampler Locations: Aq Blank location	nap1	JHJ8	3/20/2012
3/20/2012	Appendix B, Table 3: Clarification of stock standard preparation	nap1	JHJ8	3/20/2012
3/20/2012	Appendix B, Table 10: Typical sample/batch window: changed autosampler location to reflect current positions	nap1	JHJ8	3/20/2012
3/20/2012	Updated screenshots in Appendix B, Figures 1e, 1f, and 2d	nap1	JHJ8	3/20/2012
3/20/2012	Created Appendix C for "help sheets"	nap1	JHJ8	3/20/2012

3/20/2012	Method Procedures: Types of Quality Control: Removed reference to blind QC	nap1	JHJ8	3/20/2012
5/03/2012	Sample Diluent Preparation: Changed concentration of TMAH from 0.25% to 0.4%	nap1	JHJ8	5/03/2012
5/10/2012	Added Appendix A, Experiment 6: Validated extra dilutions up to 20x. Updated Reportable Range and Table 6 (descriptions of sample preparation).	EMU2	JHJ8	5/10/2012
9/10/2012	Sample Rinse Preparation: Changed concentration of TMAH from 0.25% to 0.4%	nap1	JHJ8	9/10/2012
1/22/2013	Extended calibration range S0-S8 adding a third bench QC level. Changed to weighted linear regression and dual detector mode.	JHJ8	KLC7	1/22/2013
1/22/2013	Clarified and updated handling elevated concentrations, Tables 8 – 11, Sections 7 – 11 and references. Added Figures 1 and 4.	JHJ8	KLC7	1/22/2013
1/22/2013	Added description of solutions for DRC and dual detector optimizations.	JHJ8	KLC7	1/22/2013
1/22/2013	Updated reference range Tables	JHJ8	KLC7	1/22/2013
1/22/2013	Added detail of potential MoO_2 interference on ^{130}Te	JHJ8	KLC7	1/22/2013
1/22/2013	Updated action levels	JHJ8	KLC7	1/22/2013
3/20/2013	Updated evaluating calibration curves language	nap1	JHJ8	3/20/2013
4/16/2013	Updated help sheets re: calibration std prep	nap1	JHJ8	4/16/2013
5/15/2013	Replaced references to urine with references to blood in Table of Figures, Section 7.c.ii, and Section 12. Updated reference from “Section 10a” to “Section 11a” in Section 10a. References to Tables 5, 10, and 11 updated.	JHJ8	Klc7	5/20/2013
9/15/2014	Clarified method details (esp. references to urine methods and solutions preparations). Bldblkchk to be made with S0 instead of water.	JHJ8	Klc7	9/15/2014
12/08/2015	Changed method name from Blood Metals Panel 3 (BMP3) by ICP-DRC-MS to Blood multi-element analysis by ICP-DRC-MS	JJ	KLC	12/9/2015
12/08/2015	Updated Title page to new DLS template	JJ	KLC	12/9/2015
12/08/2015	Updated Section 3 to specify not to freeze blood in blood collection tubes (esp. glass)	JJ	KLC	12/9/2015
12/08/2015	Clarified comments, updated examples, corrected typos: Increased use of active voice (eliminated ‘may’ and ‘shall’). Clarified comments in Tables 8 and 9. Renamed second “Figure 2g” to “Figure 2h”. Correct table references in Section 10.	JJ	KLC	12/9/2015

12/08/2015	Minor equipment updates: References to Digiflex pipette changed to Hamilton Microlab 625 benchtop automatic pipette and updated Table 8 volumes. Updated regulator part numbers for methane and oxygen compressed gases.	JJ	KLC	12/9/2015
12/08/2015	Updated instructions related to very elevated results. Set criteria to confirm proper washout after an elevated sample to ± 3SD limits of low bench QC wash check (Section 8.b.iv). Set criteria to confirm samples potentially affected by insufficient washout to ±10% or ±3SD of the low bench QC, whichever is greater (Section 8.b.vii.2.a). Updated extended wash details in Table 1. Added highest validated washout concentrations to Table 9. Updated Figure 4 (Flow Chart for handling an elevated result).	JJ	KLC	12/9/2015
12/08/2015	Added 2011-2012 NHANES reference level data to Table 10 and replaced statement about blood lead >10 µg/dL with statement about 5 µg/dL reference level.	JJ	KLC	12/9/2015
12/08/2015	Updated record retention in section Section 9.c to match DLS policy (3 years to 2 years).	JJ	KLC	12/9/2015
03/02/2016	Left justified text. Updated references. Removed “(esp. glass)” regarding do not freeze blood in blood tubes. Referenced highest calibrator and max extra dilution tables in reportable range section. Updated description of disinfectant. Changed “working calibration standard” to “working calibrator” throughout. Updated references to high purity water.	JJ	RLJ	03/02/2016

Laboratory Procedure Manual

Analytes: **Cadmium, Lead, Manganese, Mercury, and Selenium**

Matrix: **Whole Blood**

Method: **blood multi-element analysis by ICP-DRC-MS**

Method No: DLS 3016.8-05

As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
National Center for Environmental Health

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James L. Pirkle, M.D., Ph.D.
Director, Division of Laboratory Sciences

Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

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1. Clinical relevance & summary of test principle

a. Clinical relevance:

Metals ions affect human health in various ways. Some metals (i.e. lead, cadmium, and mercury) show only deleterious effects on human health. Some (i.e. selenium and manganese) play an essential role in the human biological system if within certain concentration ranges, while negative health implications are observed when concentrations in biological systems are in deficit or excess. Determination of a person's level of environmental exposure to chemicals through direct measurement of the substances or their metabolites in human specimens such as blood is called biomonitoring. Biomonitoring reduces the uncertainty of determining levels of exposure over making these determinations through calculations of estimated dose based on analysis of environmental samples and assumptions about exposure pathways[1]. Biomonitoring measurements are the most health-relevant assessments of exposure because they indicate the amount of the chemical that actually gets into people from all environmental sources (e.g., air, soil, water, dust, or food) combined, rather than the amount that gets into them. The laboratory method described here is a multi-element technique for monitoring the concentrations of cadmium (Cd), lead (Pb), manganese (Mn), mercury (Hg), and selenium (Se) in whole human blood for the purpose of biomonitoring.

There is no known biological role of mercury in the human body. The main sources of mercury intake in humans are fish, dental amalgams, and occupational exposures[2]. The main organs affected by mercury are the brain and the kidneys. Exposure of childbearing-aged women is of particular concern because of the potential adverse neurologic effects of Hg in fetuses. The health effects of mercury are diverse and depend on the form of mercury encountered and the severity and length of exposure. The general population is be exposed to three forms of mercury: elemental, inorganic, and organic (predominantly methyl). However, this method tests only for the total amount of mercury in the blood without regard to chemical form. In the general population, total blood mercury is due mostly to the dietary intake of organic forms which are formed through microbial action from inorganic mercury that has deposited in aquatic environments and bioaccumulated through the food chain (especially into large predatory fish)[3]. Exposure to inorganic or elemental mercury (e.g. dental amalgams or occupational exposures) is particularly reflected in urine excretion rather than blood. Psychic and emotional disturbances are the initial signs of chronic intoxication by elemental mercury vapors or salts. Those exposed are at increased risk for parasthesia, neuralgias, renal disease, digestive disturbances, and ocular lesions [4]. Massive exposure over a longer period of time results in violent muscular spasms, hallucinations, delirium, and death[5]. Except for methylmercury exposures, blood is considered useful if samples are taken within a few days of exposure. This is because most forms of mercury in the blood decrease by one-half every three days if exposure has been stopped. Thus,

mercury levels in the blood provide more useful information after recent exposures than after long-term exposures. Several months after an exposure, mercury levels in the blood and urine are much lower. Blood mercury reference ranges for the U.S. population are listed in Table 10 in Appendix B.

There is no known biological role of lead in the human body. Lead, a naturally occurring metal, has had many different commercial uses from which a person can be exposed either in the occupational / manufacturing process or by the manufactured products such as paint (paint chips, or dust and soil contaminated from deteriorating paint), solder or pipes (only now in older homes), gasoline (now outlawed for all but specialized applications), glazes on pottery, hobby uses (e.g. stained glass), commercial products (e.g. batteries, lead-containing jewelry), home remedy medicines containing lead compounds and non-Western cosmetics. Soil contains lead naturally, or from man-made uses of lead such as paint (near older homes), gasoline (near roadways), mining, manufacturing, and disposal. The main target for lead toxicity is the nervous system, both in adults and children. The developing biological systems of children are most sensitive to the effects of Pb, where effects are being recognized even at blood lead levels <5 μg/dL [6-10]. Acute, elevated lead exposure is associated with anorexia, dyspepsia, and constipation followed by diffuse paroxysmal abdominal pain. When lead exposure is high, particularly in children, the person is at increased risk for encephalopathy [11]. The alkyl lead species are highly toxic to the central nervous system[12]. The primary screening method for lead exposure is blood lead, which primarily reflects recent exposures (excretory half-life in blood is approximately 30 days)[13]. Lead in blood is primarily (99%) in the red blood cells. Blood lead reference ranges for the U.S. population are listed in Table 10 in Appendix B. The CDC now uses a reference level of 5 μg/dL to identify children with blood lead levels that are much higher than most children's levels. This new level is based on the U.S. population of children ages 1-5 years who are in the highest 2.5% of children when tested for lead in their blood. This reference value is based on the 97.5th percentile of the National Health and Nutrition Examination Survey (NHANES)'s blood lead distribution in children. CDC will update the reference value every four years using the two most recent NHANES surveys [14].

There is no known biological role of cadmium in the human body. The predominant commercial use of cadmium is in battery manufacturing. Other uses include pigment production, coatings and plating, plastic stabilizers, and nonferrous alloys. Since 2001, U.S. cadmium use has declined in response to environmental concerns. In the United States, for nonsmokers the primary source of cadmium exposure is from the food supply. People who regularly consume shellfish and organ meats will have higher exposures. In general, leafy vegetables such as lettuce and spinach, potatoes and grains, peanuts, soybeans, and sunflower seeds contain high levels of cadmium due to bioaccumulation from the soil. Tobacco leaves accumulate high levels of cadmium from the soil, and smoking is the primary non-occupational source of

cadmium exposure for smokers. Generally, the critical organ for Cd is the kidney. Kidney dysfunction is one of the most characteristic signs of exposure to Cd. Workers in an environment with high exposure levels have developed proteinuria, renal glucosuria, aminoaciduria, hypercalciuria, phosphaturia, and polyuria. Chronic obstructive lung disease of varying degrees of severities is frequently seen in Cd workers. Concentration of cadmium in blood of healthy unexposed adults are in the range 0.1 – 4 μg/L[15]. Newborn babies are practically free of Cd[16]. Exposure to high concentration of fumes appearing from heated cadmium metal or compounds has led to acute poisoning and in some cases to the death of workers[11]. Principal symptoms reported were respiratory distress due to chemical pneumonitis and edema. It has been estimated that 8 hrs. exposure to 5 g Cd/m^3 will be lethal[11]. Ingestion of high amounts of Cd puts a person at increased risk to a rapid onset with severe nausea, vomiting, and abdominal pain. Cadmium levels in blood, urine, feces, liver, kidney, hair, and other tissues have been used as biological indicators of exposure to cadmium. Blood cadmium levels are principally indicative of recent exposure(s) to cadmium rather than whole-body burdens [17-20]. Urine cadmium levels primarily reflect total body burden of cadmium, although urine levels do respond somewhat to recent exposure[21]. Blood cadmium reference ranges for the U.S. population are listed in Table 10 in Appendix B.

Manganese (Mn) is a trace element essential to humans and is associated with the formation of connective and bony tissue, growth and reproductive functions and with carbohydrate and lipid metabolism [22]. Manganese is also a known neurotoxin but little information exists about levels of manganese that cause toxicity. Symptoms of manganese toxicity are similar to Parkinson's Disease and can also include disorientation, memory impairment, anxiety and compulsive behavior [23]. There is much concern for the levels of manganese in humans whom are occupationally exposed to it [24-30]. Recently, there are growing concerns over exposure due to contamination of drinking water with manganese [31-33] and as a result of methylcyclopentadienyl mangangese tricarbonyl (MMT) used as an anti-knocking additive in gasoline [34-40]. Populations suffering from iron deficiencies are at an increased risk to manganese toxicity because iron deficiency can result in an accumulation of manganese in the central nervous system [37]. To fully understand the essentiality and toxicity of manganese, further investigations are needed regarding the levels of manganese in biological matrices. Group average levels in blood appear to be related to manganese body burden, while average urinary excretion levels appear to be most indicative of recent exposures [41]. On an individual basis the correlation between the level of workplace exposure and the levels in blood or urine has always been found to be a reliable predictor of exposure [25, 41-43]. Manganese in blood or urine are useful in detecting groups with above-average current exposure, but measurements of manganese in these body fluids in individuals are sometimes be related to exposure dose after the exposure has ceased. In addition to individual variability, another factor that limits the usefulness of measuring manganese in blood, urine, or feces as a measure of excess manganese

exposure is the relatively rapid rate of manganese clearance from the body. Excess manganese in blood is rapidly removed by the liver and excreted into the bile, with very little excretion in urine [44, 45]. Thus, levels of manganese in blood or urine are not expected to be the most sensitive indicators of exposure [46]. Typical blood manganese concentrations in humans which have been reported in the literature are listed in Table 11 of Appendix B.

Selenium is an essential element that is required to maintain good health but both selenium deficiency and excessive levels of selenium are associated with several disorders[47, 48]. Selenium is a naturally occurring mineral element that is distributed widely in nature in most rocks and soils. Most processed selenium is used in the electronics industry, but it is also used: as a nutritional supplement; in the glass industry; as a component of pigments in plastics, paints, enamels, inks, and rubber; in the preparation of pharmaceuticals; as a nutritional feed additive for poultry and livestock; in pesticide formulations; in rubber production; as an ingredient in antidandruff shampoos; and as a constituent of fungicides. Radioactive selenium is used in diagnostic medicine. In the body, selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that increase risk of chronic diseases such as cancer and heart disease[48, 49]. Other selenoproteins help regulate thyroid function and play a role in the immune system [50-53]. Human selenium deficiency is rare in the U.S. but is seen in other countries where soil concentration of selenium is low[54]. There is evidence that selenium deficiency increases the risk of a form of heart disease, hypothyroidism, and a weakened immune system[55, 56]. There is also evidence that selenium deficiency does not usually cause illness by itself. Rather, it can make the body more susceptible to illnesses caused by other nutritional, biochemical or infectious stresses[57]. Symptoms of very high exposure to selenium, a condition called selenosis, include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage[47]. Selenium can be detected in the blood, feces, urine, hair, and nails of exposed individuals, however, field studies have used primarily blood or urine levels to indicate the degree of selenium exposure[47]. Typical blood selenium concentrations in humans which have been reported in the literature are listed in Table 11 of Appendix B.

The laboratory method presented here can be used to achieve rapid and accurate quantification of five elements of toxicological and nutritional interest including cadmium (Cd), lead (Pb), mercury (Hg), manganese (Mn) and selenium (Se) in whole human blood. Use this method to screen blood when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure.

b. Test principle:

This method directly measures the Cd, Mn, Hg, Pb, and Se content of whole blood specimens using mass spectrometry after a simple dilution sample preparation step.

During the sample dilution step, a small volume of whole blood is extracted from a larger whole blood patient specimen after the entire specimen is mixed (vortexed) to create a uniform distribution of cellular components. This mixing step is important because some metals (e.g. Pb) are known to be associated mostly with the red blood cells in the specimen and a uniform distribution of this cellular material must be produced before a small volume extracted from the larger specimen will accurately reflect the average metal concentration of all fractions of the larger specimen. Coagulation is the process in which blood forms solid clots from its cellular components. If steps are not taken to prevent this process from occurring, i.e. addition of anti-coagulant reagents such as EDTA in the blood collection tube prior to blood collection, blood will immediately begin to form clots once leaving the body and entering the tube. These clots prevent the uniform distribution of cellular material in the blood specimen even after rigorous mixing, making a representative sub-sample of the larger specimen unattainable. It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots). Clotted samples are not analyzed by this method due to the inhomogeneity concerns (i.e. all results for the sample are processed as “not reportable”).

Dilution of the blood in the sample preparation step prior to analysis is a simple dilution of 1 part sample + 1 part water + 48 parts diluent. The effects of the chemicals in the diluent are to release metals bound to red blood cells making them available for ionization, reduce ionization suppression by the biological matrix, prevent clogging of the sample introduction system pathways by undissolved biological solids, and allow introduction of internal standards to be utilized in the analysis step. Tetramethylammonium hydroxide (TMAH, 0.4% v/v) and Triton X-100® (0.05%) in the sample diluent solubilizes blood components. Triton X-100® also helps prevent biological deposits on internal surfaces of the instrument’s sample introduction system and reduce collection of air bubbles in sample transport tubing. Ammonium pyrrolidine dithiocarbamate (APDC) in the sample diluent (0.01%) aids in solubilizing metals released from the biological matrix. Ethyl alcohol in the sample diluent (1%) aids solubility of blood components and aids in aerosol generation by reduction of the surface tension of the solution. The internal standards, rhodium, iridium, and tellurium, are at a constant concentration in all blanks, calibrators, QC, and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift, and sample-to-sample matrix differences.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted blood sample is forced through a nebulizer which converts the bulk liquid into small droplets in an

argon aerosol. The smaller droplets from the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6000-8000 K. The small aerosol droplets pass through a region of the plasma and the thermal energy vaporizes the liquid droplets, atomizes the molecules of the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions first pass through a focusing region, then the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In 'vented' (or 'standard') mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In 'DRC' mode, the cell is pressurized with a gas for the purpose of causing collisions and/or reactions between the fill gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion, change the ion of interest to a new mass, which is free from interference, or collisions between ions in the beam and the DRC gas can focus the ion beam to the middle of the cell and increase the ion signal. In this method, the instrument is operated in DRC mode when analyzing for manganese, mercury, and selenium. For selenium, the DRC is pressurized with methane gas (CH_4, 99.999%) which reduces the signal from $^{40}Ar_2^+$ while allowing the $^{80}Se^+$ ions to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. Manganese and mercury are both measured when the DRC is pressurized with oxygen gas (O_2, 99.999%). They are analyzed at the same flow rate of oxygen to the DRC cell to avoid lengthening analysis time due to pause delays that would be necessary if different gas flows were used for the two analytes. The oxygen reduces the ion signal from several interfering ions ($^{37}Cl^{18}O^+$, $^{40}Ar^{15}N^+$, $^{38}Ar^{16}O^1H^+$, $^{54}Fe^1H^+$) while allowing the Mn^+ ion stream to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. In the case of mercury, collisional focusing of the mercury ions occurs, increasing the observed mercury signal at the detector by approximately a factor of two (2x).

Once ions pass through the DRC cell and electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the discrete dynode detector are processed into digital information that is used to indicate the intensity of the ions. The intensity of ions detected while aspirating an unknown sample is correlated to an elemental concentration through comparison of the analyte: internal standard signal ratio with that obtained when aspirating calibration standards. This method was originally

based on the method by Lutz et al [58]. The DRC portions of the method are based on work published by Tanner et al. [59, 60].

2) Limitations of Method; Interfering Substances and Conditions

a. Interferences addressed by this method

i. Reduction of argon dimer ($^{40}Ar^{2+}$) interference on selenium ($^{80}Se^{+}$) using ICP-DRC-MS: $^{40}Ar^{2+}$ is a polyatomic ion formed in the plasma as a result of a reaction between the plasma gas (Ar) and itself. The dynamic reaction cell of the ICP-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry [60, 61]. In the reaction cell, methane (CH_4) molecules react with $^{40}Ar^{2+}$ ions through a charge transfer reaction. The products of the reaction are $^{40}Ar^{+}$ (ion at a different mass) and ^{40}Ar (neutral). The background ion signal at m/z 80 is reduced by six orders of magnitude because of this reaction.

ii. Reduction of argon nitride ($^{40}Ar^{15}N^{+}$), argon hydroxide ($^{38}Ar^{16}O^{1}H^{+}$) interference on manganese (^{55}Mn) using ICP-DRC-MS: $^{40}Ar^{15}N^{+}$ and $^{38}Ar^{16}O^{1}H^{+}$ are polyatomic ions formed in the plasma as a result of reactions between the plasma gas (Ar) and atmospheric gases (N_2, O_2) or the solvent (H_2O). The dynamic reaction cell of the ICP-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry [60, 61]. In the reaction cell, oxygen molecules react with $^{40}Ar^{15}N^{+}$ and $^{38}Ar^{16}O^{1}H^{+}$ ions through either charge transfer reactions or oxygen transfer reactions. The products of the reactions are either neutral molecules and are not detected (charge transfer), or a new ion with higher mass (oxygen transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

iii. Reduction of $^{37}Cl^{18}O^{+}$, $^{39}K^{16}O^{+}$, $^{54}Fe^{1}H^{+}$ interferences on manganese (^{55}Mn) using ICP-DRC-MS: $^{37}Cl^{18}O^{+}$, $^{39}K^{16}O^{+}$, $^{54}Fe^{1}H^{+}$ are polyatomic ions created in the plasma as a result of reactions between elements present in the blood matrix (Cl, K, and Fe) and the solvent (H_2O). Due to the high concentrations of Cl, K, and Fe in the blood matrix the resulting ion signals of $^{37}Cl^{18}O^{+}$, $^{39}K^{16}O^{+}$, and $^{54}Fe^{1}H^{+}$ interfere with the measurement of $^{55}Mn^{+}$ at m/z 55. The dynamic reaction cell of the ICP-MS is used to reduce ion signals from polyatomic ions via ion-molecule reaction chemistry [60, 61]. In the reaction cell, oxygen molecules react with $^{37}Cl^{18}O^{+}$, $^{39}K^{16}O^{+}$, $^{54}Fe^{1}H^{+}$ ions through either charge transfer reactions or oxygen transfer reactions. The products of the reactions are either neutral molecules and are not detected (charge transfer), or a new ions with higher mass (oxygen transfer). In either case, attenuation of the background ion signal at m/z 55 occurs.

b. Limitations of method (interferences remaining in method)

i. MoO_2 interference on ^{130}Te: Molybdenum will combine with oxygen in the DRC conditions used in this method for Hg analysis to form a polyatomic ion, $^{98}Mo^{16}O_2^+$, which interferes with the measurement of the internal standard $^{130}Te^+$. Increased signal at m/z 130 (due to measuring both $^{130}Te^+$ and $^{98}Mo^{16}O_2^+$) results in an erroneously low net intensity for Hg (net intensity = measured intensity for analyte isotope / measured intensity for internal standard isotope). If this interference occurs during the measurement of the calibration standards (i.e. a multi-element calibration stock standard includes high levels of Mo) it can result in a positive bias for observed mercury concentrations as a consequence of a nonlinear calibration curve having an artificially low slope. If this interference occurs during the measurement of an unknown sample, the reduced net intensity observed can result in reporting an erroneously low Hg result. This interference has been verified to be of concern (>5% effect negative bias) at blood molybdenum concentrations greater than 15 ug/L. However, typical levels of molybdenum in whole blood (0.2 – 4.6 ug/L [62, 63]) are below this. Also, levels of molybdenum in whole blood after acute exposures have been observed to be ≤15 μg/L [62]. Molybdenum concentrations below 5 μg/mL in stock calibration standard solutions do not produce an observable interference.

3) Procedures for collecting, storing, and handling specimens; criteria for specimen rejection; specimen accountability and tracking

a. Procedures for collecting, storing, and handling specimens: Specimen handling conditions, special requirements, and procedures for collection and transport are discussed in the Division of Laboratory Science's (DLS) Policies and Procedures Manual [64]. In general,

i. No fasting or special diets are required before collection of blood

ii. Specimen type – whole blood

iii. Optimal amount of specimen is 1^+ mL. Request a minimum volume of 0.25 mL. Volume for one analytical measurement is 0.05 mL.

iv. Verify sample collection devices and containers are free of significant contamination ("pre-screened") before use.

v. Draw the blood through a stainless steel needle into a pre-screened vacutainer.

vi. Do not freeze blood in blood collection tubes due to risk the tubes cracking. Transfer to plastic, pre-screened cryovials before freezing.

vii. Once received, store blood collection tubes at refrigerated temperatures (2–8 ^{o}C). Transfer to plastic, pre-screened cryovials before freezing. Specimen stability has been demonstrated for over 1 year at ≤ -20 °C.

b. Criteria for specimen rejection: The criteria for an unacceptable specimen include:

i. Contamination: Improper collection procedures, collection devices, or sample handling can contaminate the blood through contact with dust, dirt, etc. Manganese is present in the general environment, found often in combination with iron, and is present in many alloys (especially stainless steel).

ii. Low Volume: Request a minimum volume of 0.25 mL. Volume for one analytical measurement is 0.05 mL.

In all cases, request a second blood specimen.

c. Transfer or referral of specimens; procedures for specimen accountability and tracking: Location, status, and final disposition of the specimens will be tracked at least by paper document in the "Study Folder" (created before analysts receive the samples). Apart from this specimen tracking form, this folder will also contain the paper print outs of results from analysis of the specimens. Maintain records for a minimum of 3 years. Use only numerical identifiers for samples within the laboratory (e.g., case ID numbers) in order to safeguard confidentiality. Access to personal identifiers for samples will be limited to the medical supervisor or project coordinator (e.g. non-CDC personnel).

4) Safety precautions

a. General safety

i. Observe all safety regulations as detailed in the Laboratory Safety Manual and the Chemical Hygiene Plan. Participate in training regarding blood-borne pathogens prior to performing this method.

ii. Observe Universal Precautions when working with blood.

iii. Wear appropriate gloves, lab coat, and safety glasses while handling all solutions.

iv. Take special care when handling and dispensing bases and concentrated acids. Use additional personal protective equipment which protects face, neck, and front of body. **If TMAH or concentrated hydrochloric acid comes in contact with any part of the body, quickly wash with copious quantities of water for at least 15 minutes.**

v. Use secondary containment for containers holding biological or corrosive liquids.

vi. The use of the foot pedal on the benchtop automatic pipette is recommended because it reduces analyst contact with work surfaces that have been in contact with blood and also keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the tip of benchtop automatic pipette.

vii. There are many potential hazards on an operating ICP-MS instrument including ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures. This information is detailed in the ICP-MS System Safety Manual.

viii. Transport and store compressed gas cylinders with proper securing harnesses. For compressed oxygen gas, use regulators which are oil-free and are equipped with a flash arrestor.

ix. Wipe down all work surfaces at the end of the day with disinfectant. Disinfectant may be either daily remake of diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water) or an equivalent disinfectant

b. Waste disposal:

i. Autoclaving: All diluted biological specimens, original biological specimens being disposed, or consumables which come into contact with biological specimens (even diluted or aerosolized). Use sharps containers or special autoclave pans for broken glass / quartz or items which puncture autoclave bags (e.g. pipette tips).

ii. Other liquid waste

1. Waste discarded down sink: Only non-corrosive liquid waste (EPA defines as pH >2 and pH<12.5, 40CFR §261.22) from the ICP-MS instrument can be discarded at the sink. Flush the sink with copious amounts of water.

2. Waste to be picked up by CDC hazardous waste program: Submit request for hazardous waste removal of all other liquid waste generated in the CDC laboratory for this method.

5) Instrument & material sources

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively Coupled Plasma Mass Spectrometer with Dynamic Reaction Cell Technology (ELAN® DRC II) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

ii. Recirculating chiller / heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE) or heat exchanger (PolyScience 3370) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

iii. Autosampler: ESI SC4-DX autosampler (Elemental Scientific Inc., Omaha, NE) or equivalent.

iv. Computer: Computer controller provided or recommended by ICP-MS manufacturer is recommended to ensure proper communication between computer and ICP-MS. Recommend 1-2 Gb RAM and secondary internal hard disk for nightly backups (if network backups are not possible).

v. FAST sample introduction system (optional): Standard peristaltic pump on ICP-MS replaced by DXi-FAST micro-peristaltic pump / FAST actuator and valve combination unit. Like part # DXI-54-P4-F6. If DXi-FAST upgrade on ICP-MS is not used, a separate FAST actuator (built-in option on ESI SC4-DX autosampler or stand-alone FAST actuator) will be necessary to complete the FAST sample introduction system.

b. Sources for ICP-MS parts & consumables

NOTE: The minimum number of spares recommended before reordering (if owning one instrument) are listed as "# *Spares* = X amount" in the descriptions below.

i. Adapter, PEEK: Securely connects 1.6mm O.D. PFA tubing to 0.03" I.D. peristaltic tubing. Composed of three PEEK parts.

1. Female nut for 1.6mm O.D. (1/16") tubing. Like part P-420 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).
2. PEEK ferrule. Like part P-260x (10pk SuperFlangeless ferrule, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).
3. Conical Adapter Body. Like part P-692 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).

ii. Bottles (for rinse solution): Four liter screw-cap polypropylene container with built-in luer connections (2) designed for use with FAST sample introduction

system (like catalog# SC-0305-1, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

iii. Carboy and cap assembly for waste collection: 10-15 L, polypropylene wide-mouth carboy (100 mm neck size) with handles and no spigot (Like part #7BE-25126, Lab Safety Supply, Janesville, WI, www.lss.com) with cap assembly like part # N0690271 (PerkinElmer, Norwalk, CT, www.perkinelmer.com) with tubing connections built into the cap for addition of liquid waste.

iv. Coolant, for polyscience chiller or heat exchanger: Only PerkinElmer part # WE01-6558 (PerkinElmer Norwalk, CT, www.perkinelmer.com) is approved for use by PerkinElmer. *# Spares = 6*.

v. Cones: Platinum or Nickel cones have been used and tested to be comparable in performance from either PerkinElmer or Spectron. Platinum cones are more expensive, but will last longer, can be refurbished (often for free by the manufacturer), and will frequently yield higher sensitivity.

1. Sampler (nickel/platinum): PerkinElmer part # WE021140 / WE027802 (PerkinElmer Norwalk, CT, www.perkinelmer.com). *# Spares = 4*.
2. Skimmer (nickel / platinum): PerkinElmer part # WE021137 / WE027803 (PerkinElmer Norwalk, CT, www.perkinelmer.com). *# Spares = 4*.

vi. Connector (for tubing): Use to connect 1/8" I.D. PVC tubing to 0.125" I.D peristaltic pump tubing. Use part # 3140715 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. *# Spares = 4*.

vii. Detector, electron multiplier: Like part # N8125001 (PerkinElmer Norwalk, CT, www.perkinelmer.com). *# Spares = 1*.

viii. FAST accessories

1. Valve: CTFE High-flow valve head for SC-FAST (uses ¼-28 fittings). Like part # SC-0599-1010 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).
2. Stator: CTFE Stator for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-01 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).
3. Rotor: Composite rotor for 6 port SC-FAST high flow valve (¼-28 fittings). Like part # SC-0599-1010-05 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).
4. Sample Loop: 1 mL Teflon, white connector-nuts for high flow valve head(¼-28 fittings). Like part # SC-0315-10 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).
5. Probe, Autosampler: Teflon, carbon fiber support, 0.8mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 2.*

6. Probe, Carrier Solution: Teflon, carbon fiber support, 0.5mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 2.*
7. Tubing, FAST vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).
8. Tubing, connects nebulizer to valve: See "Nebulizer, PolyPro-ST micro flow"

ix. Hose, for connection to chiller: Push on hose. I.D. = ½", O.D. = ¾". Use part # PB-8 (per inch, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. Do not normally need spare hose (unless moving instrument into a new location).

x. Hose, for exhaust of ICP-MS: Available as part of ICP-MS installation kit from Perkin Elmer (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net), or equivalent. ***# Spares = 10 feet of 4" diameter and 10 feet of 6" diameter hose.***

xi. Injector, quartz with ball joint: I.D. = 2.0 mm. PerkinElmer part # WE023948 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-30 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. *# Spares = 2.*

xii. Ion lens: PerkinElmer part # WE018034 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # Spares = 3.

xiii. Nebulizer: PolyPro-ST micro flow polypropylene nebulizer with external 1/4-28 threaded connector for liquid delivery, low pressure version or equivalent. Like part # ES-4040-7010 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 1*. Different nebulizers are acceptable, however, the nebulizer gas flow rate, sample flush time, read delay time, loop fill time, loop size, blood sample dilution preparation volume, and sample-to-sample carry-over must be evaluated and optimized.

1. Gas connection:
    a. Teflon tubing: 4mm o.d., 2.4mm i.d. Teflon tubing (like part # ES-2502, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 1*.
    b. Adapter kit: Plastic adapters to connect Teflon tubing (2.4 mm i.d) to ¼" male Swagelok (compression) port on ICP-DRC-MS. Parts can be obtained as components in a "gas fittings kit for microflow nebulizer", kit like part # ES-2501-1000 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). *# Spares = 1*.

2. Liquid connection: Connects nebulizer to port #3 of high flow FAST valve head with green, 1/4- 28 fitting. Like part # SC-0317-0250 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # *Spares = 2*.

xiv. Nut: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Flanged, for 1/16" o.d. tubing, 1/4-28 threads. Use part # P-406x (pkg. of 10, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. Use a Teflon-coated Viton o-ring with this nut instead of the stainless steel washer that comes with part # P-406x*)*. # *Spares = 10.*

xv. Nut and ferrule set, 1/8" Swagelok: Such as part # SS-200-NFSET (stainless steel) or part # B-200-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xvi. Nut and ferrule set, 1/4" Swagelok: Such as part # SS-400-NFSET (stainless steel) or part # B-400-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xvii. Oil for roughing pumps:

1. Welch Directorr Gold: For roughing pumps. Available direct from manufacturer as part # 8995G-15 (1 gallon, Welch Rietschle Thomas, Skokie, IL, www.welchvacuum.com), or equivalent. # *Spares = 4.*
2. Fomblin Y14/5 fluid: PerkinElmer part # N8122265 (1 kg bottle, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares =1 per instrument.*

xviii. O-ring: (for sampler cone) PerkinElmer part # N8120511 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

xix. O-ring: (for skimmer cone) PerkinElmer part # N8120512 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

xx. O-ring: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Teflon-coated Viton o-ring, i.d. = 1/16", thickness = 1/16", o.d. = 3/16". Such as part # V75-003 (O-rings West, Seattle, WA, www.oringswest.com) or equivalent. # *Spares = 20.*

xxi. O-ring: (for injector support).

1. Internal o-rings: ID = ¼", OD = 3/8", thickness = 1/16". Need 2 o-rings per injector support setup. PerkinElmer part # N8122008 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-010, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*
2. External o-rings: ID = 3/8", OD = 1/2", thickness = 1/16". Need 2 o-rings for each injector support setup. PerkinElmer part # N8122009 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as

part # V75-012, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*

xxii. O-ring (for inside nebulizer port on standard PerkinElmer cyclonic quartz spray chamber for the ELAN): Such as part # 120-56 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). Additional o-rings can sometimes be obtained free of charge or at reduced price when acquired while purchasing spray chambers. # *Spares = 20.*

xxiii. O-ring: (for inside of bayonet torch mount): Part # WE017284 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Do not substitute. The PerkinElmer o-ring is specially metal impregnated to minimize RF leakage though the torch mount. # *Spares = 2.*

xxiv. Photon stop: PerkinElmer part # WE018278 (PerkinElmer, Shelton, CT, www.perkinelmer.com). # *Spares = 1.*

xxv. Plugs, quick change for roughing pump oil: These plugs will only work on the Varian roughing pumps which come standard on ELAN DRC II ICPMS instruments. These plugs will not fit the Leybold pumps which come standard on the ELAN DRC Plus instruments. Part # W1011013 (PerkinElmer, Shelton, CT, www.perkinelmer.com). No spares typically needed.

xxvi. Probes

1. for ESI autosampler: Teflon, carbon fiber support, 0.8 mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # *Spares = 2.*
2. for carrier solution of FAST sample introduction system: Teflon, carbon fiber support, 0.5mm i.d., orange marker, 1/4-28 fittings. Like part number SC-5037-3501 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # *Spares = 2.*

xxvii. RF coil: PerkinElmer part # WE02-1816 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xxviii. Spray chamber, quartz concentric: PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-20 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

xxix. Torch, quartz: PerkinElmer part # N812-2006 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *New Spares = 2.*

xxx. Tubing and adapter, for SC autosampler rinse station drain: Tygon tubing and adapter to attach to back of SC autosampler for draining rinse station waste (like part # SC-0303-002, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxi. Tubing and adapters, for SC autosampler rinse station filling: Teflon tubing and adapters (to attach to back of SC autosampler for filling rinse stations and

to attach to rinse containers). Like part # SC-0302-0500, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxii. Tubing and nut, for FAST carrier solution: 0.5 mm i.d. Teflon tubing (orange marker) with red ¼-28 male nut. Connects to high flow FAST valve head, port #2. Like part # SC-0316-0500 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxiii. Tubing, FAST vacuum: Vacuum line for SC-FAST high flow valve, connects to port #6, black nut for connection to valve head, natural brown color nut on other end for connection to SC autosampler vacuum port. Like part # SC-0321 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxiv. Tubing, main argon delivery to instrument: I.D. = 1/8", O.D. = ¼". Like part # C-06500-02 (pkg. of 100ft, polypropylene, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent. *# Spares = 50 ft*.

xxxv. Tubing, PFA: I.D. = 0.5 mm, O.D. = 1.59 mm (1/16"). Used to transfer liquidbetween rinse solution jug and peristaltic pump tubing

The Perfluoroalkoxy (PFA) copolymer is a form of Teflon®. Like part # 1548 (20ft length, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent.*# Spares = 20ft.*

xxxvi. Tubing, peristaltic, 0.03" i.d. (carrier solution for ESI autosampler): *use either*

1. Standard PVC, 2-stop (black / black) peristaltic pump tubing, i.d. = 0.03". PerkinElmer part # 09908587 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 6 packs of 12 tubes.*
2. Standard PVC, 3-stop (black/ black/black) peristaltic pump tubing, i.d. 0.76 mm. Spectron part # SC0056 (Spectron, Ventura, CA, www.spectronus.com) or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xxxvii. Tubing, peristaltic, 0.125" i.d. (spray chamber drain): *use either*

1. Standard PVC, 2-stop (black / white) peristaltic pump tubing, i.d. = 0.125" or equivalent. PerkinElmer part # N812-2012 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. *# Spares = 6 packs of 12 tubes.*
2. Standard Santoprene, 3-stop (grey/ grey/ grey) peristaltic pump tubing, i.d. 1.30 mm. Spectron part # SC0311 (Spectron, Ventura, CA, www.spectronus.com) or equivalent. #Spares = 6 packs of 12 tubes. *Use this type of tubing with ESI DXi micro-peristaltic pump.*

xxxviii. Tubing, PVC, i.d. = 1/8", o.d. = 3/16". Used to transfer liquid

1. between spray chamber waste port and peristaltic pump
2. between peristaltic pump and liquid waste jug

Like part # 14-169-7A (pkg. of 50 ft, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent. # *Spares = 20ft*.

xxxix. Tubing, Stainless Steel, o.d. = 1/8", wall thickness = 0.028": Used to connect gas cylinders to NexIONUCT gas ports. Like part # SS-T2-S-028-20 (20ft, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 20 ft.*

xl. Tubing, Teflon, corrugated, ¼" o.d.: Connects to the auxiliary and plasma gas side-arms of the torch. Part # WE015903 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xli. Tubing, vinyl (argon delivery to nebulizer): Vinyl Tubing, 1/8" ID x 1/4" OD. Like part # EW-06405-02 (Cole Parmer, Vernon Hills, Illinois, www.coleparmer.com) or equivalent. # *Spares = 10 ft*.

xlii. Union elbow, PTFE ¼" Swagelok (ELAN bayonet mount): Connects argon tubing to torch auxiliary gas sidearm on bayonet mount NEXION ICP-MS instruments. Like part # T-400-9 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

xliii. Union tee, PTFE, ¼" Swagelok (ELAN bayonet mount): Connects argon tubing to torch plasma gas sidearm and holds igniter inside torch sidearm on bayonet mount NEXION ICP-MS instruments. Like part # T-400-3 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

c. \Sources for ICP-MS maintenance equipment & supplies

i. Anemometer: Like digital wind-vane anemome*ter (Model* 840032, SPER Scientific LTD., Scottsdale, AZ, www.sperscientific.com) or equivalent. Use to verify adequate exhaust ventilation for ICP-MS (check with hoses fully disconnected).

ii. Pan, for changing roughing pump oil: Like part # 53216 (United States Plastics Corporation, Lima, OH, www.usplastic.com) or equivalent.

iii. Container, to hold acid baths for glassware: Polypropylene or polyethylene containers with lids (must be large enough for torch, injector, or spray chamber submersion). Available from laboratory or home kitchen supply companies.

iv. Cotton swabs: Any vendor. For cleaning of cones and glassware.

v. Cutter (for 1/8" o.d. metal tubing): Terry tool with 3 replacement wheels. Like part # TT-1008 (Chrom Tech, Inc., Saint Paul, MN, www.chromtech.com) or equivalent.

vi. Getter regeneration Kit: Part # WE023257 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Use this as needed (at least annually) to clean the getter in the pathway of channel A DRC gas.

vii. Magnifying glass: Any 10x + pocket loupe for inspection of cones and other ICP-MS parts. Plastic body is preferred for non-corrosion characteristics. Like part # 5BC-42813 (Lab Safety Supply, Janesville, WI, www.labsafety.com).

viii. Ultrasonic bath: Like ULTRAsonik™ Benchtop Cleaners (NEYTECH, Bloomfield, CT, www.neytech.com) or equivalent.

d. Sources for general laboratory equipment and consumables

i. Bar code scanner: Like Code Reader 2.0 (Code Corporation, Draper, UT, www.codecorp.com) or equivalent. For scanning sample IDs during analysis setup. Any bar code scanner capable of reading Code 128 encoding at a 3 mil label density can be substituted.

ii. Carboy (for preparation of blood quality control pool and waste jug for ICPMS sample introduction system): Polypropylene 10-L carboy (like catalog # 02-960-20C, Fisher Scientific, Pittsburgh, PA, www.fischersci.com) or equivalent. Carboys with spouts are not advised due to potential for leaking.

iii. Containers for diluent and rinse solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., www.fishersci.com, or equivalent) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com, or equivalent) have both been used. Acid rinse before use.

iv. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor).

v. Paper towels: For general lab use, any low-lint paper wipes such as KIMWIPES®EX-L Delicate Task Wipers or KAYDRY®EX-L Delicate Task Wipers (Kimberly-Clark Professional, Atlanta, GA, www.kcprofessional.com). For sensitive applications in cleanrooms, use a wipe designed for cleanrooms such as the Econowipe or Wetwipe (Liberty, East Berlin, CT, www.liberty-ind.com).

vi. Pipette, benchtop automatic (for preparation of blood dilutions to be analyzed): Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 5.0 mL left syringe, a 250 µL right syringe, a 12 gauge Concorde CT probe dispense tip, the Microlab cable management system and a foot pedal. Alternatives are acceptable, including the Micromedic Digiflex™ (Titertek, Huntsville, AL, http://www.titertek.com/) equipped with 10.0-mL dispensing syringe, 200 µL sampling syringe, 0.75-mm tip, and foot pedal.

vii. Pipettes (for preparation of intermediate stock working standards & other reagents): Like Brinkmann Research Pro Electronic pipettes (Brinkmann Instruments, Inc., Westbury, NY, http://www.brinkmann.com/home/). 5-100 µL (catalog #4860 000.070), 20-300 µL (catalog #4860 000.089), 50-1000 µL (catalog #4860 000.097), 100-5000 µL (catalog #4860 000.100). Note: pipette catalog numbers are without individual chargers. Can purchase individual chargers (pipette catalog numbers will differ) or a charging stand that will hold four pipettes (catalog #4860 000.860). When purchasing pipette tips (epTips), purchase one or more boxes, then “reloads” for those boxes after that: 5-100

μL (box catalog # 22 49 133-4, reload catalog # 22 49 153-9), 20-300 μL (box catalog # 22 49 134-2, reload catalog # 22 49 154-7), 50-1000 μL (box catalog # 22 49 135-1, reload catalog # 22 49 155-5), 100-5000 μL (box catalog # 22 49 138-5, reload catalog # 22 49 198-9, bulk bag catalog # 22 49 208-0). Equivalent pipettes and tips can be substituted.

viii. Tubes for sample analysis (for autosampler): Like polypropylene 15-mL conical tubes, BD Falcon model #352097 (Becton Dickinson Labware, FranklinLakes, NJ, www.bd.com) or equivalent. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

ix. Tubes for storage of intermediate working stock standards: Like polypropylene 50-mL conical tubes, BD Falcon model #352098 (Becton Dickinson Labware, FranklinLakes, NJ, www.bd.com) or equivalent. For use in storage of intermediate working stock standards. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

x. Vortexer: Like MV-1 Mini Vortexer (VWR, West Chester, PA, www.vwr.com). Used for vortexing blood specimens before removing an aliquot for analysis. Equivalent item can be substituted.

e. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Veritas™ double-distilled grade, 30–35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com) or equivalent. This is referred to as “concentrated” hydrochloric acid in this method write-up. For use in preparation of intermediate working stock standards.

ii. Acid, nitric acid: Veritas™ double-distilled grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in cleaning any bottles, vials, tubes, and flasks. This is referred to as “concentrated” nitric acid in this method write-up.

iii. Blood, whole (human or bovine): Bags of human blood can be purchased from various sources such as American Red Cross (http://www.redcross.org) or Tennessee Blood services (Memphis, TN, http://tennesseebloodservices.com/). Request that human blood be screened for infectious diseases such as Hepatitis B and HIV. Source for bovine blood includes the Wisconsin State Laboratory of Hygiene (WSLH, Madison, WI, http://www.slh.wisc.edu).

iv. Ethanol (EtOH): USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

v. Ammonium pyrrolidine dithiocarbamate, laboratory grade (Fisher Scientific, Fairlawn, NJ) or equivalent.

vi. Argon gas (for plasma & nebulizer) and regulator: High purity argon (>99.999% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsgas.com) for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250 L). Bulk tank (1500^{+}L is preferred).

1. Regulator for argon (at dewar): Stainless steel, single stage, specially cleaned regulator with 3000 psig max inlet, 0–200 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Part number "KPRCGRF415A2/AG10-AR1" (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. # *Spares = 1*.
2. Regulator for argon (between bulk tank and PerkinElmer filter regulator): Single Stage 316SS Regulator, with 0-300 psi Inlet Gauge, 0-200 psi Outlet Gauge, Outlet Spring Range, 0-250 psi, ¼" Swagelok Inlet Connection, ¼ turn Shut off Valve on Outlet with ¼" Swagelok Connection and Teflon Seals. Part number KPR1GRF412A20000-AR1 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. # *Spares = 1*.
3. Regulator for argon (filter regulator on back of ICP-MS): Argon regulator filter kit. Catalog number N812-0508 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

vii. Disinfectant, for work surfaces: Daily remake of diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water), or an equivalent disinfectant.

viii. Methane: Methane (Research Grade 5.0, 99.99% purity), for DRC channel A. Typically purchased in cylinder size 200 (part # ME R200, Airgas South, Atlanta, GA, www.airgas.com).

1. Regulator for methane: Stainless steel, two stage, specially cleaned regulator with 3000 psig max inlet, 0-25 outlet pressure range, CGA 350 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Like part number KCYADPF412A2AD10 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. # *Spares = 1*.
2. Flash Arrestor: Like part # 6104a (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com) or equivalent.

ix. Oxygen: Oxygen ("Research Grade Research Grade 5.0", 99.9999% purity) for DRC channel B. Like part # OX R33A (Airgas South, Atlanta, GA, www.airgas.com).

1. Regulator for oxygen: Stainless steel, two stage regulator for use with high purity oxygen (cleaned to be free of all oils). Maximum inlet pressure 3600-5000 psi. Inlet gauge pressure 0-5000 psi (no oil in gauge). Maximum delivery pressure 50–100 psi with a 0-30 psi outlet gauge (no oil in gauge). CGA 540 cylinder connector on inlet side and an angle pattern (90 degree) stainless steel needle valve on the delivery side terminating in

a 1/8" stainless steel Swagelok connector. Like part # GEORG/KCYCFR/ORS2/540 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent.

2. Flash arrestor: Like part # 6104A (Matheson Tri Gas, Montgomeryville, PA, www.mathesontrigas.com), or equivalent. # *Spares = 1.*

x. Standard, iridium: Like 1,000 µg/mL, item #CGIR1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com). Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

xi. Standard, multi-element stock calibration standard: Item number SM-2107-042 (High Purity Standards, Charleston, SC, http://www.hps.net/). Standard must be traceable to the National Institute for Standards and Technology.

xii. Standard, rhodium: Like 1,000 mg/L, item # PLRH3-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com). Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

xiii. Standard, single element stock standards for preparation of calibrators and blood quality control pools: National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs): 3108 (Cd), 3132 (Mn), 3128 (Pb), 3133 (Hg), 3149 (Se). (Gaithersburg, MD, www.nist.gov). Standard must be traceable to the National Institute for Standards and Technology.

xiv. Standard, tellurium: Like 1,000 mg/L, item #CGTE1-1 (Inorganic Ventures, Christiansburg, VA http://www.inorganicventures.com).Used as an internal standard in diluent. Standard must be traceable to the National Institute for Standards and Technology.

xv. Tetramethylammonium hydroxide, 25% w/w, or equivalent (AlfaAesar, 30 Bond St., Ward Hill, MA 01835).

xvi. Triton X-100™ surfactant: Like "Baker Analyzed" TritonX-100™ (J.T. Baker Chemical Co., www.jtbaker.com).

6) Preparation of reagents and materials

a. Internal standard intermediate mixture:

i. Purpose: Preparation of single intermediate solution containing all internal standards simplifies the addition of the internal standard(s) into the final diluent solution. This solution can be purchased rather than prepared.

ii. Preparation: To prepare 50 mL of 20 mg/L Rh, Ir, Te in 1% v/v HNO_3:

1. If not previously dedicated to this purpose, acid wash a 50 mL volumetric flask (PP, PMP, or Teflon™). For example, with 1% (v/v) HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.

2. Partially fill the 50 mL volumetric flask with 1% v/v HNO_3 (approximately 25-40 mL).
3. Add 1 mL of 1,000 μg/mL Rh standard, 1 mL of 1,000 μg/mL Ir standard, and 1 mL of 1,000 μg/mL Te standard. If initial Rh, Ir, or Te standard concentration is different, adjust volume proportionally.
4. Fill to mark (50 mL) with 1% v/v HNO_3 and mix thoroughly.
5. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

b. Intermediate Triton X-100® solution:

i. Purpose: To ease daily preparation of the diluent and rinse solutions by first preparing an intermediate Triton X-100® solution.

ii. Preparation: To prepare 1 L of 20% Triton x-100®

1. If not previously dedicated to this purpose, acid wash a 200 mL volumetric flask (PP, PMP, or Teflon™). For example, with 1% (v/v) HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
2. Add 200 mL of Triton X-100® to the 1L container that is partially filled with ≥18 Mohm·cm water.
3. Fill to 1 L with ≥18 Mohm·cm water and mix until the Triton X-100® has completely dissolved into solution (overnight). A magnetic stirring plate can be used to assist mixing by adding an acid-washed Teflon®coated stirring bar to the bottle.
4. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

c. Sample diluent and carrier

i. Purpose: This solution will be used in the preparation of all samples and calibrators during the dilution process prior to analysis. Make all samples, standards, blanks, QC, etc. . . in a run from the same diluent solution so that the concentration of the internal standards will be the same among all calibrators and samples in the run. When using a flow-injection component in the sample introduction system (i.e. the Elemental Scientific SC4-FAST autosampler), use the same solution for the the 'carrier' and sample diluent. The diluent is an aqueous solution of 5 μg/L internal standard mixture (Rh, Ir, Te), in 0.4% v/v tetramethyl ammonia hydroxide (TMAH), 1% ethyl alcohol, 0.01% APDC, and 0.05% v/v Triton X-100®. Larger volumes of these solutions can be prepared by adjusting component volumes proportionally.

ii. Preparation: To prepare 2L of 5 μg/L Rh, Ir and Te, 0.01% APDC in 0.4% v/v TMAH, 1% ethanol, and 0.05% v/v Triton X-100:

1. If not previously dedicated to this purpose, acid wash a 2L container (PP, PMP, or Teflon™). For example, with 1% (v/v) HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
2. Partially fill the 2L container with ≥18 Mohm·cm water.
3. Add 0.2 g of APDC , 8 mL of 25% v/v TMAH, 20 mL of ethanol, and 5 mL of 20% Triton X-100®.
4. Dilute to volume (2L) with ≥18 Mohm·cm water.
5. Spike 500 µL of 20 mg/L Rh, Ir, Te to the final diluent.
6. Invert bottle a few times to insure thorough mixing. Allow to sit for several hours or overnight before using.
7. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

d. ICP-MS rinse solution

i. Purpose: The rinse solution used in this method is an aqueous solution of 0.01% APDC in 0.4% v/v TMAH, 1% ethanol, and 0.05% v/v Triton X-100. This solution will be pumped through the autosampler rinse station, probe, and sample loop between sample analyses to prevent carry-over of analytes from one sample measurement to the next.

ii. Preparation: To Prepare 4 L of 0.01% APDC in 0.4% v/v TMAH, 1% ethanol, and 0.05% v/v Triton X-100:

1. If not previously dedicated to this purpose, acid wash a 4L container (PP, PMP, or Teflon™). For example, with 1% v/v HNO_3 and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
2. Partially fill the 4 L bottle with ≥18 Mohm·cm water (approximately 2-3 L). Use of volumetric flask is not required.
3. Add 0.4 g of APDC
4. Add 16 mL of TMAH
5. Add 40 mL of ethyl alcohol,
6. Add 10mL of 20% Triton X-100®, (See Section 6.b for details on preparation)
7. Fill to 4 L using ≥18 Mohm·cm water.
8. Store at room temperature and prepare as needed. To prepare volumes other than specified here, add proportionally larger or smaller volumes of the solution constituents.

9. Invert bottle a few times to ensure thorough mixing. Allow to sit for several hours or overnight before using.
10. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

e. Standards, calibrators, base blood and QC

i. Multi-element stock calibration standards

1. Purpose: This multi-element stock standard will be used to prepare the intermediate working calibration standards.
2. Purchase & Storage:
   a. Purchasing from vendors: Whether purchased or prepared in-house, the starting materials must be NIST-traceable. Matrix and concentrations of Pb, Cd, Hg, Mn and Se are listed in Table 3 of Appendix B.
   b. Storage: Store at room temperature and label appropriately. Expiration is as defined by the manufacturer or 1 year from date of opening, whichever comes first.

ii. Diluent for intermediate calibration standard preparations:

1. Purpose: This diluent is used to dilute stock and intermediate stock calibration standards, not to prepare working calibrators or blood samples for analysis.
2. Preparation: To prepare 2L of 3% v/v HCl:
   a. If not previously dedicated to this purpose, acid wash a 2L container (PP, PMP, or Teflon™). For example, with 3% HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate.
   b. In the 2 L flask, add 1-1.5L ≥18 Mohm·cm water.
   c. Add 60 mL high purity concentrated HCl.
   d. Fill to the mark and mix thoroughly.
   e. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

iii. Multi-element intermediate stock calibration standard

1. Purpose: This multi-element intermediate stock standard will be used to prepare the intermediate working calibration standards.
2. Preparation: To prepare 3% v/v HCl solutions containing Cd, Pb, Hg, Se, and Mn with concentrations listed in Table 4 of Appendix B:
   a. Acid-rinse one 100 mL, PP (or PMP) volumetric flask. For example, with 3% HCl and ≥18 Mohm·cm water (at least 3 times each) and verify

cleanliness through analysis of rinsate. Mark flask according to intended use. Dedicate to purpose.

b. Partially fill (50-75% full) the 100 mL flask with the 3% (v/v) HCl diluent prepared in Section 6.e.ii.

c. Using the volume listed in Table 4 of Appendix B, pipette the appropriate volume of the multi-element stock calibration standard solution into the volumetric flask. Dilute to the volumetric mark with the 3% HCl (v/v) diluent using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 4 of Appendix B.

d. Once mixed, transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage.

e. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

iv. Intermediate working calibration standards

1. Purpose: Used each day of analysis to prepare the final working calibrators that will be placed on the autosampler.

2. Preparation: To prepare 3% v/v HCl solutions containing Cd, Pb, Hg, Se, and Mn with concentrations listed in Table 3 of Appendix B:

a. Acid-rinse eight 100 mL, PP (or PMP) volumetric flasks and one 2 L PP (or PMP) volumetric flasks. For example, with 3% HCl and ≥18 Mohm·cm water (at least 3 times each) and verify cleanliness through analysis of rinsate. Mark each flask according to intended use. Dedicate to purpose.

b. Fill each 100 mL flask 50-75% with the 3% (v/v) HCl diluent prepared in Section 6.e.ii.

c. Using the volumes listed in Table 5 of Appendix B, pipette the appropriate volume of the multi-element intermediate stock calibration standard solutions into each of the volumetric flasks. Dilute each to the volumetric mark with the 3% HCl diluent using a pipette for the final drops. Mix each solution thoroughly. Final concentrations are listed in Table 5 of Appendix B.

d. Once mixed, transfer to acid-cleaned, labeled, 50 mL containers (PP, PMP, or Teflon™) for storage.

e. Store at room temperature and label appropriately. Expiration is 1 year from date of preparation.

f. Pour aliquots of each standard into clean 15mL polypropylene tubes and label for daily use.

v. Working calibrators

1. Purpose: The working calibrators will be analyzed in each run to provide a signal-to-concentration response curve for each analyte in the method. The concentration of the analyte of interest in a patient blood sample dilution is determined by comparing the observed signal ratio (element/internal standard) from the dilution of the patient blood sample to the signal ratio response curve from the working calibrators.
2. Content: Dilutions (1:50) of the corresponding eight intermediate working calibration standards with base blood and sample diluent.
3. Preparation: Mix with base blood and diluent (Section 6.c) using a benchtop automatic pipette to make 1:50 dilutions of the corresponding eight intermediate working calibration standards immediately prior to analysis (see Table 8 of Appendix B).

vi. Base blood

1. Purpose: This blood pool material will be mixed with the intermediate working calibrators just prior to analysis to matrix-match the calibration curve to the blood matrix of the unknown samples.
2. Preparation: To prepare a mixture of multiple blood sources collected from anonymous donors to approximate an average blood matrix:
   a. Purchase several bags of whole blood.
   b. Screen each individual bag of blood for concentration of analytes of interest. See Table 2 in Appendix B for minimum acceptable values
   c. Once screened, mix the acceptable blood together in a larger container (i.e. acid washed polypropylene (PP), polymethylpentene (PMP), or Teflon™) and stir for 30+ minutes on a large stir plate (acid wash large Teflon™ stir bar before use).
   d. Store long-term as smaller portions for daily use (e.g. 2 mL cryovials) according the same storing and handling criteria described in Section 3.

vii. Internal quality control materials ("bench" QC)

1. Purpose: Internal (or "bench") quality control (QC) materials are used to evaluate the accuracy and precision of the analysis process, and to determine if the analytical system is "in control" (is producing results that are acceptably accurate and precise). They are included in the beginning and at the end of each analytical run.
2. Preparation: To prepare pooled animal or human blood at low-normal and high-normal concentrations:

   Both purchased or in-house prepared quality control materials are suitable for this purpose if volumes, concentrations meet method requirements and

any spikes of elemental levels are traceable to the National Institute for Standards and Technology (NIST).

3. Screening blood: Screen bags of blood for analyte of interest concentration before mixing together to make 2 separate base blood pools (for preparing the low and high bench QC materials). Samples can be screened individually
    a. Keep blood refrigerated whenever possible to minimize microbial growth.
    b. Because this is only a quick screen of the analyte of interest concentration, the number of replicates in the blood method can be reduced to one in order to reduce analysis time.
    c. Select blood for the low bench QC pool which has analyte concentrations in the low-normal population range. Select blood for the high and elevated bench QC pools which has analyte concentrations less than some pre-selected target concentration values in the high normal population range. See Table 2 in Appendix B for recommended concentration ranges.
4. Combining collected blood: The goal is for combining samples is to approach an 'average' matrix for each pool.
    a. Graduate four acid-washed 10 L carboys (PP or PMP) in 0.5 L increments (two will be used for decanting into).
    b. Combine collected blood samples into two separate acid-washed 10 L carboys (PP or PMP), according to their concentrations, for the low bench and high bench QC pools.
    c. Mix each blood pool using carboy stirrers and large stir plates. Keep blood refrigerated whenever possible.
5. Spiking of blood
    a. Analyze three samples of each blood pool. Record these results for future recovery calculations.
    b. Use these results to determine target analyte concentrations possible for the pools
    c. Calculate the volume of single element standards needed to spike each pool to the desired concentrations. See Table 2 in Appendix B for recommended concentration ranges.
    d. While stirring the pools on large stir plates, spike each pool with calculated volumes of single element standards (all spiking standards used must be traceable to NIST).
    e. Continue to stir pools overnight after spiking, then reanalyze.

f. Repeat steps 4 and 5 until all analytes reach target concentrations keeping track of the total volume of spiking solution added to each blood pool.

6. Dispensing and storage of blood

a. Container types: Dispense blood into lot screened containers (i.e. – 2 mL polypropylene tubes). If possible, prepare tubes of QC which have only enough volume for one typical run + 1 repeat analysis. This allows for one vial of QC to be used per day of analysis, reducing chances of contamination of QC materials due to multi-day use.

b. Labels: Place labels on vials after dispensing and capping if the vials are originally bagged separately from the caps. This minimizes the chance for contamination during the process. Include at least the name of QC pool (text and bar code), date of preparation, and a vial number on the labels.

c. Dispensing: Dispensing can be accomplished most easily using a benchtop automatic pipette in continuous cycling dispense mode. Dispense the pools in a clean environment (i.e. a class 100 cleanroom area or hood).

1. Allow blood to reach room temperature before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials).

2. Replace the tubing attached to the dispensing syringe (left when looking at front of the benchtop automatic pipette) with a length of clean Teflon™ tubing long enough to reach into the bottom of the 10 L carboy while it is sitting on the stir plate.

3. Check cleanliness of the benchtop automatic pipette before use by analyzing 1-2% (v/v) HNO_3 which has been flushed through the benchtop automatic pipette with a portion of the same solution which has not been through the benchtop automatic pipette.

4. Approximately one hour before dispensing begins,

a. With the large stir plate close to the left side of the benchtop automatic pipette, begin stirring the blood pool to be dispensed.

b. Also during this time, flush the benchtop automatic pipette with blood from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of blood so that blood won't be used up during this process. Be sure to secure both ends

of tubing in the carboy with Parafilm so they will not come out during the flushing process.

5. After dispensing the blood into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

d. Homogeneity test: Check homogeneity of analyte concentrations in pool aliquots.

e. Storage: Store long-term as smaller portions for daily use (e.g. 2 mL cryovials) according the same storing and handling criteria described in Section 3.

f. Optimization solutions

i. DRC optimization:

1. Purpose: For periodic testing of the DRC cell parameters. Procedure requires at a minimum a blank (i), an analyte solution (ii), a blank with interference (iii), and an analyte and interference containing solution (iv). For Se, only the blank (i), an analyte solution (ii) are needed because the interference on Se is plasma based.

2. Content:

Diluent in this section refers to sample diluent (5 μg/L internal standard mixture (Rh, Ir, Te), 0.4% v/v tetramethyl ammonia hydroxide (TMAH), 1% ethyl alcohol, 0.01% APDC, and 0.05% v/v Triton X-100® as described in Section 6c.

a. Solutions for testing elimination of $^{54}Fe^{1}H$ interference on ^{55}Mn:

i. Base blood in diluent (1 + 49)
ii. Base blood in diluent (1 + 49) + 4.5 μg/L Mn
iii. Base blood in diluent (1 + 49) + 500 μg/L Fe
iv. Base blood in diluent (1 + 49) + 4.5 μg/L Mn + 500 μg/L Fe

b. Solutions for testing elimination of $^{40}Ar_2$ interference on ^{80}Se:

i. Base blood in diluent (1 + 49)
ii. Base blood in diluent (1 + 49) + 90 μg/L Se

3. Preparation & storage: Prepare different volumes, if needed, by adding proportionally larger or smaller volumes of solution constituents. Interference concentrations can be prepared higher as needed by adjusting the volume of this spike. Keep interference spike volume small (<0.3 mL) using a high concentration stock solution (i.e. 1000 mg/mL). Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

a. Solutions for testing elimination of $^{54}Fe^{1}H$ interference on ^{55}Mn:
   i. Base blood in diluent (1 + 49)
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 6 (multiply volumes by 5).
   ii. Base blood in diluent (1 + 49) + 4.5 μg/L Mn
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 6 (multiply volumes by 5).
   iii. Base blood in diluent (1 + 49) + 500 μg/L Fe
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 6 (multiply volumes by 5).
      2. Add 0.025 mL of 1000 mg/mL Fe.
   iv. Base blood in diluent (1 + 49) + 4.5 μg/L Mn + 500 μg/L Fe
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 6 (multiply volumes by 5).
      2. Add 0.025 mL of 1000 mg/mL Fe.

b. Solutions for testing elimination of $^{40}Ar_2$ interference on ^{80}Se:
   i. Base blood in diluent (1 + 49)
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 0 as described in Table 6 (multiply volumes by 5).
   ii. Base blood in diluent (1 + 49) + 90 μg/L Se
      1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 50 mL portion of working calibrator 2 as described in Table 6 (multiply volumes by 5).

c. Store at room temperature and prepare as needed.
d. Label appropriately (see Section 6.f.i.2), “Store at room temperature”, preparation date, expiration date one year from preparation date, and preparer’s initials.

ii. Dual detector calibration:

1. Purpose: Use as necessary to perform the dual detector calibration.
2. Content: Aqueous dilutions of single element stock standard solutions in 2% (v/v) nitric acid. Current solution in use contains: Pb with a final concentration of 200 ug/L.

3. Preparation & storage: Prepare different volumes, if needed, by adding proportionally larger or smaller volumes of solution constituents.
   a. To prepare a total of 50 mL: In a 50 mL lot screened polypropylene tubes, spike in 0.01 mL of 1000 mg/mL single element stock solution for each element desired in the final solution.
   b. Dilute to the 50 mL mark with 2% (v/v) nitric acid.
   c. Store at room temperature and prepare as needed.
   d. Label appropriately, e.g. "200 ug/L Pb in 2% (v/v) HNO3", "Store at room temperature", preparation date, expiration date one year from preparation date, and preparer's initials.

7) Analytical instrumentation setup

(see Section 5 for details on hardware used, including sources)

a. Instrumentation and equipment setup:

i. Configuration for liquid handling

1. FAST valve setup: See Appendix B, Figure 1 for diagram and Section 5.b "FAST / ESI SC4-DX autosampler accessories" for source information.
   a. Port 1: sample loop (white nut).
   b. Port 2: 0.5 mm ID probe (red nut) for carrier solution.
   c. Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.
   d. Port 4: sample loop (white nut).
   e. Port 5: 0.8 mm ID probe (blue nut) for diluted samples.
   f. Port 6: vacuum line (black nut).

2. Carrier solution uptake: Use peristaltic pump to control uptake flow rate of carrier solution to the SC-FAST valve. Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.d. tubing when making connections (see consumables descriptions in Section 5.b).

3. Spray chamber waste removal

   Use of a 'peristaltic to Teflon tubing adapter' for prevents damage to small i.d. tubing when making connections (see consumables descriptions in Section 5.b).

   a. Between spray chamber and peristaltic tubing:
      i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.

ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (e.g. 1/8" i.d. x ¼" o.d.).

b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the white / black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Place the free end of the PVC tubing through the lid of the waste jug (be sure it is secure). Place waste container in a deep secondary containment tray in case of overflow.

4. Rinse solution for autosampler:

a. Rinse solution jug: Leave one of the caps on the top of the rinse jug loose to allow air venting into the jug as liquid is removed. Otherwise the jug will collapse on itself as the liquid is removed and a vacuum is created inside. Use secondary containment tray.
b. Rinse solution uptake to autosampler rinse station: Use tubing of different lengths and inner diameters between the rinse solution container and the autosampler rinse station to control uptake rate of rinse solution. These can be obtained from the autosampler manufacturer, their distributors, or custom built in the lab. Optimize these factors along with fill time in the software so that waste of rinse solution is minimized and rinse station does not go empty.
c. Autosampler rinse station waste removal: Gravity drain of waste to the waste container is sufficient. Use minimum drain tubing to make this connection. If this tube is too long, the rinse station will not drain properly.

ii. Gas delivery and regulation

1. ICP-MS modifications:
   a. Plastic tubing between mass flow controllers and dynamic reaction cell have been replaced with stainless steel. Stainless steel tubing is preferred between the reaction gas cylinder / regulator and the back of the ICP-MS instrument.
   b. A second mass flow controller will be needed (channel B) that does not send the DRC gas through a 'getter'.

2. Argon gas: Used for various ICP-MS functions including plasma and nebulizer.

   a. Regulator for argon source (if a dewar): Set delivery pressure of this regulator at least 10 psi higher than the delivery pressure of the step-

down regulator to allow for pressure drop across tubing that stretches to the instrument.

b. Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to 10 psig above the delivery pressure of the filter regulator on the ICP-MS.

c. Filter regulator at ICP-MS: Single stage "argon regulator filter kit" supplied with the ICP-DRC-MS. Set the delivery pressure depending on the instrument setup:

i. ELAN with a 0-60psi gauge on the filter regulator: 52±1 psi when plasma is running (need 0-150 psi regulator if using a PolyPro or PFA nebulizer made by Elemental Scientific Inc).

ii. ELAN with a 0-150psi gauge on the filter regulator: 90-100 psi when plasma is running.

3. Methane (99.99%) gas: Used for dynamic reaction cell interference removal from selenium isotopes.
   a. Connect to DRC channel A
   b. Set the delivery pressure of regulator to 5-7 psig when gas is flowing. See section 5.e for part numbers and details.

4. Oxygen (99.999^{+}%) gas: Used for dynamic reaction cell interference removal from manganese isotopes.
   a. Connect to DRC channel B.
   b. Set the delivery pressure of regulator to 5-7 psig when gas is flowing. See Section 5.e for part numbers and details.
   c. Use a brass flash arrestor on outlet side of regulator. See Section 5.e for part numbers and details.

iii. Chiller / heat exchanger: If using refrigerated chiller, set temperature control to approximately 18 °C.

b. Instrument and method parameters: See Tables and Figures in Appendix B for a complete listing of the instrument and method parameters and software screen shots.

8) The run: quality, execution, evaluation, and reporting

a. Bench QC, reference materials and calibration verification:

i. Bench "QC": Analysis of bench QC permits assessment of methodological imprecision, determination of whether the analytical system is 'in control' during the run, and assessment of time-associated trends. Before QC materials can be used in the QC process, they must be characterized by at least twenty (20) analytical runs to determine appropriate QC parameters.

Bench QC pool analyte concentrations in this method span the analyte concentration range of the calibrators including “low-normal” (‘Low QC’), “high-normal” (‘High QC’), and “above-normal” (‘Elevated QC’) concentrations.

In each analytical run the analyst will test each of the three bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibration standards but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 40-50 patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 6 in Appendix B are both acceptable ways to analyze multiple consecutive “runs”.

ii. Reference materials: Use standard reference materials (SRM) from the National Institute of Standards and Technology (NIST) (i.e. SRM 955c Levels 1-4) to verify method accuracy. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable.

iii. Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibration standards. These calibrators, along with the QCs and blanks, are used to verify that the test system is performing properly.

b. Perform, evaluate and report a run

i. Starting the equipment for a run

1. Power on the computer, printer, and autosampler, and instrument computer controller.
2. Peristaltic pump: Set proper tension on peristaltic pump tubing.
3. Software: Start software for the ICP-MS and autosampler control.
4. Daily pre-ignition maintenance checks: Perform and document daily maintenance checks (e.g., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.).
5. Place probe in adequate volume of carrier or rinse solution: If using an ESI FAST, manually place carrier probe into carrier solution. If not, send the autosampler probe to a rinse solution (e.g. autosampler rinse station).
6. Start the plasma

7. Start the peristaltic pump: Start the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.
8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (e.g. RF generator) after igniting the plasma. There will be another warm-up time (or "stability time") for the DRC later in this procedure.
9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

   Save new parameters to the "default.tun" and "default.dac" files.
10. DRC stability time: Best analyte-to-internal standard ratio stability is typically observed after 1-1.5 hours of analysis of diluted blood samples using the DRC mode method (~15 measurements of the 5 element panel can be made in 1 hour). Prepare 50mL^{+} of a calibration standard (e.g. standard 2) to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific and learned from performance of runs. See Table 7 in Appendix B for example of setup in the Samples / Batch window and Table 8 in Appendix B for details of making a working standard.
11. Readying the instrument for quick-start analysis: Leave the plasma running to eliminate the need for an initial instrument warm-up period and / or a DRC stabilization period as long as appropriate planning is made for sufficient solution supply and waste collection. Analysis of conditioning samples (diluted blood matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (e.g. one 5 element analysis with a 1500s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning. If running a DRC-only method during these scheduled analyses, the ICP-MS will remain in DRC-mode for approximately 45 minutes without depressurizing the cell.
12. Software setup for analysis:
    a. Workspace (files & folders): Verify & set up the correct files and data directories for your analysis (See Table 1 in Appendix B for defaults).
    b. Samples / batch window: Update the software to reflect the current sample set. Use a bar code scanner to input data whenever possible. See Table 1 in Appendix B for times and speeds.

        1. Blood vs. aqueous method files:
            a. The difference: There are two method files for this one method (see Table 1 in Appendix B). It is necessary to use both to accomplish each run because the current PerkinElmer software will not allow for more than one blank

per method file. The ONLY DIFFERENCE between these two files is on the Sampling tab where one lists the autosampler positions of the blood blank and blood-based calibrators (the "bldblk" method file) and the other lists the autosampler position of the aqueous blank (the "aqblk" method file).

b. Use: The ONLY TIME when it matters which of these files is used is when the measurement action *includes* "Run blank" or "Run standards". When the measurement action is only 'run sample', it does not matter whether the "bldblk" or "aqblk" method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample. See Table 7 in Appendix B.

i. The *"bldblk" method file:* *U*se to analyze the initial blood blank (blank for the calibration curve), the blood calibrators, and the blood blank checks at the very beginning of the run. The blood blank method defines the autosampler location of the blood blank and the blood calibration standards.

ii. The "*aqblk*" method file must be used to analyze all QC materials and patient samples. The aqueous blank method defines the aqueous blank in autosampler location.

ii. Preparation of samples for analysis (See Table 6 in Appendix B)

1. Thaw blood samples; allow them to reach ambient temperature.
2. Prepare the following solutions into pre-labeled containers using the benchtop automatic pipette or other volumetric sample transfer device. See Table 8 in Appendix B for a summary.

a. *Aqueous Blank*: Prepare a minimum of two aqueous blanks. One will be the actual aqueous blank and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank is unusable.

b. *Calibrators*: Prepare the working calibrators (S0-S8). Prepare S0 in triplicate. One of these S0 preparations will be the zero calibrator (blood blank) for the calibrators; the other two will be analyzed twice after the last calibrator to collect run blank data that can be used in calculating method limit of detection (LOD).

c. *Patient & QC Samples*: Before taking an aliquot for analysis, homogenize the sample thoroughly.

After preparation, mix and cover. Place prepared dilutions on the autosampler of the ICP-MS in the order corresponding to the sequence setup in the ICP-MS software.

Room temperature is acceptable for the original samples for the work day.

NOTE: ***Samples must be analyzed within 24 hours of preparation to obtain valid results for selenium***. The method has been validated to produce valid results for other Pb, Cd, Hg, and Mn even 48 hrs after sample preparation. See critical parameter test results in Appendix A for details.

iii. Start the analysis using the ICP-MS software.

iv. Monitor the analysis in real-time as much as possible. If necessary, leave the run to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

Monitor the analysis for the following:

1. *Verify proper operation of the instrument* (proper loop filling, sample reaching nebulizer in correct timing, autosampler arm moving properly, etc . . .).
2. Verify that background signal from instrument and reagents are low. Helpful checks when diagnosing high background problems include:
   a. Water to be used in Aq Blank Checks and dilutions.
   b. Diluent before and after being flushed through the benchtop automatic pipette.

      If contamination is observed from the pipette, flush the pipette with ≥500 mL of nitric acid solution (≤ 5% v/v HNO_3) and retest.

   c. Comparison with other instruments.
3. *Verify analyte / internal standard ratio stability*

   The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC can be evaluated to determine the readiness of the system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run.
4. *Verify calibration curves* meet R^2 requirements (minimum of 0.98, typically 0.99 to 1.000).
5. *Verify bench QC results are within the acceptable limits*.

   If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then the following steps are recommended:

   a. Evaluate the blank results.
   b. Evaluate the reproducibility of the 3 replicates within the measurements.
   c. Evaluate the consistency of the internal standard across the measurements (esp. the calibrators).

d. Evaluate calibration curves. If a particular calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte between or including S2 and S7 can be removed from the curve (Do not drop S0, S1 or S8). Follow up problems with calibration standards with appropriate corrective actions (e.g. re-preparation of intermediate working standards or troubleshooting instrument parameters).

e. Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.

f. Prepare a fresh dilution of the failing QC material (unused vial) and analyze it to see if the QC vial had become compromised.

g. Prepare and analyze new working calibrators.

h. Test a different preparation of intermediate working calibration standards.

If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions.

6. *Verify good precision among replicates* of each measurement.

7. *Verify consistent measured intensities of the internal standards*.

Some sample-to-sample variations are to be expected, however, intensities drifting continuously in one direction resulting in failing results for ending QC indicate the instrument needs additional pre-conditioning before the run or environmental conditions are changing too much around the instrument.

8. Verify elevated patient results.

Refer to Figure 4 in Appendix B for flowchart.

a. Confirming an elevated concentration: Repeat for confirmation any sample having a concentration greater than the 1UB threshold. See Table 9 in Appendix B.

b. Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibration standard to bring the observed result within the concentration range of the calibrators. See Table 7 in Appendix B for validated extra dilutions.

c. Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if a sample concentration is greater than the highest concentration validated for washout (see Table 9 of Appendix B), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

i. Stop run following elevated sample

ii. Verify that the run is still in control for lower concentration samples before proceeding with analysis. Analyze 2 blood blank checks followed by a low bench QC washout check. If the low bench QC wash check is not in control (within ± 3SD limits), repeat these 3 check samples until washout is verified before proceeding with analysis.

Example:
3016 BldBlkChk Wash1
3016 BldBlkChk Wash2
LBXXXXX Wash

iii. If the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8.b.vii.2. for directions.

v. Overnight operation or using auto stop: The run may be left to complete itself unattended as long as appropriate planning is made (e.g. sufficient solution supply and waste collection). Turn on the AutoStop feature of the ICP-MS software. Delay the shutdown at least 10 minutes (use peristaltic pump speed approximately that of the method wash) to rinse the sample introduction system of blood matrix before turning off the plasma. It will be necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight. Enable “Auto Start / Stop” is on the “AutoStop” tab of the Instrument window.

vi. Records of results: Run results will be documented after each run in both electronic and paper form.

1. Electronic records: Transfer data electronically to the laboratory information system. When keyboard entry must be used, proofread transcribed data after entry.

a. Export data from the ICP-MS software using “original conditions” or files and folders used during the analysis. Use descriptive report filenames (e.g. 2005-0714a_group55.txt). In the ICP-MS software under “Report Format” (METHOD window, REPORT tab) choose the “Use Separator” option, and under the “File Write” Section choose “Append.”

b. Move the generated .TXT data file to the appropriate subdirectory on the network drive where exported data are stored prior to import to the laboratory information management system.

c. Import the instrument file into the laboratory information system with appropriate documentation (e.g. instrument ID, analyst, calibration standards lot number, and run or sample specific comments).

2. Paper records: Printed run sheets must be documented with

i. Analyst initials

ii. Instrument ID

iii. Date of analysis and run # for the day

vii. Analyst evaluation of run results:

1. Bench quality control: After completing a run, and importing the results into the laboratory information system, evaluate the run bench QC according to laboratory QC rules. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is in control until statistically reviewed.

a. Rules for bench quality control evaluation: The following are the CDC DLS QC rules for three QC pools per run with two or more QC results per pool.

i. If all three QC run means are within $2S_m$ limits and individual results are within $2S_i$ limits, then accept the run.

ii. If one of the three QC run means is outside a $2S_m$ limit - reject run if:

1. Extreme Outlier – Run mean is beyond the characterization mean ± $4S_m$
2. 3S Rule - Run mean is outside a $3S_m$ limit
3. 2S Rule – Two or more of the run means are outside the same $2S_m$ limit
4. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

iii. If one of the QC individual results is outside a $2S_i$ limit - reject run if:

1. Extreme Outlier – One individual result is beyond the characterization mean ± $4S_m$
2. R 4S Rule – 2 or more of the within-run ranges in the same run exceed $4S_w$ (i.e., 95% range limit)

Note: Since runs have multiple results per pool for 3 pools, the R 4S rule is applied within runs only.

Abbreviations:

S_i = Standard deviation of individual results.

S_m = Standard deviation of the run means.

S_w = Within-run standard deviation.

b. Implications of QC failures: If the DLS SAS program declares the run “out of control” for any analyte, use the following to determine the implications on usability of the data from the run.

i. For 1 or 2 analytes: ONLY the analytes which were "out of control" are invalid for reporting from the run.

ii. For 3 or more analytes: All results, regardless of analyte, are invalid for reporting from the run.

2. Patient results:

a. Elevated concentrations: Refer to Figure 5 in Appendix B for flowchart.

i. Boundaries requiring confirmatory measurement:

1. Results greater than the first (1UB) or second (2UB) upper boundaries.

The concentrations assigned to 1UB and 2UB for an element is determined by study protocol but default concentrations are in Table 9 in Appendix B.

a. Results greater than the first upper boundary (1UB): Confirm by repeat analysis of a new sample preparation concentrations observed greater than the "first upper boundary" (defined in the laboratory database as the "1UB"). Report the first analytically valid result, as long as the confirmation is within 10%. Continue repeat analysis until a concentration can be confirmed.

b. Analyst reporting of elevated results: Report any patient results confirmed to be greater than the second upper boundary (2UB) as an "elevated result".

2. Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with minimum extra dilution in duplicate to bring the observed result within the calibration range (≤ S8). Report the first analytically valid result (i.e. the first one within the calibration range), as long as the confirmation is within 10%. Continue repeat analysis until a concentration can be confirmed.

ii. Concentrations requiring verification of washout: Following a result greater than the highest concentrations validated for washout (see Table 9 of Appendix B) do the following:

1. If the run was determined to be in-control for low concentration samples before the next samples were analyzed, no further action is required.

2. If the run was not determined to be in-control for low concentration samples before the next samples were analyzed confirm by re-analysis the results for the 2 samples immediately following the elevated sample. Report the results if they confirm the initial results within ±10% or ±3SD of the low bench QC, whichever is greater.

b. Unacceptable reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria listed in Table 9 in Appendix B ***and*** the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

viii. Submitting final work for review: All analyses must undergo quality control and quality assurance review. After appropriately documenting the run in the laboratory information system (e.g. sample and run QC, and run and sample comments), inform the first level reviewer of the completed work and submit any printed documentation.

9) Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

a. Equipment maintenance: Analysts are expected to regularly evaluate the need for, and when necessary perform, cleaning, replacement, or re-positioning of components in ICP-MS the sample introduction system, interface, ion optics region, and equipment required resources (e.g. autosampler, exhaust, compressed gases, and coolant). Frequency of equipment maintenance will be dependent on instrument throughput.

b. Parameter optimizations: Analysts are expected to optimize instrument parameters.

i. Dual detector calibration: Perform dual detector calibration regularly for any element exceeding 1,000,000 cps for calibration standard 8. This is typically only Pb. Dual detector calibration solution is described in Section 6.f.ii. Frequency of dual detector calibration is typically monthly when throughput requires multiple analytical runs per week, or as needed for optimized linearity.

ii. DRC optimizations: DRC conditions (cell gas flow rate and RPq value) can be verified by analyzing the DRC optimization solutions (see Section 6.f.i) as needed to ensure proper reduction of potential ICP-MS interferences.

c. Data backup: Data on the instrument computer will be backed up via two backup routines. Files used and produced by the ICP-MS in analyzing samples will be backed up and kept a minimum of two years after analysis.

i. Daily backups to secondary hard drive: Program automatic backups of the relevant computer files to occur each night onto a secondary hard drive to prevent loss of data from failure of primary hard drive.

ii. Weekly backup: Backup relevant computer files weekly either to secondary hard drive which is remote to the laboratory or to removable media which will be placed remote to the laboratory for retrieval in the case of catastrophic data loss elsewhere.

10) Reporting thresholds

a. Reportable range: Blood elemental concentrations are reportable in the range between the method LOD and the highest calibrator (see 'calibrator concentrations' in Table 1) times the maximum validated extra dilution (see Table 8). Above the highest concentration verified, extra dilutions are made of the blood sample to bring it within the reportable range.

b. Reference ranges (normal values): In this method the 95% reference ranges (see Appendix B, Table 10) for these elements in blood fall within the range of the calibrators.

c. Action levels: Report concentrations observed greater than the "second upper boundary" (defined in the laboratory database as the "2UB") to the QC reviewer as an "elevated result". The concentration assigned to the 2UB for an element is determined by study protocol but default concentrations are listed in Table 9 in Appendix B. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. But typically,

   i. Lead: Levels of lead in blood of children ages 1-5 are considered elevated above 5 μg/dL and chelation treatment is recommended at blood lead levels ≥45 μg/dL[65]. The Occupational Safety and Health Administration regulations use a blood lead level of 40 μg/dL as cause for written notification and a medical exam, and a blood lead level of 60 μg/dL as cause for medical removal from exposure[66].

   ii. Cadmium: Levels of concern for cadmium in blood is >5 μg/L[67, 68].

   iii. Mercury: The American Conference of Governmental Industrial Hygienists has a biological exposure index (BEI) of 15 μg/L for inorganic mercury in blood (end of shift at end of work week)[68].

   iv. Manganese: Insufficient data to establish an action level.

   v. Selenium: >500 μg/L [69, 70]

11) Method Calculations

a. Method limit of detection (LODs): The method detection limits for elements in blood specimens are defined as 3 times $s_{0,}$ where s_0 is the estimate of the standard deviation at zero analyte concentration. S_0 is taken as the y-intercept of a linear or 2nd order polynomial regression of standard deviation versus concentration (4 concentration levels of the analytes in blood each measured 60 times across at least a 2-month timeframe). Method LODs are re-evaluated periodically.

b. Method limit of quantitation (LOQ): The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits [71].

c. QC Limits: Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers,

instruments, and analysts to best mimic real-life variability. The statistical calculations are performed using the SAS program developed for the Division of Laboratory Sciences (DLS_QC_compute_char_stats.sas).

12) Alternate methods for performing test and storing specimens if test system fails:

If the analytical system fails, the analysis may be setup on other ICP-MS instruments in the laboratory. If no other instrument is available, store the specimens at ~4 °C until the analytical system can be restored to functionality. If interruption longer than 4 weeks in anticipated, then store blood specimens at ≤ -20 °C.

Appendix A: Critical parameter test results

Critical parameter test #1: Testing scenario of something preventing a set of prepared samples from being analyzed immediately.

Test details:

Day 1: Prepared a set of dilutions (calibrators, blanks, reference material, fake samples) for analysis in triplicate. Analyzed set 1 immediately (normal practice). Cap sets 2 and 3 and leave at room temperature for later analysis.

Day 2: Prepared run set 4 and analyzed it sequentially with run set 2

Day 3: Prepared run set 5 and analyzed it sequentially with run set 3

Table 1. Ruggedness testing results: Evaluating the significance of time from preparation to analysis on sample stability. Test performed 12/6-8/10 by Deanna Jones. Results are the average of the beginning and ending QC results for each analytical run.

ID	Time, prep to analysis	Hg (µg/L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
LB08707_WB2	*target mean and 3SD range*	0.585 0.318 – 0.852	2.12 1.99 – 2.25	0.488 0.353 – 0.623	7.98 6.38 – 9.59	
	0 hr	0.418	2.03	0.399	6.09	
	24 hr (fresh)	0.504 (0.522)	1.99 (2.18)	0.419 (0.47)	7.06 (7.88)	
	48 hr	0.396 (0.418)	2.04 (2.03)	0.509 (0.40)	7.82 (6.09)	
HB08708_WB2	*target mean and 3SD range*	6.19 5.74 – 6.63	10.1 9.73 – 10.4	3.14 2.84 – 3.44	14.9 12.8 – 17.1	
	0 hr	5.86	10.0	3.03	12.5	
	24 hr	5.46 (5.7)	9.5 (10.7)	2.85 (3.17)	13.6 (14.7)	
	48 hr	2.64 (5.9)	9.2 (10.0)	2.79 (3.03)	13.5 (12.5)	
QMEQAS 07B-03*	*target mean and 3SD range*					228 206 – 251
	0 hr					192
	24 hr					202 (217)
	48 hr					56 (192)
QMEQAS 10B-06*	*target mean and 2SD range*					239 215 – 253
	0 hr					212
	24 hr					221 (238)
	48 hr					62 (212)

*samples purchase from Le centre de toxicology du Quebec (Quebec, Canada)

Conclusion: Samples which have been diluted 1+1+48 for analysis up to one (1) day previously can still be analyzed.

Appendix A: Critical parameter test results (continued)

Critical parameter test #2: This test evaluated the significance of the RF Power setting of the ICP when analyzing blood samples for whole blood metals.

Test details:
1. Prepare a set of dilutions (calibrators, blanks, reference material, dummy samples) for analysis in triplicate (three separate sets of tubes).
2. Analyze them in three separate runs on the same day, same instrument.
3. Change the RF Power across the runs
4. Allow 15 minutes equilibration time between runs for RF Power to stabilize

Table 2. Ruggedness testing results: Evaluating the significance of RF Power setting on sample stability. Test performed on December 6 and December 10, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	RF power (W)	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
LB08707_W B2	*target mean and 2SD range*	0.585 0.407 – 0.763	2.12 2.03 – 2.21	0.488 0.398 – 0.578	7.98 6.91 – 9.05	
	1150 W	0.517	2.09	0.432	7.35	
	1450 W (default)	0.512	2.03	0.369	6.76	
	1600 W	0.529	2.02	0.418	7.17	
HB08708_W B2	*target mean and 2SD range*	6.19 5.89 – 6.48	10.1 9.84 – 10.3	3.14 2.94 – 3.34	14.9 13.5 – 16.4	
	1150 W	5.90	10.0	2.93	13.7	
	1450 W (default)	6.23	10.2	2.90	12.8	
	1600 W	5.99	10.1	3.07	13.3	
QMEQAS08 B-02*	*target mean and 2SD range*					293 273 - 313
	1150 W					269
	1450 W (default)					288
	1600 W					314
QMEQAS08 B-08*	*target mean and 2SD range*					165 154 - 176
	1150 W					179
	1450 W (default)					147
	1600 W					146

*samples purchase from Le centre de toxicology du Quebec (Quebec, Canada)

Conclusion: Results are not compromised by changes in RF power within the range of 1150W to 1600W.

Appendix A: Critical parameter test results (continued)

Critical parameter test #3: This test evaluated the significance of the dynamic reaction cell gas flow rate of the reaction gas (oxygen and methane) while analyzing blood samples for elements analyzed in DRC mode (Hg, Mn, and Se). The cell gas flow rate for Mn and Hg is oxygen (O_2) and the per method setting is 1.2 mL/min. The cell gas flow rate for Se is methane (CH_4) and the per method setting is 0.84 mL/min.

Test details:
1. Prepare a set of dilutions (calibrators, blanks, reference material, dummy samples) for analysis in triplicate (three separate sets of tubes).
2. Analyze them in three separate runs on the same day using the same instrument.
3. Change the cell gas flow rate.

Table 3. Ruggedness testing results: Evaluating the significance of dynamic reaction cell gas flow rate on sample stability. Test performed on December 6, 2010 and January 4, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	cell gas flow rate	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
LB08707_WB2	*target mean and 2SD range*	0.585 0.407 – 0.763	2.12 2.03 – 2.21	0.488 0.398 – 0.578	7.98 6.91 – 9.05	See Table 4
	0.96 mL/min O_2; 0.7 mL/min CH_4	0.457	2.10	0.471	8.49	
	1.2 mL/min O_2; 0.84 mL/min CH_4	0.479	2.10	0.438	8.15	
	1.44 mL/min O_2; 1.0 mL/min CH_4	0.555	2.11	0.457	8.12	
HB08708_WB2	*Target Mean and 2SD Range*	6.19 5.89 – 6.48	10.1 9.84 – 10.3	3.14 2.94 – 3.34	14.9 13.5 – 16.4	
	0.96 mL/min O_2; 0.7 mL/min CH_4	4.71	10.0	3.19	14.4	
	1.2 mL/min O_2; 0.84 mL/min CH_4	5.45	10.1	2.92	15.2	
	1.44 mL/min O_2; 1.0 mL/min CH_4	5.34	10.3	3.04	14.6	

Conclusion: Accuracy of Mn and Hg results are not compromised by changes in cell gas flow rate within the range tested (0.96 – 1.44 mL/min).

Appendix A: Critical Parameter Test Results (Continued)

Table 4. Ruggedness testing results: Evaluating the significance of dynamic reaction cell gas flow rate on sample stability. Test performed on December 6, 2010 and January 4, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	cell gas flow rate	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
QMEQAS07B-09*	target mean and 2SD range	See Table 3, Appendix A				157 146 - 168
	0.96 mL/min O_2; 0.7 mL/min CH_4					187
	1.2 mL/min O_2; 0.84 mL/min CH_4					186
	1.44 mL/min O_2; 1.0 mL/min CH_4					191
QMEQAS08B-02*	target mean and 2SD range					293 273 - 313
	0.96 mL/min O_2; 0.7 mL/min CH_4					328
	1.2 mL/min O_2; 0.84 mL/min CH_4					334
	1.44 mL/min O_2; 1.0 mL/min CH_4					339

*samples purchase from Le centre de toxicology du Quebec (Quebec, Canada)

Conclusion: Accuracy of Se results are not compromised by changes in cell gas flow rate within the range tested (0.7 – 1.0 mL/min).

Appendix A: Critical parameter test results (continued)

Critical parameter test #4: This test evaluated the significance of the RPq value while analyzing blood samples for Se, Mn and Hg. The RPq value setting per method for Mn and Hg is 0.6, and for Se it is 0.65. The reduced and elevated RPq values for Mn and Hg are 0.48 and 0.72, respectively. The reduced and elevated RPq values for Se are 0.52 and 0.78, respectively. The results are presented in Tables 5 and 6.

Test details:
1. Prepare a set of dilutions (calibrators, blanks, reference material, fake samples) for analysis in triplicate (three separate sets of tubes).
2. Analyze them in three separate runs on the same day, using the same instrument.
3. Change the RPq value.

Table 5. Ruggedness testing results: Evaluating the significance of RPq value on sample stability. Test performed on December 21, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	RPq	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
LB08707_WB2	*Target Mean and 2SD Range*	0.585 0.407 – 0.763	2.12 2.03 – 2.21	0.488 0.398 – 0.578	7.98 6.91 – 9.05	See Table 6
	0.48 Mn and Hg; 0.52 Se	0.455	1.97	0.361	7.86	
	0.6 Mn and Hg; 0.7 Se	0.418	2.03	0.399	6.09	
	0.72 Mn and Hg; 0.78 Se	0.402	2.07	0.402	7.99	
HB08708_WB2	*Target Mean and 2SD Range*	6.19 5.89 – 6.48	10.1 9.84 – 10.3	3.14 2.94 – 3.34	14.9 13.5 – 16.4	
	0.48 Mn and Hg; 0.52 Se	5.54	9.4	2.79	14.4	
	0.6 Mn and Hg; 0.7 Se	5.86	10.0	3.03	12.5	
	0.72 Mn and Hg; 0.78 Se	5.53	9.7	2.88	14.9	

Conclusion: Accuracy of Mn and Hg results are not compromised by changes in RPq settings within the range tested (0.48 – 0.72).

Appendix A: Critical Parameter Test Results (Continued)

Table 6. Ruggedness testing results: Evaluating the significance of RPq value on sample stability. Test performed on December 21, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	RPq	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
QMEQAS07B-09*	*target mean and 2SD range*	See Table 5, Appendix A				293 273 – 313
	0.48 Mn and Hg; 0.52 Se					262
	0.6 Mn and Hg; 0.7 Se					250
	0.72 Mn and Hg; 0.78 Se					277
QMEQAS08B-02*	*target mean and 2SD range*					361 337 - 385
	0.48 Mn and Hg; 0.52 Se					347
	0.6 Mn and Hg; 0.7 Se					349
	0.72 Mn and Hg; 0.78 Se					364

*samples purchase from Le centre de toxicology du Quebec (Quebec, Canada)

Conclusion: Accuracy of Se results are not compromised by changes in RPq settings within the range tested (0.52 – 0.78 for Se).

Appendix A: Critical parameter test results (continued)

Critical parameter test #5: This test evaluated the significance of the Axial Field Voltage (AFT) while analyzing blood samples for whole blood metals. The Axial Field Voltage may vary on each instrument. The Axial Field Voltage was increased and decreased by 20%. The results are presented in Table 7.

Test details:
1. Prepare a set of dilutions (calibrators, blanks, reference materials, fake samples) for analysis in triplicate (three separate sets of tubes).
2. Analyze them in three separate runs on the same day, same instrument.
3. Change the AFV value +/- 100 V.

Table 7. Ruggedness testing results: Evaluating the significance of Axial Field Voltage on sample stability. Test performed on December 20, 2010 by Deanna Jones. Results below are the average of the beginning and ending QC results for each analytical run.

ID	axial field voltage	Hg (µg /L)	Pb (µg /dL)	Cd (µg /L)	Mn (µg /L)	Se (µg /L)
LB08707_ WB2	Target Mean and 2SD Range	0.585 0.407 – 0.763	2.12 2.03 – 2.21	0.488 0.398 – 0.578	7.98 6.91 – 9.05	
	(optimized - 100V)	0.511	2.00	40.415	7.77	
	(optimized)	0.461	2.04	0.394	6.36	
	(optimized + 100V)	0.414	2.01	0.376	6.95	
HB08708_ WB2	Target Mean and 2SD Range	6.19 5.89 – 6.48	10.1 9.84 – 10.3	3.14 2.94 – 3.34	14.9 13.5 – 16.4	
	(optimized - 100V)	5.50	9.8	2.91	14.3	
	(optimized)	5.62	9.8	2.84	12.0	
	(optimized + 100V)	5.75	10.1	2.99	12.8	
QMEQAS 07B-09*	Target Mean and 2SD Range					157 146 – 168
	(optimized - 100V)					139
	(optimized)					147
	(optimized + 100V)					138
QMEQAS0 9B-08*	Target Mean and 2SD Range					548 511 - 585
	(optimized - 100V)					501
	(optimized)					556
	(optimized + 100V)					532

*samples purchase from Le centre de toxicology du Quebec (Quebec, Canada)

Conclusion: Accuracy of Mn, Hg and Se results are not compromised by changes in AFV settings within the range tested (optimized setting +/- 100V).

Appendix A: Critical parameter test results (continued)

Parameter test #6: Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibration standards.

Test details:

1. A large blood sample was spiked to elevated concentrations, and mixed well. The spiked sample was then prepared for analysis at various extra dilution levels and the observed results compared to results obtained with no extra dilution performed.

normalized concentration ± 1RSD

Dilution level	Mn	Hg	Se	Cd	Pb
No Extra (N=8)	1.00	1.00	1.00	1.00	1.00
2x dilution (N=8)	1.00 ± 0.01	1.03 ± 0.05	1.02 ± 0.03	1.00 ± 0.01	1.01 ± 0.01
5x dilution (N=6)	1.01 ± 0.01	1.06 ± 0.06	1.01 ± 0.02	1.01 ± 0.01	1.02 ± 0.01
10x dilution (N=8)	1.01 ± 0.03	1.04 ± 0.06	1.04 ± 0.06	1.00 ± 0.02	1.02 ± 0.02
20x dilution (N=8)	1.02 ± 0.04	1.09 ± 0.05	1.06 ± 0.08	1.01 ± 0.03	1.02 ± 0.02

Conclusion: Results show that all analytes of the method (Pb, Cd, Hg, Mn, and Se) can be analyzed at up to a 20x extra dilution without significant effect (> ± 10% error) to the observed concentration.

Appendix B

Table 1. Instrument and method parameters.	
Instrument: PerkinElmer ELAN DRC II ICP-MS ESI SC4 autosampler with (optional) PC3 Peltier cooled spray chamber	
Optimization window parameters	
RF power	1450 W
Plasma Gas Flow (Ar)	15 L/min
Auxiliary Gas Flow (Ar)	1.2 L/min
Nebulizer Gas Flow (Ar)	~0.90 – 1.0 L/min (optimized as needed for sensitivity)
Ion Lens Voltage(s)	AutoLens (optimized as needed for sensitivity)
AFV, QRO, CRO, CPV, Discriminator Threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of x-y alignment, nebulizer gas flow, AutoLens voltages, mass calibration, dual detector calibration and detector voltages are optimized regularly. Optimization file name = default.dac.	
Configurations window parameters	
cell gas changes pause times	Pressurize Delay (From Standard to DRC mode) = 60 Exhaust Delay (From DRC to Standard mode) = 30 Flow Delay (Gas changes while in DRC mode) = 30 Channel Delay (Gas channel change in DRC mode) = 30
File names & directories	
method file names	*calibration curve (programmed for blood blank)* CDC_DLS3016_bldblk.mth *For QC & patient sample analysis (programmed for aqueous blank)* CDC_DLS3016_aqblk.mth
dataset	Create a new dataset subfolder each day. Name as “2011-0820” for all work done on August 20, 2011
sample file	Create for each day’s work
report file name	*For sample results printouts* cdc_quant comprehensive.rop *For calibration curve information* CDC_Quant Comprehensive (calib curve info).rop
tuning	Default.tun
optimization	Default.dac
calibration	N/A
polyatomic	elan.ply
report options template (transferring results to the database)	CDC_Database Output.rop *Report Format Options: select only “Use Separator”* *File Write Option: Append* *Report File name: make descriptive including date (e.g. 2005-0311b_DRC2A_HM-0364.txt)*

Table 1. Instrument and method parameters.	
Method Parameters	
Method Parameters: Timing Page (see Figures 1a, 2a and 2d in Appendix B)	
sweeps/reading	30
readings/replicate	1
replicates	3
enable qc checking	On
isotopes monitored and internal standard associations (exact mass)	use ^{103}Rh, ^{130}Te, ^{193}Ir as internal standards ^{103}Rh (102.905): ^{55}Mn (54.93805) ^{130}Te(129.907): ^{202}Hg (201.971), ^{80}Se(79.9165) 193Ir(192.963):^{208}Pb(207.977), ^{114}Cd(113.904)
dwell times	100 ms for ^{55}Mn,^{202}Hg, ^{80}Se, ^{208}Pb, and ^{114}Cd 50 ms for ^{130}Te,^{103}Rh, and ^{193}Ir
scan mode	Peak Hopping for all isotopes (1 MCA channel)
DRC channel A gas flow rate	99.999% methane (5-7 psig delivery pressure) typically 0.84 L/min (0.7 – 1.0) * *optimized per instrument, and periodically verified
DRC channel B gas flow rate	99.99% oxygen (5-7 psig delivery pressure) typically 1.2 L/min (0.96 – 1.44) * *optimized per instrument, and periodically verified
RPa	0 for all isotopes
RPq	Typically* 0.6 (0.48 – 0.72) for ^{103}Rh,^{55}Mn,^{130}Te, and ^{202}Hg. 0.65 (0.52 – 0.78) for ^{130}Te and ^{80}Se. 0.25 for ^{193}Ir, ^{208}Pb, and ^{114}Cd Use the same RPQ for each analyte and its IS. (* Optimize per instrument, and periodically verified)
Method parameters: processing page (see Figures 1b in Appendix B)	
detector mode	Dual
process spectral peak	N/A
autolens	On
isotope ratio mode	Off
enable short settling time	Off
blank subtraction	After internal standard
measurement units	cps
process signal profile	N/A
Method parameters: equations page (see Figure 1c in Appendix B)	
equations	+Hg 200 -0.027250 * Sn118 +Pb 206 +Pb 207

Table 1. Instrument and method parameters.	
Method parameters: calibration page (see Figures 1d in Appendix B)	
calibration type	external std.
curve type	weighted linear
sample units	"μg/L" or "ppb"
calibrator concentrations (μg/L)	Mn (μg /L): 1.5, 4.5, 10.5, 15, 30, 75, 225, 600 Cd and Hg (μg /L): 0.5, 1.5, 3.5, 5, 10, 25, 75, 200 Pb (μg /dL): 1, 3, 7, 10, 20, 50, 150, 400 Se (μg /L): 30, 90, 210, 300, 600, 1500, 4500, 12000
Method parameters: sampling page (see Figures 1e and 1f in Appendix B)	
"peristaltic pump under computer control"	On
autosampler tray port sampling device	***If using ESI autosampler*** Autosampler Type: AS-93plus Tray Name: esi.try Sampling Device: None If using other autosampler, refer to user guide.
sample flush	default is 4s at 1.5 rpm (~160 uL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to adequately fill the FAST loop. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). As a matter of lab practice, set this time to equal the loop fill time in the ESI FAST program. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
read delay	60s at 1.5 rpm (~160 uL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized as needed to reach signal stability before beginning analysis. Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate). As a matter of lab practice, set this time equal to the total time required for the signal to reach stability minus the loop fill time. As long as the combined time of sample flush + read delay is equal to the time required for signal to reach stability, analytical measurement will be good.
wash	30s at 1.5 rpm (~160 uL/min, ESI DXi peristaltic pump, FAST sample introduction system) Time can be optimized to allow for changes in FAST loop rinsing (must be greater than total time of steps in FAST program after the initial "on rinse" command). Time and rpm can be optimized as needed to using a different style peristaltic pump (maintaining approximate liquid flow rate).

Table 1. Instrument and method parameters.

extra wash (via ICP-MS software QC checking)	For sample concentrations greater than these, setup the ICP-MS software's 'QC checking' feature to "Wash for X and continue" **Analyte** **Concentration** **Extra Rinse Time** Cd 200 μg/L 200s Hg 200 μg/L 200s Mn 600 μg/L 200s Pb 400 μg/dL 200s Se 1200 μg/L 200s
autosampler locations of blanks and standards	*For calibration curve (points to blood blank)* CDC_DLS3016_bldblk.mth Calibration Stds 0 – 8 in autosampler positions 105 – 113 by default, but can be customized. *For QC & patient sample analysis (points to aqueous blank)* CDC_DLS3016_aqblk.mth Aqueous Blank in autosampler position 117 by default, but can be customized.
FAST parameters: See Figures 4a through 4h in Appendix B for details	
configuration file	default.sc (saved at C:\Program Files\ESI\ESI-SC\)
FAST program	cdc_dls3016_5element_loop1ml_scfast.txt
Potential Emergency Response Modifications:	
mercury:	Analyze mercury in standard mode with tellurium as the internal standard. Set dwell time to 100ms, DRC gas flow to 0, and RPq to 0.25.
Non-FAST sample introduction system:	If the FAST sample introduction system is not available on any instruments, the method can still be implemented, but these changes will need to be made in the ELAN (and ESI software if present). Peristaltic pump speeds are for DXi pump; adjust accordingly if another pump is installed. • sample flush: Default is ~30s at -16 rpm. Set so that solution reaches nebulizer. • read delay: Default is 45s at -5 rpm. Set for best reproducibility of replicate measured intensities. • wash: Default is 60s at -11rpm. Set to prevent significant carry-over from one sample to the next. • If using ESI autosampler without FAST, disable FAST in the ESI software before running analysis.

Appendix B (continued)

Table 2. Suggested concentrations for base blood	
analyte (units)	**suggested concentration**
Cd (µg/L)	<0.5
Hg (µg/L)	<0.5
Mn (µg/L)	< 8
Pb (µg/dL)	<2
Se (µg/L)	<200

Table 3. Stock calibration standard concentrations	
Analyte	**Stock calibration concentration (mg/L)** High Purity Standards Item # SM-2107-042 10% v/v HCl
Cd	50
Hg	50
Mn	150
Pb	1000
Se	3000

Appendix B (continued)

Table 4. Preparation of intermediate stock calibration standard	
volume of flask (mL)	100
volume of spike of stock standard solution	2
	concentrations (mg **/**L**)**
Cd	1
Hg	1
Mn	3
Pb (mg /dL)	20
Se	60

Table 5. Preparation of intermediate working standards								
Standard #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
volume of flask (mL)	100	100	100	100	100	100	100	100
volume spike of stock std. (mL)						0.05	0.15	0.4
volume spike of int. stock Std. (mL)	0.05	0.15	0.35	0.50	1.00			
concentrations (µg **/**L**) ***								
Cd	0.5	1.5	3.5	5	10	25	75	200
Hg	0.5	1.5	3.5	5	10	25	75	200
Mn	1.5	4.5	10.5	15	30	75	225	600
Pb (µg /dL)	1	3	7	10	20	50	150	400
Se	30	90	210	300	600	1500	4500	12000

* These same concentrations are entered in the ICP-MS software's calibration page to describe the concentrations of the working calibrators (preparations analyzed during a run). This eliminates the need to multiply ICP-MS observed results by a dilution factor except for the case of extra dilutions (see Table 8).

Appendix B (continued)

Table 6. Acceptable ways to perform two consecutive analytical runs, bracketing with bench quality control samples.

setup 1	**setup 2**
Run #1 calibration standards low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC *Run #2* low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC	*Run #1* calibration standards low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC *Run #2* *calibration standards* low bench QC high bench QC elevated bench QC patient samples low bench QC high bench QC elevated bench QC

Appendix B (continued)

Table 7. A typical SAMPLE/BATCH window.

AS Location*	Sample ID	Measurements Action	Method
233	DRCstability1	Run sample	…DLS3016_bldblk.mth
233	DRCstability2	Run sample	…DLS3016_bldblk.mth
233	DRCstability3	Run sample	…DLS3016_bldblk.mth
233	DRCstability4	Run sample	…DLS3016_bldblk.mth
Continue DRC stability samples . . .			
233	DRCstability9	Run sample	…DLS3016_bldblk.mth
233	DRCstability10	Run sample	…DLS3016_bldblk.mth
114	3016 BldBlkChk1	Run blank, standards, and sample **	…DLS3016_bldblk.mth
115	3016 BldBlkChk2	Run sample	…DLS3016_bldblk.mth
116	3016 AQBLK	Run blank and sample ¥	…DLS3016_aqblk.mth
125	L Bench QC	Run sample	…DLS3016_aqblk.mth
126	H Bench QC	Run sample	…DLS3016_aqblk.mth
127	E Bench QC	Run sample	…DLS3016_aqblk.mth
137	Sample 1	Run sample	…DLS3016_aqblk.mth
138	Sample 2	Run sample	…DLS3016_aqblk.mth
125	L Bench QC	Run sample	…DLS3016_aqblk.mth
126	H Bench QC	Run sample	…DLS3016_aqblk.mth
127	E Bench QC	Run sample	…DLS3016_aqblk.mth

* The exact autosampler positions of QCs and patient samples do not have to be those shown above. QC samples do not have to be run in the order of low, then high, then elevated.

** When executing this row, the ELAN will first analyze the standard 0 (blood blank) at AS position 105, then standards 1-8 at autosampler positions 106-113, then the “3016 BldBlkChk1” sample at A/S position 114. The sampling information about AS positions 105-113 are stored in the “bldblk” method file.

¥ When executing this row, the ELAN will first analyze the aqueous blank at AS position 117, then the “Aq blank ” at AS position 103. The sampling information about AS positions 117 is stored in the “aqblk” method file.

Appendix B (continued)

Table 8. Preparation of samples, working calibrators, and QC materials for analysis *

If a different total volume is prepared, adjust the volumes for each component proportionally.

* These directions are written with the expectation of a 5,000 μL syringe on the left side and a 250 μL syringe on the right side of the benchtop automatic pipette.

Description	Water (μL)	Base Blood (μL)	AQ Intermediate Working Standard (μL)	Patient or QC blood sample (μL)	Diluent (μL)**
Working Calibrators (S0-S8) and Bldblkchk (S0)	-	50 x 1	50 x 1	-	2,400 (1,200 x 2)
AQ Blank	100 x 1	-	-	-	2,400 (1,200 x 2)
Patient blood or Blood-Based QC	50 x 1	-	-	50 x 1	2,400 (1,200 x 2)
Patient Blood *2x Extra Dilution* [H]	150 x 1	-	-	50 x 1	4,800 (2,400 x 2)
Patient Blood *5x Extra Dilution* [H]	450 (225 x 2)			50 x 1	12,000 (4,000 x 3)
Patient Blood *10x Extra Dilution* [H]	950 (190 x 5)			50 x 1	24,000 (4,000 x 6)
Patient Blood *20x Extra Dilution* [H]	1950 (195 x 10)			50 x 1	48,000 (4,000 x 12)

** By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely. For example, when preparing a working calibrator, do the preparation in two steps: in step 1, dispense 2400 μL diluent + 50 μL; in step 2, dispense 2400 μL diluent + 50 μL base blood to prepare a 2.5 mL total volume dilution.

[H] Extra dilution is performed on urine samples whose concentration is greater than the highest calibrator listed in the 'calibrator concentrations' section of Table 1 in the Appendix B.

Maximum extra dilution (see Appendix A, ruggedness test #6 for details)
20x for Cd, Hg, Mn, Pb, and Se

Any extra level of dilution up to 20x (see Appendix A, Experiment 6) can be prepared as long as the 4.8:5 ratio of diluent to total dilution volume is maintained. Use of the lowest possible dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into the documented linearity range).

Appendix B (continued)

Table 9. Boundary concentrations for whole blood concentrations

analyte (units)	1st upper boundary ("1UB") *	2nd upper boundary ("2UB") **	range maximum ("Lim Rep Delta") †	Highest Concentration Validated for Washout
Mn (μg/L)	20	35	2.0	600
Pb (μg/dL)	5.0	5.0	1.0	400
Cd (μg/L)	5.0	5.0	1.0	200
Hg (μg/L)	10.0	10.0	1.0	200
Se (μg/L)	400	400	20	12,000

* Typically, the 1st upper boundary (1UB) is the 99th percentile of non-weighted concentration results from the NHANES 1999-2000 subset groups, a concentration significant to public health, or a concentration defined by study protocol. The default 1UB concentrations are listed in this table.

** The 2nd upper boundary (2UB) may be 2x the 1UB, a concentration significant to public health, or defined by study protocol.

† Range maximum (Lim Rep Delta) is the allowed limit to the range of the three replicate readings for a single sample analysis.

Appendix B (continued)

Table 10. Reference ranges for blood concentrations [72].

analyte (units)	survey years	geometric mean	50th	75th	90th	95th	N
Cd (μg/L)	07-08	0.315	0.270	0.500	1.00	1.52	8266
	09-10	0.302	0.260	0.480	0.960	1.40	8793
	11-12	0.279	0.250	0.460	0.960	1.50	7920
Hg (μg/L)	07-08	0.769	0.740	1.48	2.95	4.64	8266
	09-10	0.863	0.790	1.68	3.43	5.13	8793
	11-12	0.703	0.640	1.38	2.87	4.40	7920
Pb (μg/dL)	07-08	1.27	1.22	1.90	2.80	3.70	8266
	09-10	1.12	1.07	1.70	2.58	3.34	8793
	11-12	0.973	0.930	1.52	2.38	3.16	7920
Mn (μg/L)	11-12	9.35	9.22	11.5	14.4	16.7	7920
Se (μg/L)	11-12	190	190	206	223	236	7920

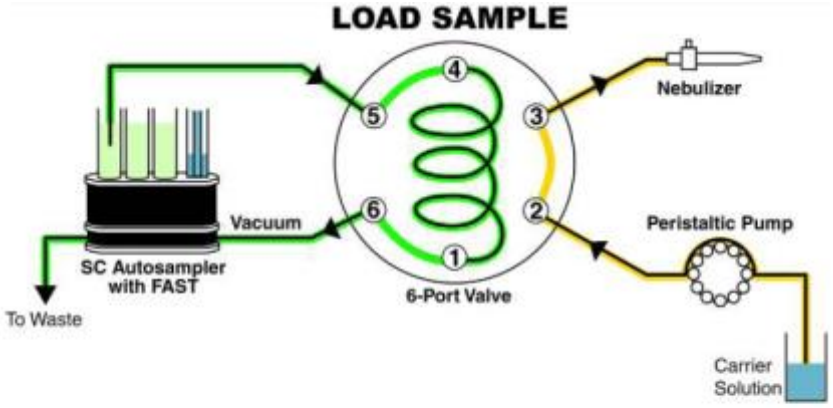
Table 11. Reference concentrations from published literature for blood Mn and Se.

analyte (units)	published concentrations
Se (μg/L)	157 – 265 μg/L [73]
Mn (μg /L)†	Non-exposed 4 – 14 (μg /L) [46]
	Exposed workers (adults) 3.2 – 101 μg /L [28]
	Children receiving long term parenteral nutrition 33.8 – 101 μg /L [74]
	Ohio adults (N=49) residing near a refinery (possible Mn emission): Mean (range) 9.4 (4.2-21.7) μg/L [30]
	Mexican infants Age 1, mean (SD) = 24.3 (4.5) μg/L, median = 23.7 μg/L, N=270 Age 2, mean (SD) = 21.1 (6.2) μg/L, median = 20.3 μg/L, N=430 [75]
	Japanese women (N = 1420) GM 13.2 μg/L overall, Range of median (max) across 8 regions 12.0-14.3 (25.0-33.4) μg/L [76]
	South African children, ages 8-10 years old (n = 49) Mean (SD) 8.48 (2.45) μg/L, range 4.58-18.20 μg/L. [34]

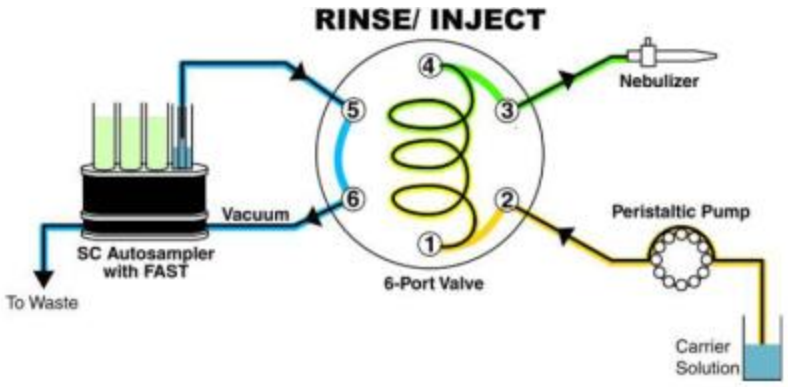
Appendix B (continued)

Figure 1. Configuration of tubing and devices for liquid handling using FAST sample introduction.

Below shows the correct connections to the 6-port FAST valve. The two diagrams show the differences in liquid flow directions when the valve changes from “Load” to “Inject” This change is internal to the valve. The shift of the valve cannot be seen, but it can be heard, and felt (with hand on the valve). The light indicators on the actuator body also indicate the valve position.



Teflon vacuum pump loads sample into loop while carrier solution is nebulized

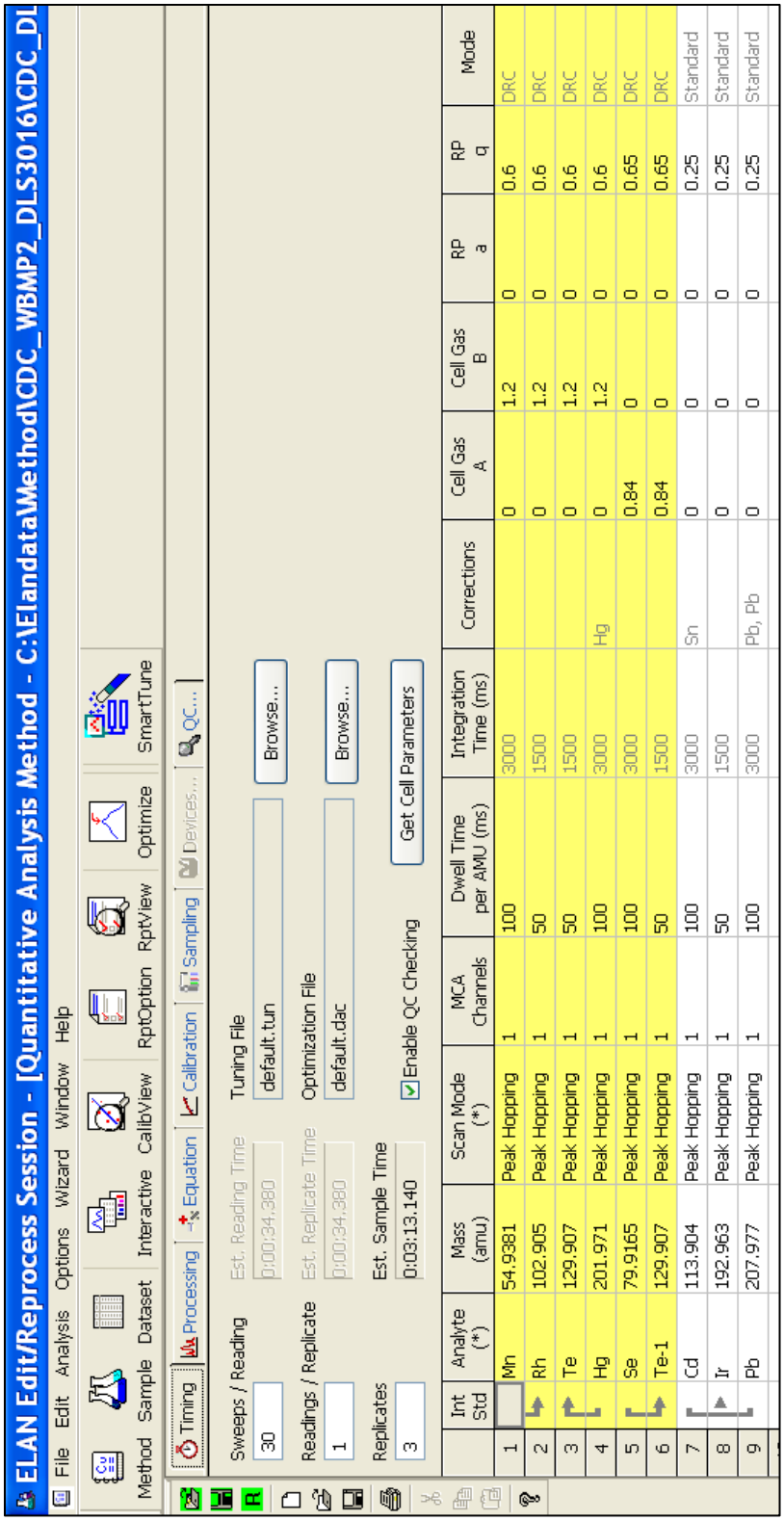


Carrier solution pushes sample into nebulizer at the same time sample line is rinsed

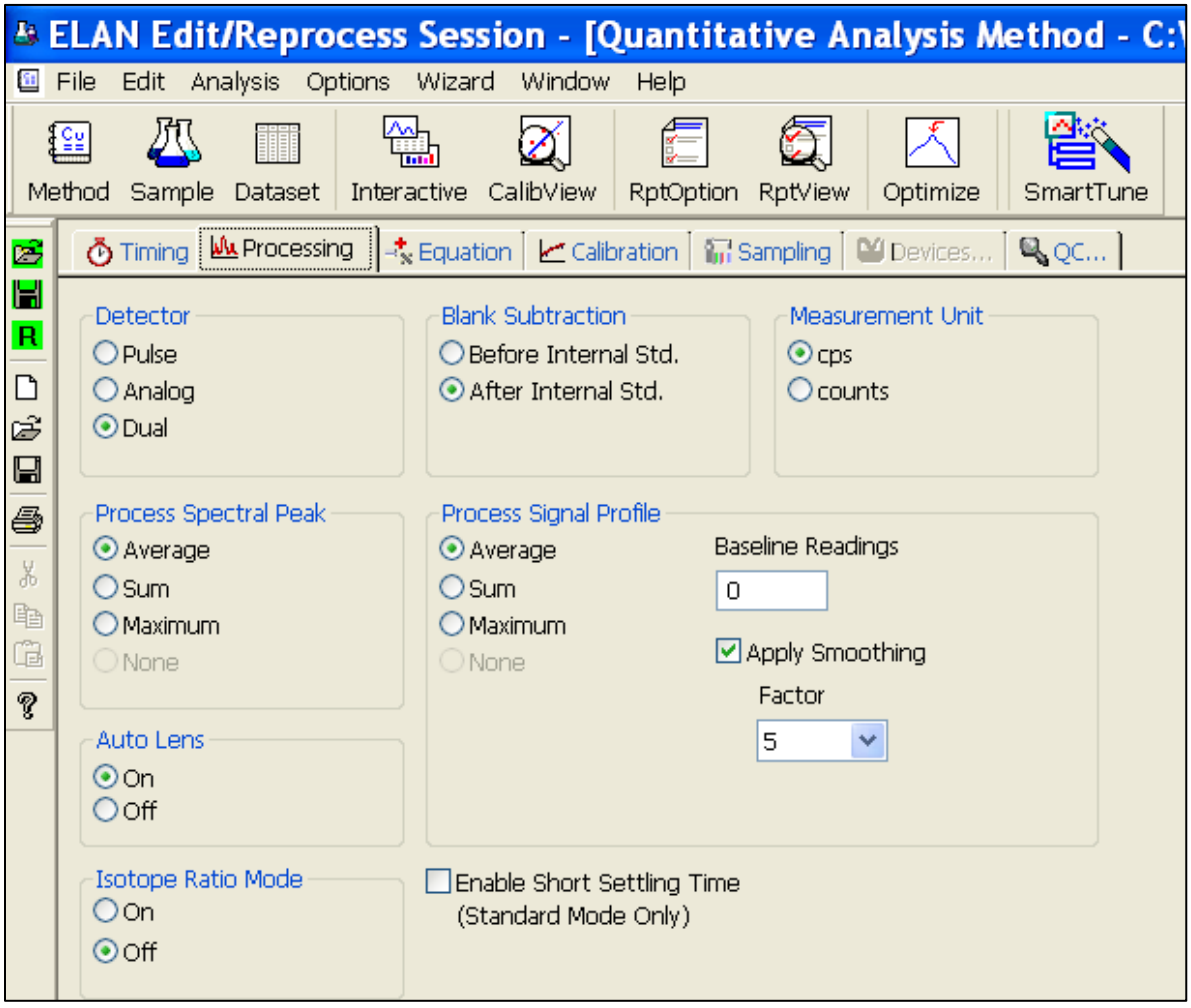
The connections to the valve are color-coded (see Section 7.a.i).

Enable the FAST program in the ESI software before running the method, but optimizations can be done in either FAST or non-FAST mode.

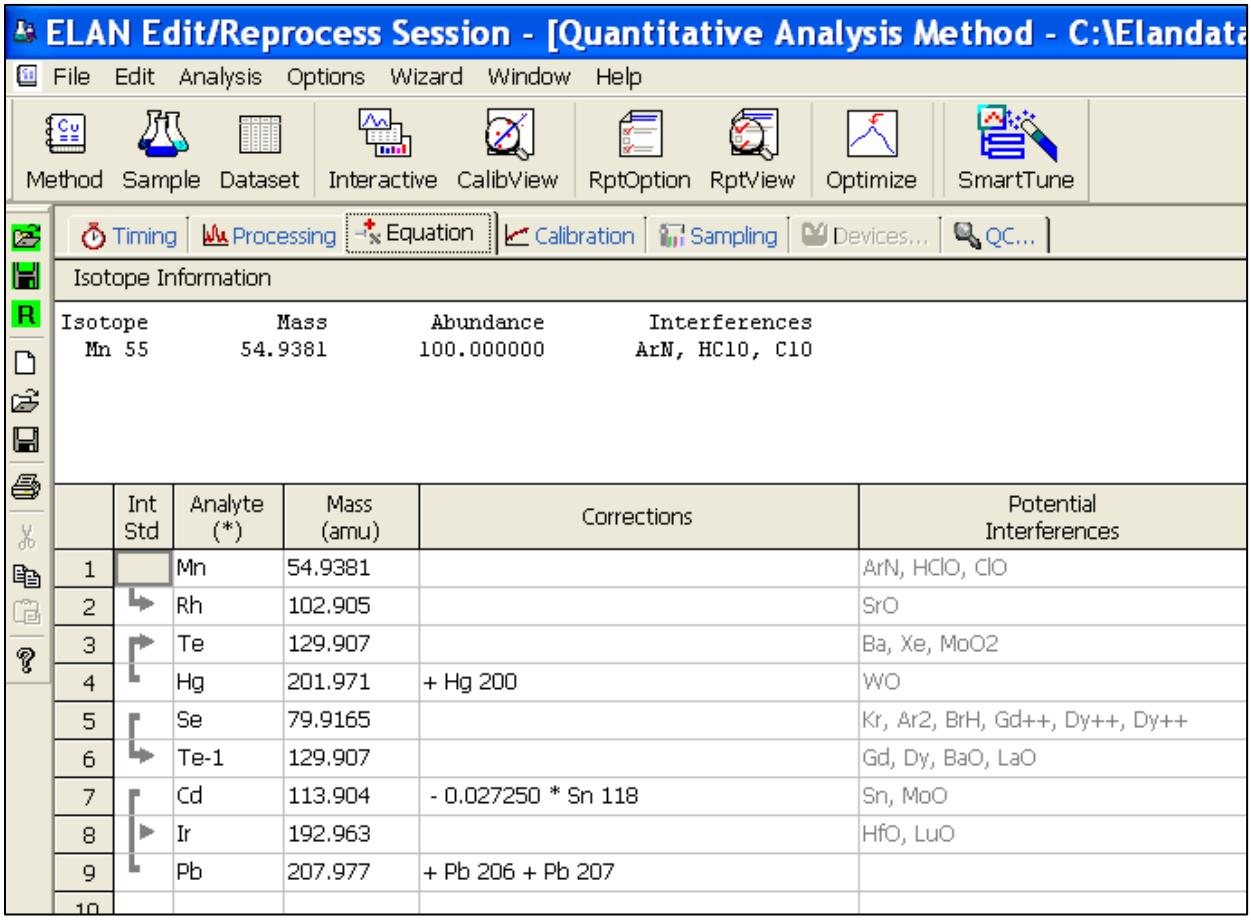
Appendix B (continued)
Figure 2a. ELAN ICP-MS method screen shots (timing page).



Appendix B (continued)
Figure 2b. ELAN ICP-MS method screen shots (processing page).

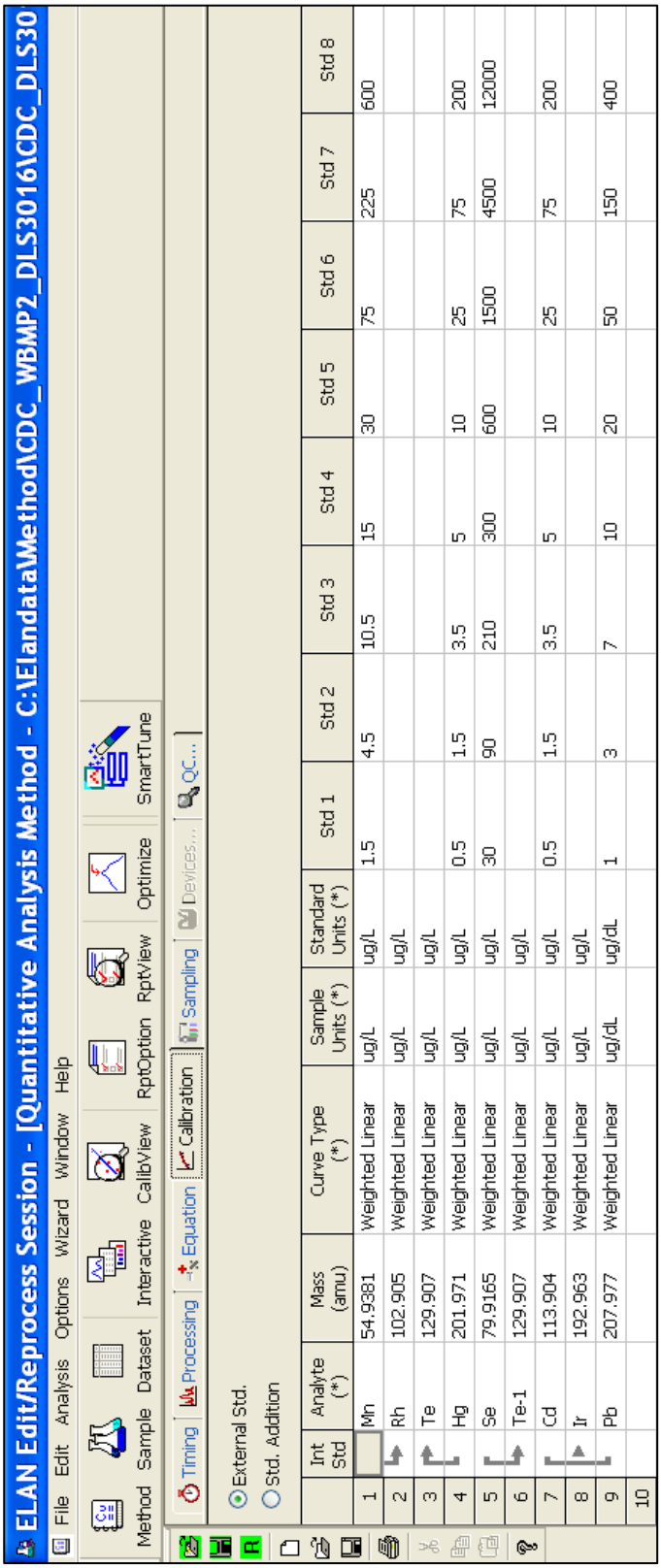


Appendix B (continued)
Figure 2c. ELAN ICP-MS method screen shots (equation page).



	Int Std	Analyte (*)	Mass (amu)	Corrections	Potential Interferences
1		Mn	54.9381		ArN, HClO, ClO
2		Rh	102.905		SrO
3		Te	129.907		Ba, Xe, MoO2
4		Hg	201.971	+ Hg 200	WO
5		Se	79.9165		Kr, Ar2, BrH, Gd++, Dy++, Dy++
6		Te-1	129.907		Gd, Dy, BaO, LaO
7		Cd	113.904	- 0.027250 * Sn 118	Sn, MoO
8		Ir	192.963		HfO, LuO
9		Pb	207.977	+ Pb 206 + Pb 207	
10					

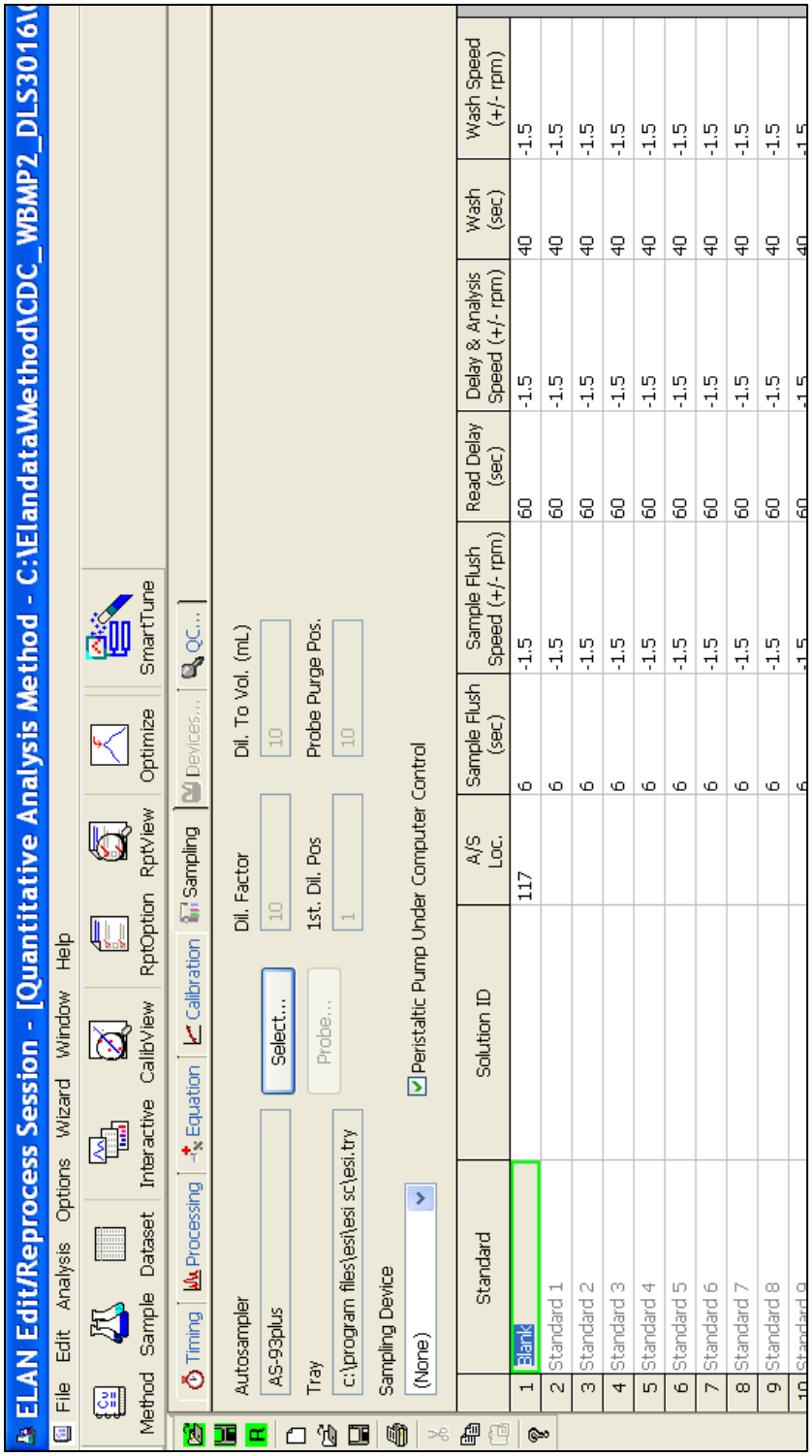
Appendix B (continued)
Figure 2d. ELAN ICP-MS method screen shots (calibration page).



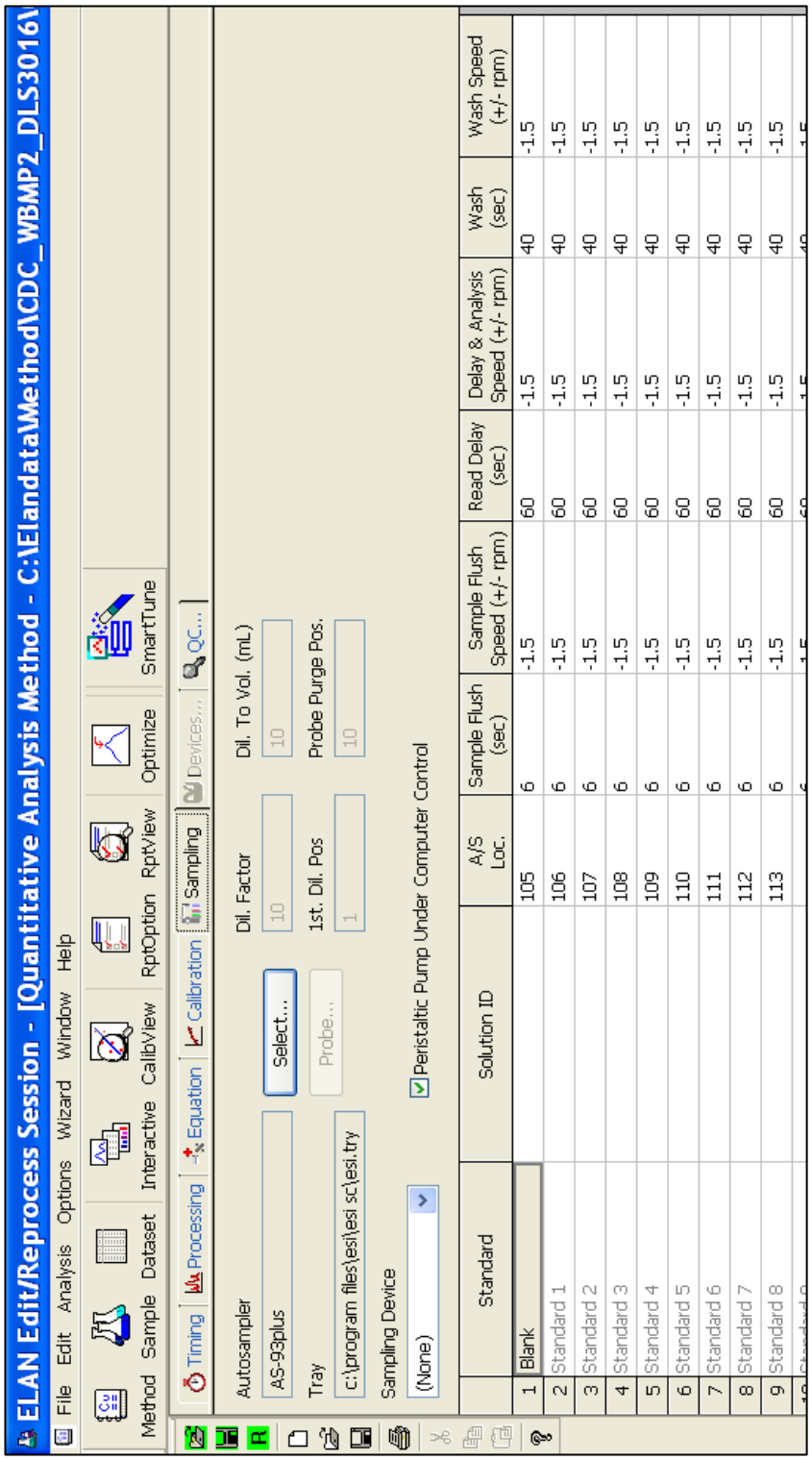
	Int Std	Analyte (*)	Mass (amu)	Curve Type (*)	Sample Units (*)	Standard Units (*)	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
1		Mn	54.9381	Weighted Linear	ug/L	ug/L	1.5	4.5	10.5	15	30	75	225	600
2		Rh	102.905	Weighted Linear	ug/L	ug/L								
3		Te	129.907	Weighted Linear	ug/L	ug/L								
4		Hg	201.971	Weighted Linear	ug/L	ug/L	0.5	1.5	3.5	5	10	25	75	200
5		Se	79.9165	Weighted Linear	ug/L	ug/L	30	90	210	300	600	1500	4500	12000
6		Te-1	129.907	Weighted Linear	ug/L	ug/L								
7		Cd	113.904	Weighted Linear	ug/L	ug/L	0.5	1.5	3.5	5	10	25	75	200
8		Ir	192.963	Weighted Linear	ug/L	ug/L								
9		Pb	207.977	Weighted Linear	ug/dL	ug/dL	1	3	7	10	20	50	150	400
10														

Appendix B (continued)

Figure 2e. ELAN ICP-MS method screen shots (sampling page, AqBlank method).

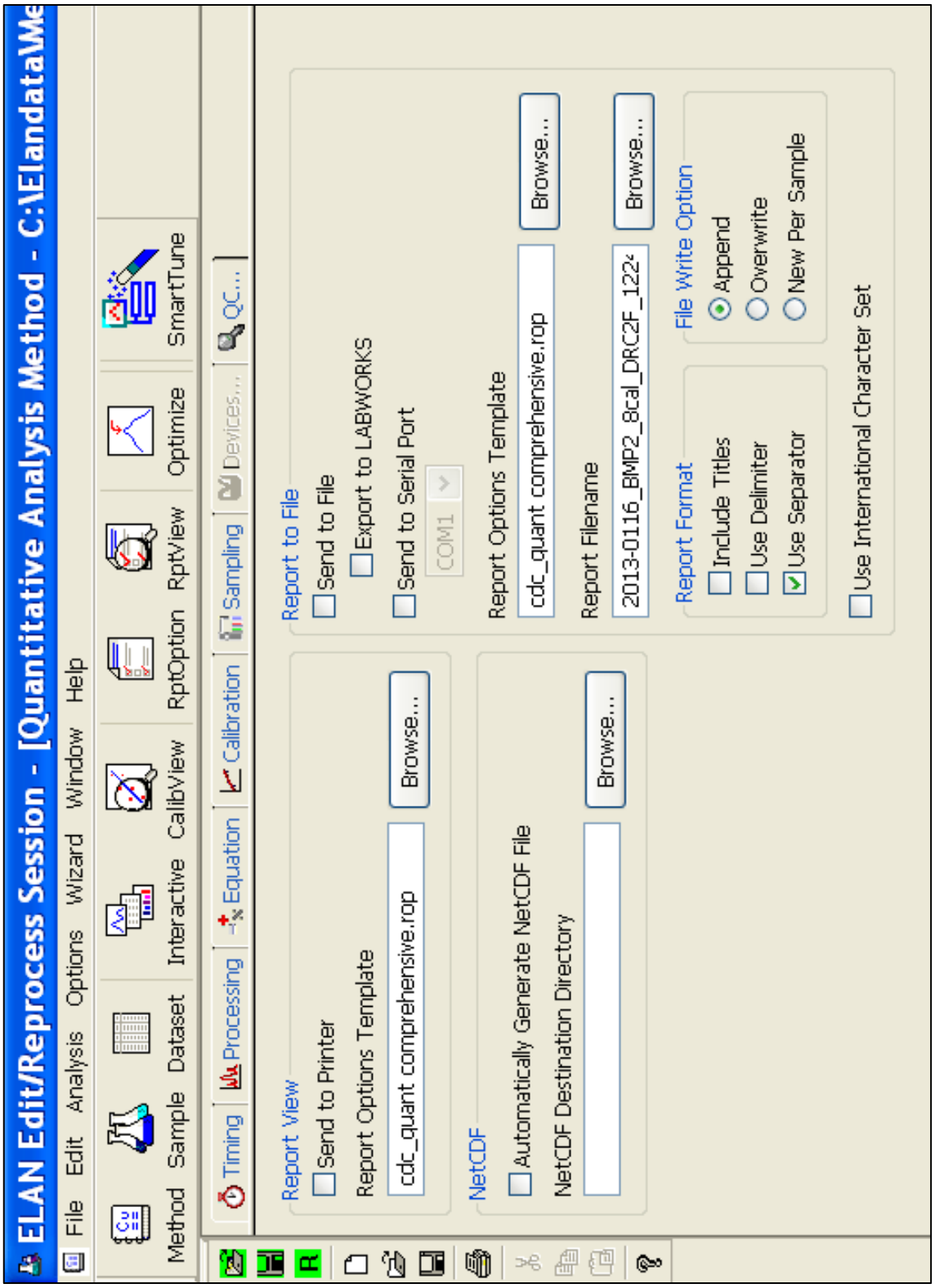


Appendix B (continued)
Figure 2f. ELAN ICP-MS method screen shots (sampling page, BldBlank method).

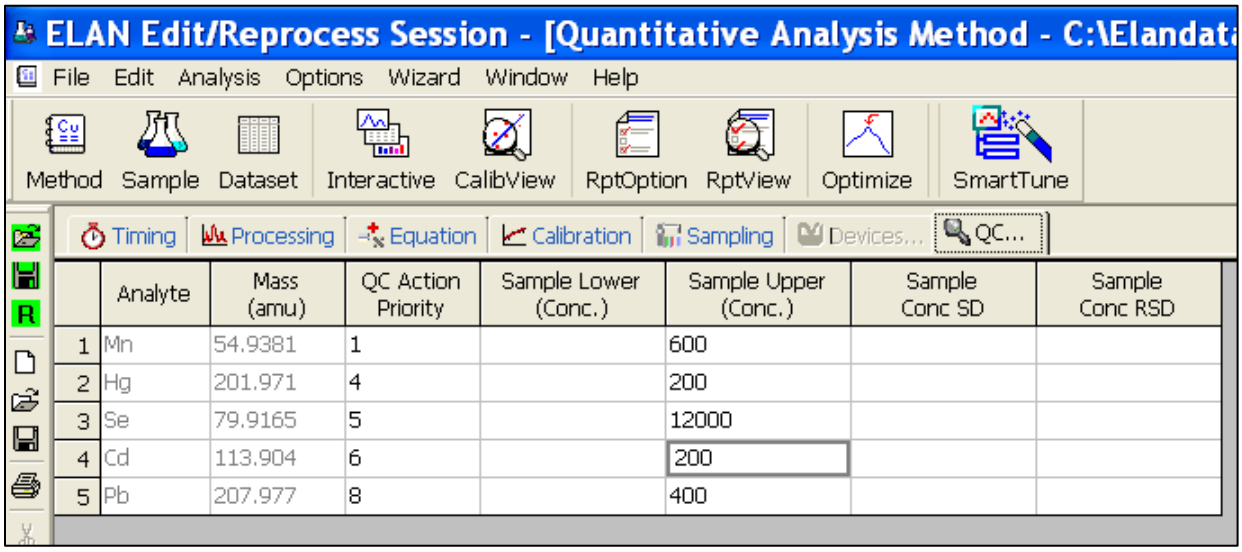


	Standard	Solution ID	A/S Loc.	Sample Flush (sec)	Sample Flush Speed (+/- rpm)	Read Delay (sec)	Delay & Analysis Speed (+/- rpm)	Wash (sec)	Wash Speed (+/- rpm)
1	Blank		105	6	-1.5	60	-1.5	40	-1.5
2	Standard 1		106	6	-1.5	60	-1.5	40	-1.5
3	Standard 2		107	6	-1.5	60	-1.5	40	-1.5
4	Standard 3		108	6	-1.5	60	-1.5	40	-1.5
5	Standard 4		109	6	-1.5	60	-1.5	40	-1.5
6	Standard 5		110	6	-1.5	60	-1.5	40	-1.5
7	Standard 6		111	6	-1.5	60	-1.5	40	-1.5
8	Standard 7		112	6	-1.5	60	-1.5	40	-1.5
9	Standard 8		113	6	-1.5	60	-1.5	40	-1.5

Appendix B (continued)
Figure 2g. ELAN ICP-MS method screen shots (report page).



Appendix B (continued)
Figure 2h. ELAN ICP-MS method screen shots (QC / Sample page).



	Analyte	Mass (amu)	QC Action Priority	Sample Lower (Conc.)	Sample Upper (Conc.)	Sample Conc SD	Sample Conc RSD
1	Mn	54.9381	1		600		
2	Hg	201.971	4		200		
3	Se	79.9165	5		12000		
4	Cd	113.904	6		200		
5	Pb	207.977	8		400		

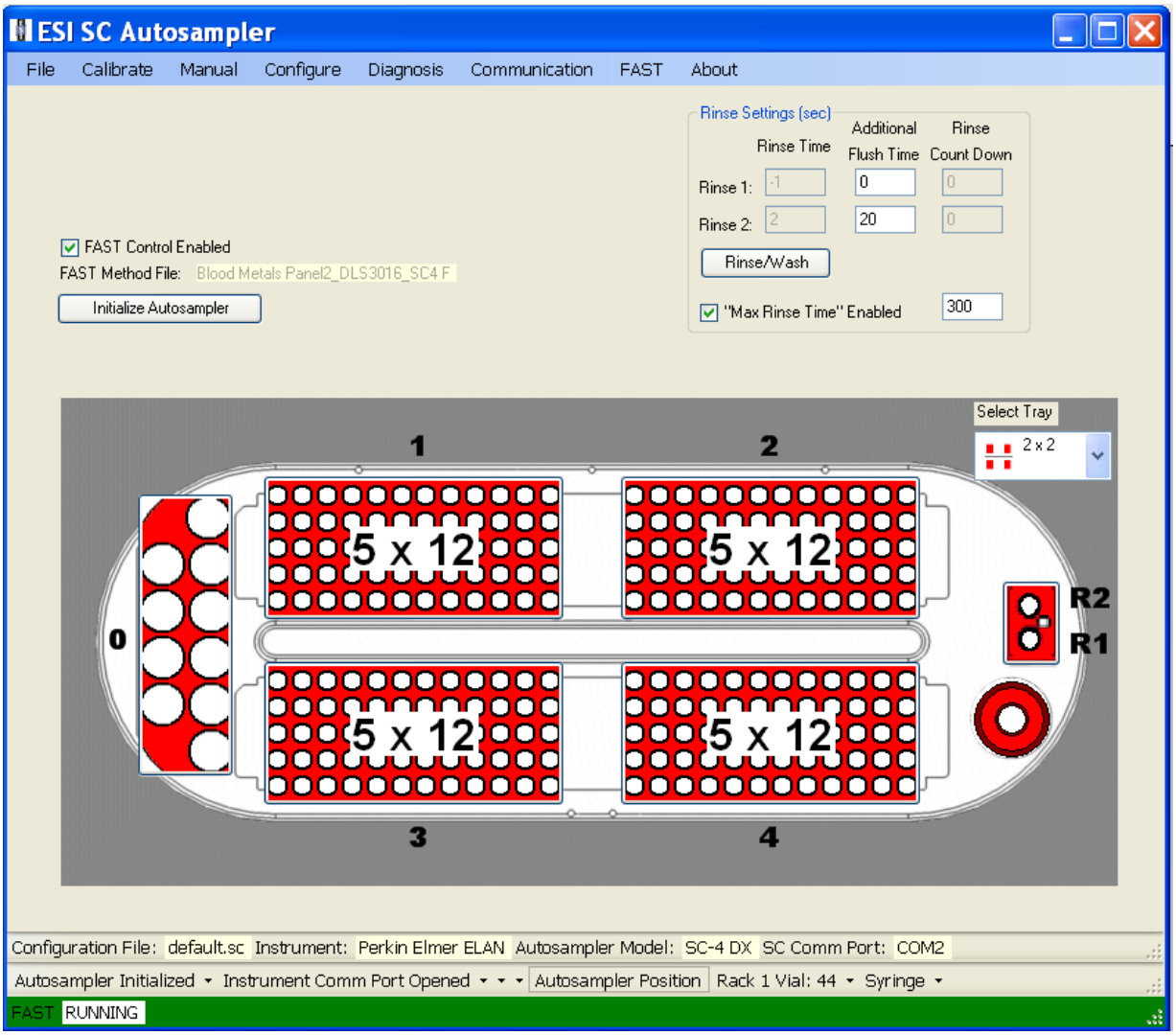
	Measurement	Action 1 (*)	Action 1 Data	Action 2 (*)	Action 2 Data
1	Mn 55 Lower	Continue		Continue	
2	Mn 55 Upper, S, EEE	Wash for X and Continue	200 seconds	Continue	
3	Mn 55 Std Dev	Continue		Continue	
4	Mn 55 RSD	Continue		Continue	
5	Hg 202 Lower	Continue		Continue	
6	Hg 202 Upper, S, EEE	Wash for X and Continue	200 seconds	Continue	
7	Hg 202 Std Dev	Continue		Continue	
8	Hg 202 RSD	Continue		Continue	
9	Se 80 Lower	Continue		Continue	
10	Se 80 Upper, S, EEE	Wash for X and Continue	200 seconds	Continue	
11	Se 80 Std Dev	Continue		Continue	
12	Se 80 RSD	Continue		Continue	
13	Cd 114 Lower	Continue		Continue	
14	Cd 114 Upper, S, EEE	Wash for X and Continue	200 seconds	Continue	
15	Cd 114 Std Dev	Continue		Continue	
16	Cd 114 RSD	Continue		Continue	
17	Pb 208 Lower	Continue		Continue	
18	Pb 208 Upper, S, EEE	Wash for X and Continue	200 seconds	Continue	
19	Pb 208 Std Dev	Continue		Continue	
20	Pb 208 RSD	Continue		Continue	

Appendix B (continued)

Figure 3a. ESI SC4 autosampler screen shots (main page). Additional flush times and "Max Rinse Time" are approximate. Optimize these for best reduction of elemental carry-over between samples. Tray types can be changed to allow for different volumes of diluted sample digests. 'FAST control' must be enabled before start of method, but does not need to be used in instrument optimization (pre-analysis) steps. Rinse and additional flush times for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution.

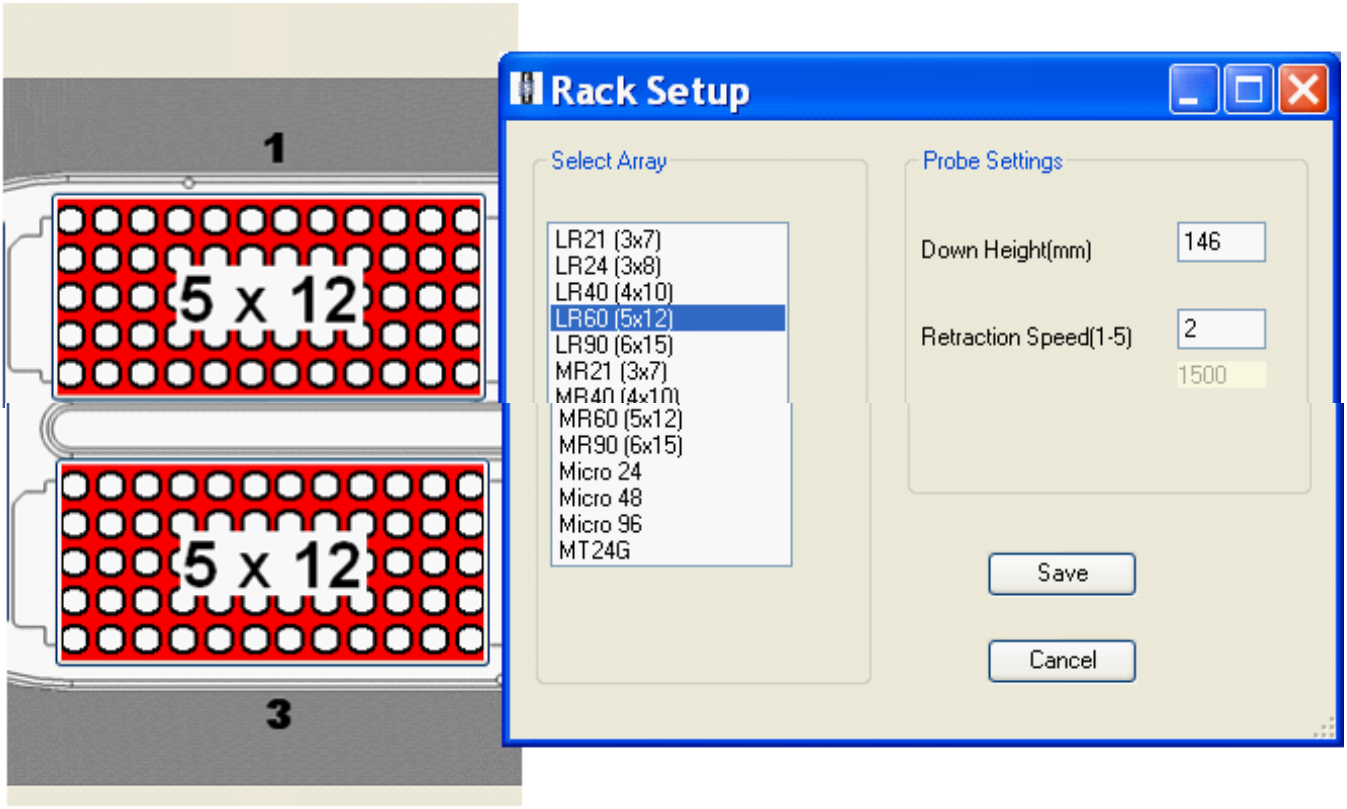
A rinse time of -1 causes the rinse station to be skipped.
A rinse time of 0 causes the probe to only dip into the station, but spends no time there.

Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made in the laboratory.



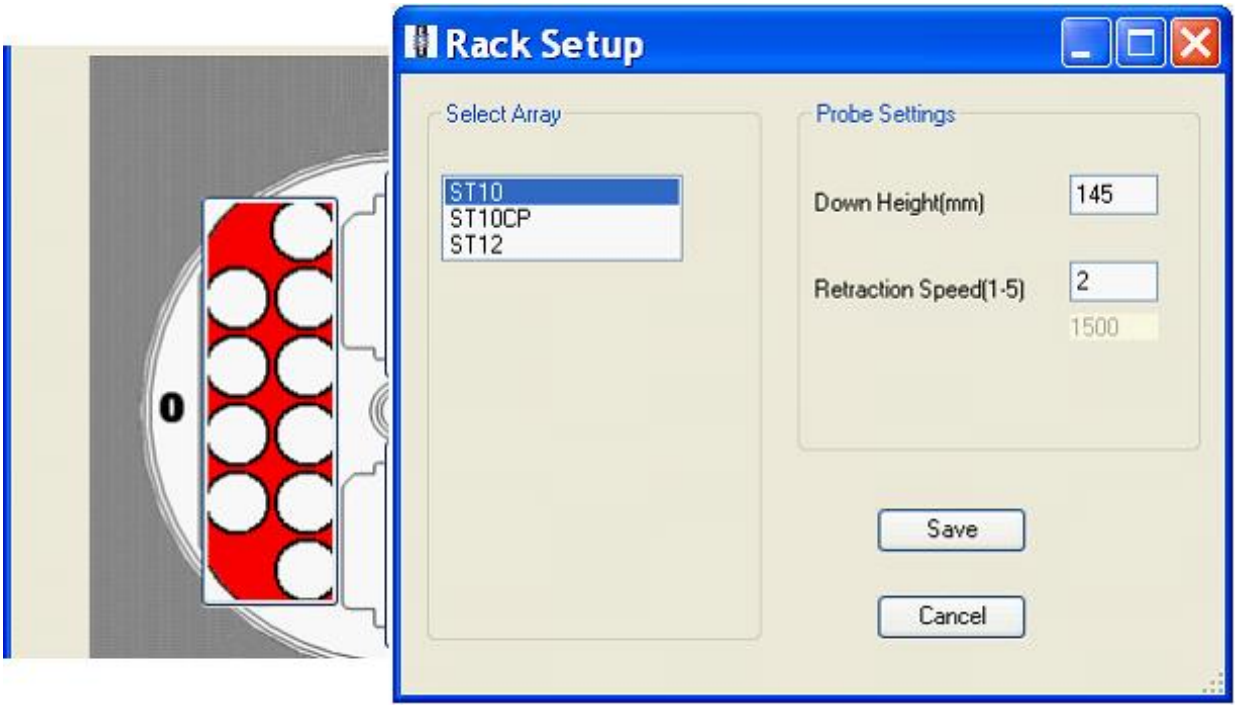
Appendix B (continued)

Figure 3b. ESI SC4 autosampler screen shots (5x12 rack setup window). Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.



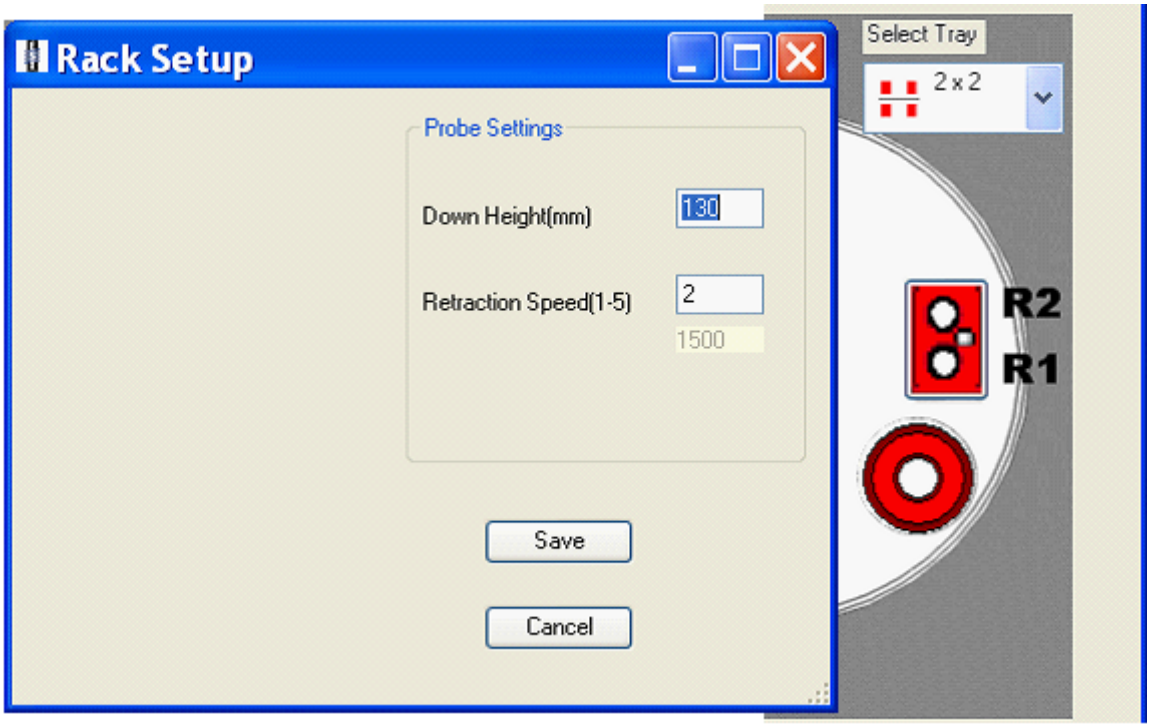
Appendix B (continued)

Figure 3c. ESI SC4 autosampler screen shots (50mL tube rack setup window). Settings are approximate. To be sure the loop is filled, set the probe to go close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.



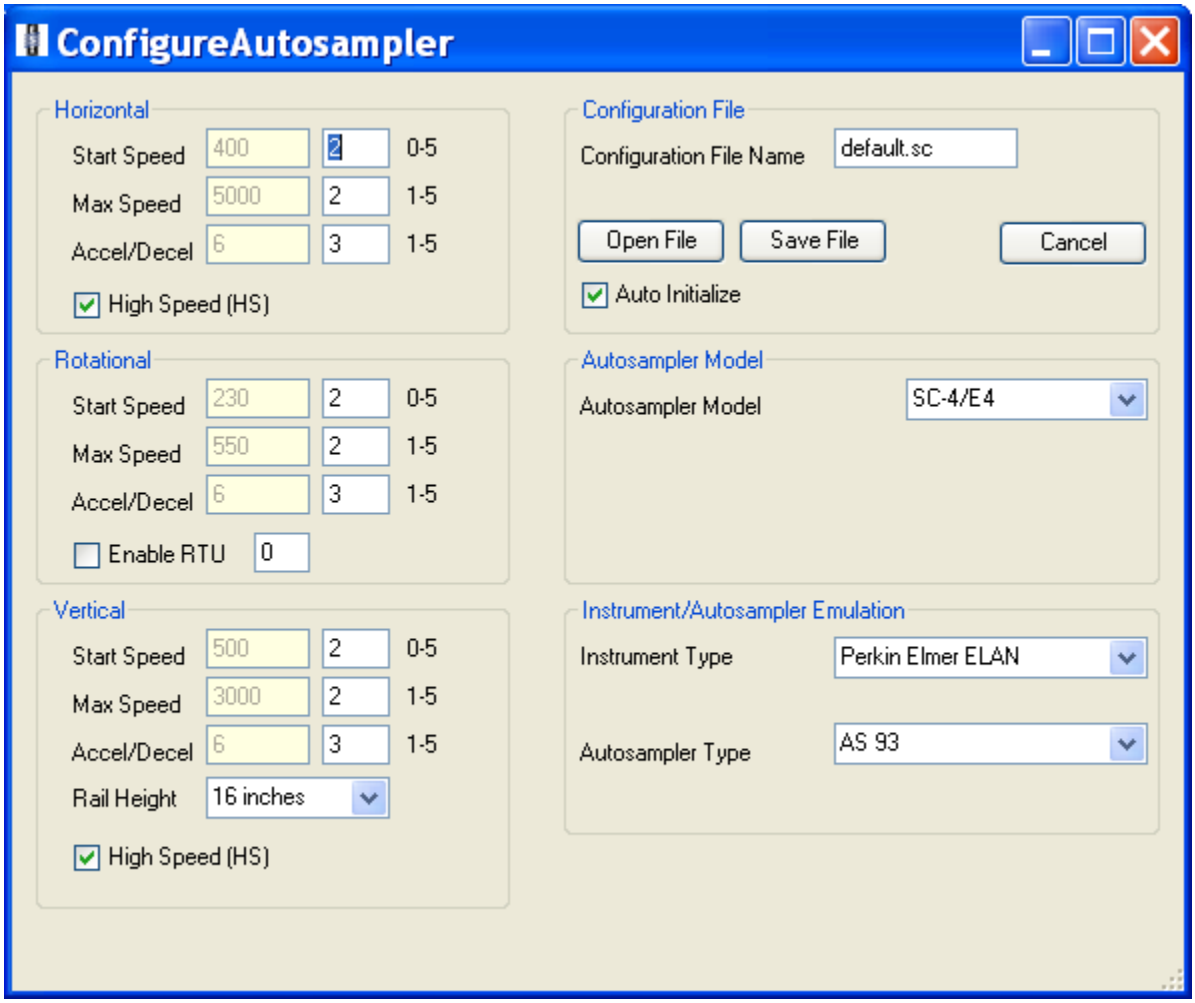
Appendix B (continued)

Figure 3d. ESI SC4 autosampler screen shots (rinse station rack setup window). Settings are approximate. Optimize down height for best probe cleaning, and retraction speed for least droplet splatter.



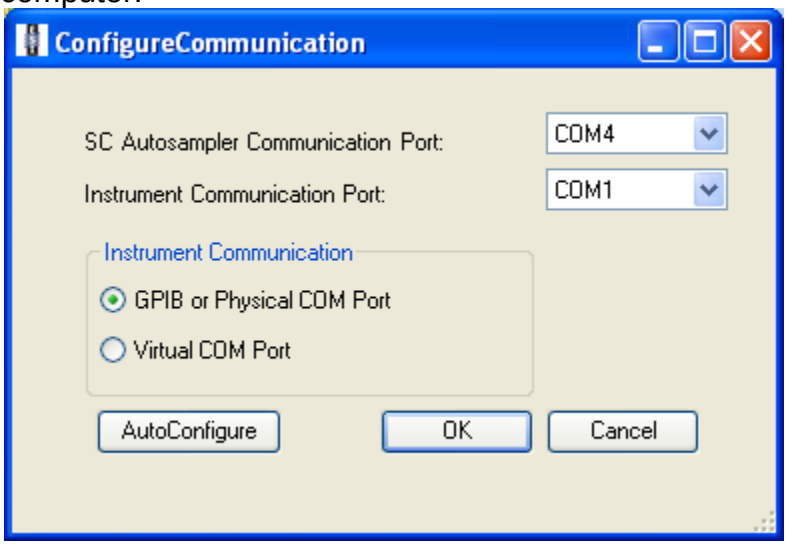
Appendix B (continued)

Figure 3e. ESI SC4 autosampler screen shots ("Configure" page). "High Speed" option is to only be used for 'High Speed' models of the SC4 (look for "HS" in serial number). Speeds and accel / decel values can be optimized per analyst preference and to minimize droplet splatter off of probe.



Appendix B (continued)

Figure 3f. ESI SC4 autosampler screen shots ("Communication" page).
Communication ports will differ depending on available ports on instrument control computer.

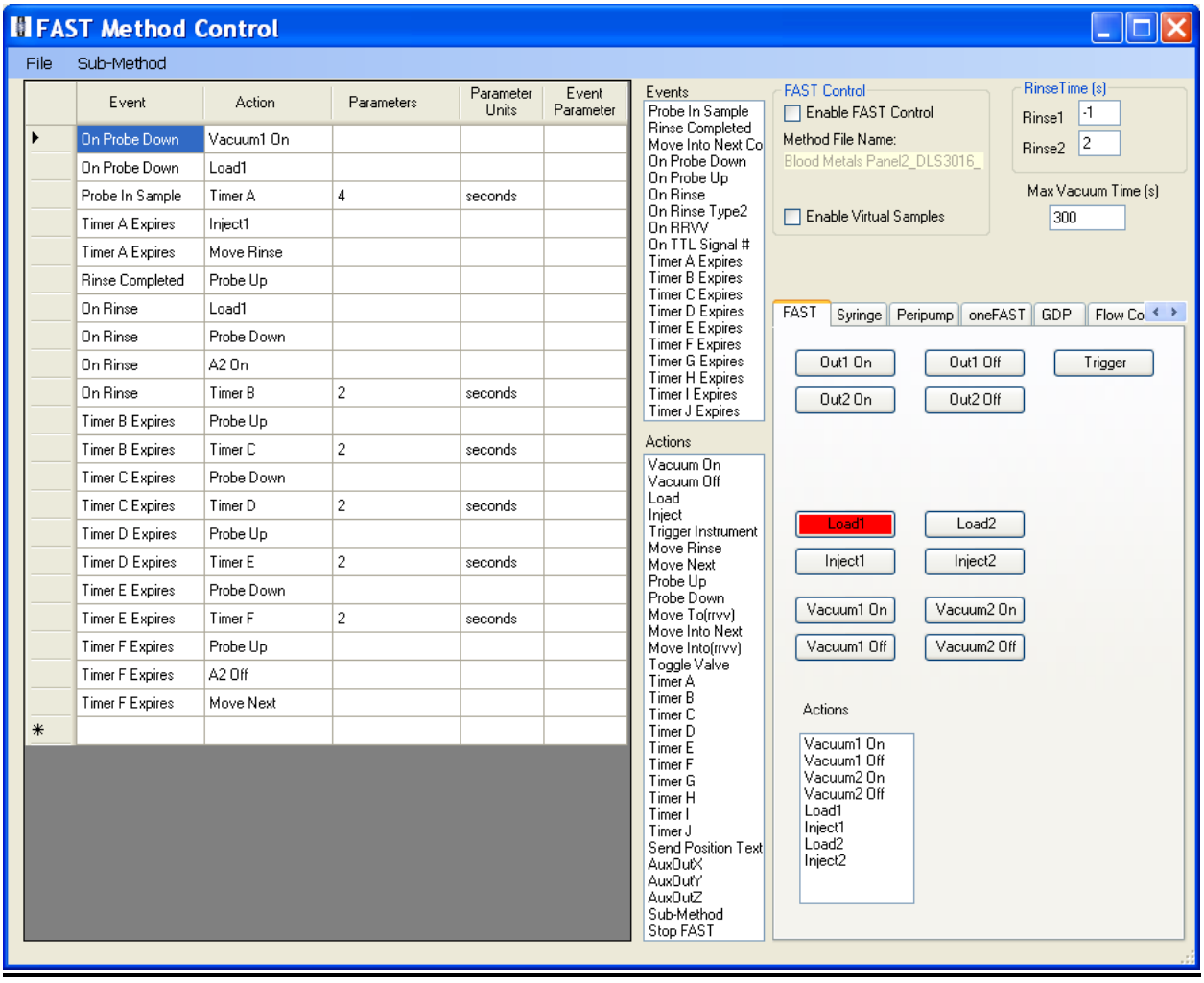


Appendix B (continued)

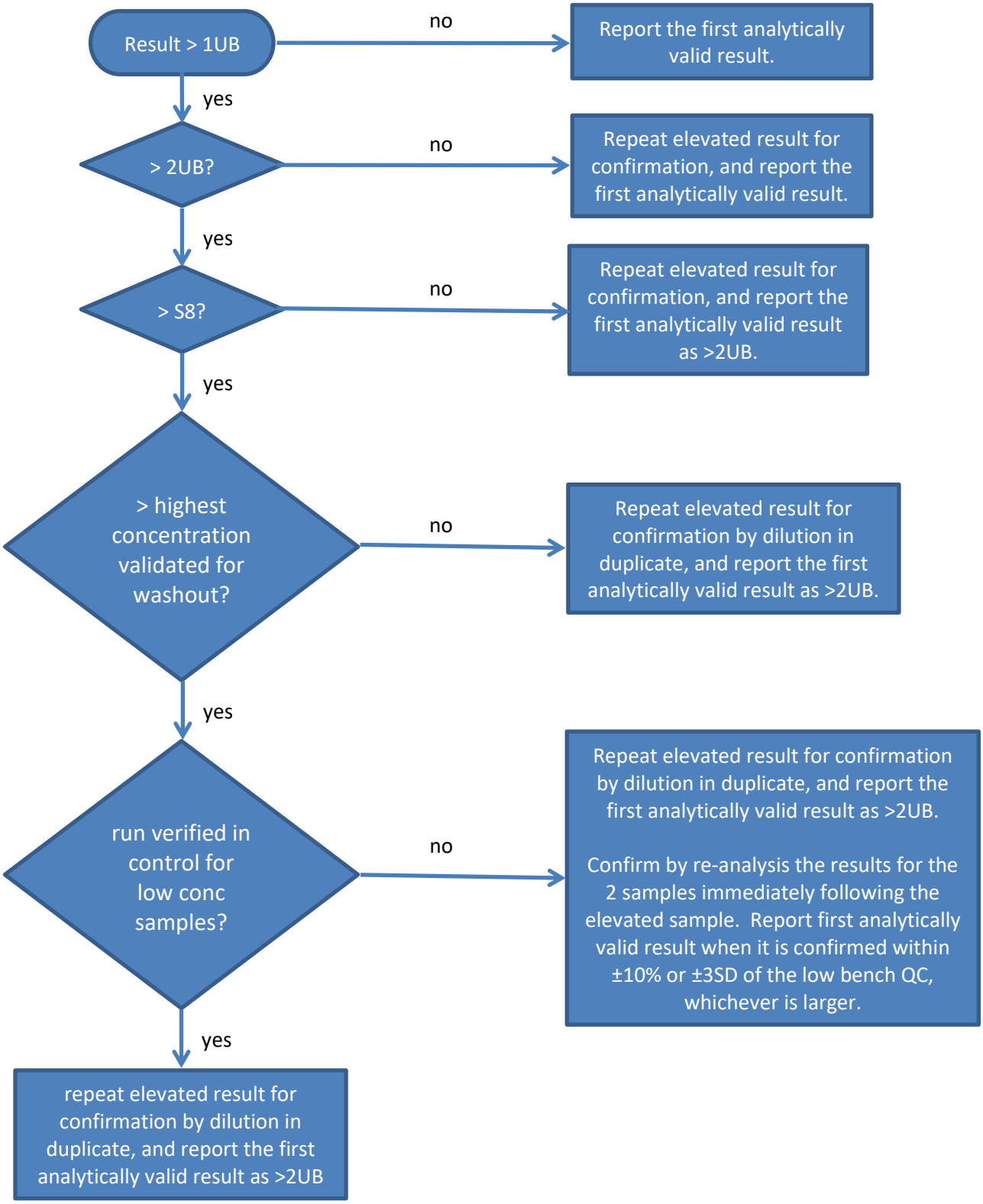
Figure 3g. ESI SC4 autosampler screen shots ("FAST" page). Timer A can be optimized to achieve proper filling of loop with diluted sample digestate. Timers B, C, D, E, and F control rinsing the loop after analysis and can be optimized for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution. Save the file with the name "DLS 3016.8 FAST parameters.txt". It can be found in the directory C:\Program Files\ESI\ESI-SC\.

Manually clicking the "Load" button prior to starting analysis will ensure the position of the actuator is always the same at the beginning of the analysis.

Manually clicking the "Vacuum On" button prior to starting the analysis will help initial sample uptake to be consistent (the vacuum pump may be slow to start for the first sample if this is not done, possibly resulting in loop filling inconsistencies).



Appendix B (continued)
Figure 4. chart for handling an elevated result



Appendix C: Help Sheets

Reagent Preparation (page 1 of 3)

NOTE:
mg/L = ppm
ug/L = ppb
ug/mL = ppm

Rinse solution
(0.4% TMAH, 0.05% Triton X-100, 1% ethyl alcohol, 0.01% APDC)

1. Partially fill a 4 liter bottle with ≥18 Mohm·cm water.
2. Add 0.4 grams of APDC.
3. Add 16 mL of TMAH (Tetramethylammonium hydroxide, 25% w/w ((CH3)4NOH).
4. Add 40 mL of ethyl alcohol (C2H5OH, 200 proof)
5. Add 200 mL of 1% Triton X-100 (OR add 10mL of 20%Triton X-100).
6. Add enough ≥18 Mohm·cm water to bring to 4 liter mark.
7. Mix well by gently inverting several times.

Sample diluent
(0.4% TMAH, 0.01% APDC, 0.05% Triton X-100, 1% Ethanol, 5ppb Te, Rh, Ir)

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 0.2 gram of APDC.
3. Add 8 mL of TMAH.
4. Add 20 mL of ethyl alcohol.
5. Add 500 uL of a 20 mg/L stock solution of Te, Rh, and Ir.
8. Add 100 mL of 1% Triton X-100 (OR, if using a 20% Triton X-100 solution, add 5mL)
9. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
10. Mix well by gently inverting several times.

0.5% HNO3
(Carrier solution for optimization)

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 10 mL of conc. HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Mix well by gently inverting several times.

Appendix C: Help Sheets (continued)

Reagent Preparation (page 2 of 3)

1% v/v HNO_3

1. Partially fill a 10 liter bottle with ≥18 Mohm·cm water.
2. Add 100 mL of conc. HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 10 liter mark.
4. Mix well by gently swirling several times.

5% v/v HNO_3

1. Partially fill a 2 liter bottle with ≥18 Mohm·cm water.
2. Add 100 mL of conc. HNO_3.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Mix well by gently inverting several times.

20% Triton X-100

1. Partially fill a 1 liter bottle with ≥18 Mohm·cm water.
2. Add 200 mL of Triton X-100.
3. Add enough ≥18 Mohm·cm water to bring to 1 liter mark.
4. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved).
5. Mix well by gently inverting several times.

1% Triton X-100

1. Partially fill a 1 liter bottle with ≥18 Mohm·cm water.
2. Add 10 mL of Triton X-100.
3. Add enough ≥18 Mohm·cm water to bring to 1 liter mark.
4. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved).
5. Mix well by gently inverting several times.

20 ppm Rh, Te and Ir internal standard solution

1. Partially fill an acid rinsed, 50 mL flask with 1% v/v HNO_3.
2. Add 1mL of Rh from 1000ppm stock standard.
3. Add 1mL of Te from 1000ppm stock standard.
4. Add 1mL of Ir from 1000ppm stock standard.
5. Add enough 1% v/v HNO_3 to fill to 50mL mark.
6. Mix well by gently inverting several times.
7. Pour the standard solution over into an appropriately labeled 50mL polypropylene tube.

Appendix C: Help Sheets (continued)

Reagent Preparation (page 3 of 3)

Daily solution (1ppb) in 2% v/v HNO_3

1. Partially fill a 1 liter volumetric flask with ≥18 Mohm·cm water.
2. Add 1mL of High Purity Standard: SM-2107-018 (or current lot #)
3. Add 20mL of concentrated HNO_3
4. Add enough ≥18 Mohm·cm water to bring to 1 liter mark.
5. Mix well by gently inverting several times.

Stability test solution (1 liter bulk prep)

1. Use a 1 liter bottle dedicated to stability test solution preparation
2. Add 960 mL of Sample Diluent
3. Add 20 mL of “junk” whole blood
4. Add 20 mL of Intermediate Working Calibration Standard (may use S1 or S2)
   OR add 1.5mL of Intermediate Stock Calibration Standard.
5. Mix well by gently inverting several times.
6. Store in the refrigerator (when not using).

Appendix C: Help Sheets (continued)

Standard Preparation (page 1 of 1)

(from single element stock standards)

Prepare 3% HCl v/v solution:

1. Partially fill a clean 2 liter bottle with ≥18 Mohm·cm water.
2. Using a clean 50 mL polypropylene tube to measure, add 60 mL of high purity concentrated HCl.
3. Add enough ≥18 Mohm·cm water to bring to 2 liter mark.
4. Gently invert to mix.

Prepare intermediate stock standard (see Table 4 in Appendix B):

1. Partially fill a 100 mL volumetric flask with 3% v/v HCl solution.
2. Label as: “HgPbCdMnSe Intermediate Stock Std”
3. Add 2 mL of HgPbCdMnSe multi-element stock solution.
4. Add enough 3% v/v HCl to bring to 100 mL mark.
5. Mix well by gently inverting several times.

Prepare intermediate working standards (see Table 5 in Appendix B):

1. Partially fill each of eight, 100 mL volumetric flasks with 3% v/v HCl solution.
2. Label as: Intermediate Working Std “S1”, “S2”, “S3” and “S4”, “S5”, “S6”, “S7” and “S8”.
3. For “S1 Intermediate Working Std”: add 50 uL of the Intermediate Stock Std.
4. For “S2 Intermediate Working Std”: add 150 uL of the Intermediate Stock Std.
5. For “S3 Intermediate Working Std”: add 350 uL of the Intermediate Stock Std.
6. For “S4 Intermediate Working Std”: add 500 uL of the Intermediate Stock Std.
7. For “S5 Intermediate Working Std”: add 1mL of the Intermediate Stock Std.
8. For “S6 Intermediate Working Std”: add 50 uL of the Multi-Element Stock Std.
9. For “S7 Intermediate Working Std”: add 150 uL of the Multi-Element Stock Std.
10. For “S8 Intermediate Working Std”: add 400 uL of the Multi-Element Stock Std.
11. Add enough 3% v/v HCl solution to bring to 100 mL mark.
12. Mix well by gently inverting several times.
13. These intermediate working standards may be poured over into clean 15 mL Falcon tubes for daily use (NOTE: “S0 Intermediate Working Std” is 3% HCl only).

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Division of Laboratory Sciences

Laboratory Protocol

Analytes: Copper, Selenium, and Zinc

Matrix: Serum

Method: Multi-Elements in Serum by ICP-DRC-MS

Method code: DLS 3006.8-03

Branch: Inorganic and Radiation Analytical Toxicology

Prepared By:	Jeffery M. Jarrett MS Author's name	 Signature	3/9/18 Date
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Supervisor:	Kathleen L. Caldwell PhD Supervisor's name	 Signature	3/5/18 Date
Branch Chief:	Robert L. Jones PhD Branch Chief's name	 Signature	3/9/18 Date

Date current version of method first used in lab: 3/26/18
Date

Director's Signature Block:

Reviewed:  3/20/18
Signature Date

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Procedure Change Log

Procedure: Multi-Elements in Serum by ICP-DRC-MS **DLS Method Code:** 3006.8-03

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
11/25/2011	Use of concentrated nitric acid during preparation of calibrators and calibration verification standards documented.	GM	JJ	11/25/2011
3/8/2012	Removed requirement for blind QC in each run	GM	JJ	3/8/12
7/26/2012	Revised maximum suggested concentrations of analytes in base serum, Table 2 of Appendix C.	KW	JJ	7/27/12
7/27/2012	Updated page number references for normal population ranges	KW	JJ	7/27/12
7/27/2012	Updated Appendix C Figure 2e. Method screenshot Sampling page	KW	JJ	7/27/12
8/8/2012	Updated Table 1: Instrument and Method Parameters (expanded typical nebulizer gas flow range and updated pump speeds and delay times for flush, read, and wash)	KW	JJ	8/8/12
12/18/2014	Defined top end of reportable range as S5 times the maximum validated extra dilution factor rather than by highest calibration verification check analyzed. Updated Tables 1 and 4. Added ruggedness testing for extra dilutions (parameter #6).	KW	JJ	12/18/2014
12/18/2014	Updated Table 7. Changed to preparing serum blank (and checks) with S0 rather than water. Clarified splitting volumes in use of Digiflex.	KW	JJ	12/18/2014
12/18/2014	Corrected volumes of Triton X-100 in intermediate Triton solution and revised default volume prep of diluent to 2L.	KW	JJ	12/18/2014
12/18/2014	Added description of solutions for DRC and dual detector optimizations.	KW	JJ	12/18/2014
12/18/2014	Added reference to SOP DLS3500 for handling corrosive liquid waste to the instrument waste disposal guidelines.	KW	JJ	12/18/2014
12/18/2014	Updated instrumentation and equipment sources for vendor names and part numbers.	KW	JJ	12/18/2014
12/18/2014	Added clarifying instruction of running water for ~30min between consecutive runs.	KW	JJ	12/18/2014
12/18/2014	Clarified and updated table of contents, section headings, table numbers and naming, and figure numbering and naming. Updated instrument software screen shots.	KW	JJ	12/18/2014
12/18/2014	Updated 1LB and 2LB for copper from 10 ug/dL to 50 ug/dL.	KW	JJ	12/18/2014
12/18/2014	Updated sections 8, 9, 10, 11 for clarity and to match other laboratory methods.	KW	JJ	12/18/2014
4/6/2015	Added Appendix D "Help sheets"	ZF	JJ	4/7/2015
4/6/2015	Updated the minimum R^2 of calibration curves to 0.98 to match DLS Policy and Procedures	JJ	JJ	4/7/2015
4/7/2015	Updated cover page per DLS Office of Director	JJ	JJ	4/7/2015

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
12/08/2015	Updated Title page to new DLS template.	ZF	JJ	12/08/2015
12/08/2015	Clarified comments, updated examples, corrected typos: Removed passive language (e.g. may, shall, should) throughout. Clarified instruction of standards preparation in Section 6.e. Removed dual detector solution prep instructions (Section 6.f.). Updated Section 8.b.ii.2 to use junk serum during DRC stability time rather than standard 2. Clarified data backup instructions in Section 9.b. Clarified comments in Table 7 (prep of samples) and 8 (boundary conditions).	ZF	JJ	12/08/2015
12/08/2015	Minor equipment updates: References to Digiflex pipette changed to Hamilton Microlab 625 benchtop automatic pipette.	ZF	JJ	12/08/2015
12/08/2015	Updated expiration dates of optimization solutions and base serum in Section 6.e.	JJ	KLC	12/9/2015
12/08/2015	Added recommendation for baseline checks before run in Section 8.b.iv.	ZF	JJ	12/08/2015
12/08/2015	Updated instructions related to very elevated results. Set criteria to confirm proper washout after an elevated sample to ± 3SD limits of low bench QC wash check (Section 8.b.iv). Set criteria to confirm samples potentially affected by insufficient washout to ±10% or ±3SD of the low bench QC, whichever is greater (Section 8.b.vii.2.a). Added highest validated washout concentrations to Table 9 in Appendix C. Added extended wash details for As in Table 1. Added Figure 2g (Method Screen Shot, QC / Sample page) and Figure 4 (Flow Chart for handling an elevated result)	JJ	KLC	12/9/2015
12/08/2015	Updated record retention in section Section 9.c to match DLS policy (3 years to 2 years).	JJ	KLC	12/9/2015
12/08/2015	Updated Appendix D: Help sheets	ZF	JJ	12/08/2015

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
4/20/2016 (3006.8-03)	Updated instructions on disposal of carboy waste with the addition of Clorox bleach or equivalent prior to disposal and changed pH requirements to Dekalb County limits pH>5 pH<11.5.	DS	JJ	3/7/2018
3/7/2018 (3006.8-03)	Clarified section 10.a on the reportable range of the method and in section 8.b.iv.8.b on diluting a sample to within the calibration range. Added Table 8 (Reportable range concentrations), changing "Boundary concentrations" from Table 8 to Table 9, and "Reference ranges" from Table 9 to Table 10. Clarified references to Tables 8-10 throughout document.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Clarified how to homogenize the sample dilution in Section 8.b.ii. Added "(e.g. vortex for 3-5 seconds, or invert 5-10 times)."	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Changed figures in Appendix C from 2a-2g numbering to figures 2-8. Changed figures 3a-3e numbering to figures 9-13.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Updated source information for cones, handheld pipettes, barcode scanner, and added part information for Hamilton diluter PEEK valves and volumetric flasks.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Added an index of tables for the ruggedness tables.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Updated references including: formatting, new (v6.0) DLS Policies and Procedures manual, and New (Jan 2017) Report on Human Exposure to Environmental Chemicals. Updated reference ranges to include NHANES 11-14.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Added Section 13 (Method performance documentation) and supporting data in Appendix A. Previous Appendix A (Ruggedness testing) became Appendix B. Previous Appendix B (Method tables and figures) became Appendix C. Previous Appendix C (Help sheets) became Appendix D. Updated references throughout document.	KW	JJ	3/7/2018

Date	Changes Made	By	Rev'd By (Initials)	Date Rev'd
3/7/2018 (3006.8-03)	Added Microsoft Word captions to all Tables and Figures. Added 'Title" in Microsoft Word 'Alt Text' for every Table and Figure. Added an Index of Tables for Ruggedness Tables. Updated the numbering of all figures, eliminating letter designations for straight numbering.	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Updated Cross reference to DLS CLIA and Policy and Procedure	KW	JJ	3/7/2018
3/7/2018 (3006.8-03)	Updated specimen stability statement in section 3.a and section 6.e.vi to match stability data in Appendix A.	JJ	RLJ	3/8/2018
3/7/2018 (3006.8-03)	Added a total volume column to Table 7 in Appendix C.	JJ	RLJ	3/8/2018
3/9/2018 (3006.8-03)	Clarified prescreening of acceptable containers and acceptable container materials (from 'polyethylene' to 'like polyethylene and polypropylene') in Section 3.a.vi.	JJ	RLJ	3/8/2018
3/9/2018 (3006.8-03)	Changed 2 L to 1 L, and reduced 40 mL to 20 mL regarding making of 2% v/v nitric acid in Section 6.e.ii.2 to match with Help Sheets in Appendix D.	JJ	RLJ	3/8/2018
3/9/2018 (3006.8-03)	Changed "room temperature" to "ambient temperature" throughout.	JJ	RLJ	3/8/2018
3/9/2018 (3006.8-03)	Added reference to Figure 7 and Figure 8 in Table 1 of Appendix C.	JJ	RLJ	3/8/2018

Laboratory Procedure Manual

Analytes: **Zinc, Copper and Selenium**

Matrix: **Serum**

Method: **Serum Multi-Element ICP-DRC-MS**

Method No: DLS 3006.8-03

Revised: 3/9/2018

As performed by: Inorganic Radiation Analytical Toxicology
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Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Table of Contents

Cross reference to DLS CLIA and Policy and Procedures policy

1. Summary of test principle and clinical relevance
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2. Safety precautions
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3. Computerization; data system management
   5.a, 8.b, 9.c
4. Specimen collection, storage, and handling procedures; criteria for specimen rejection
   3
5. Procedures for microscopic examinations; criteria for rejection of inadequately prepared slides
   - As no microscope used in this process there are no procedures for microscopic examinations; and as no slides are prepared for this analysis there is no criteria for rejection of inadequately prepared slides
6. Preparation of reagents, calibrators (standards), controls, and all other materials; equipment and instrumentation
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1) Clinical relevance and summary of test principle

a. Clinical Relevance:

This method is used to achieve rapid and accurate quantification of three elements of toxicological and nutritional interest including Zinc (Zn), Copper (Cu) and Selenium (Se). The method is useful to screen serum when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure.

b. Test Principle:

Inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) is a multi-element analytical technique capable of trace level elemental analysis [1-4]. This ICP-DRC-MS method is used to measure the entire panel of 3 elements, or any subgroup. Liquid samples are introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. By coupling radio-frequency power into flowing argon, a plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6,000-8,000 K. The sample passes through a region of the plasma and the thermal energy atomizes the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10^{-5} torr). The ions pass through a focusing region, the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In ‘vented’ (or ‘standard’) mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In ‘DRC’ mode, the cell is pressurized with a molecular gas for the purpose of causing collisions and/or reactions between the molecular gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to eliminate an interfering ion by changing the ion of interest to a new mass which is free from interference, or causing collisions between ions in the beam and the DRC gas which can focus the ion beam to the middle of the cell thus increasing the ion signal. In this method, the instrument is operated in ‘DRC’ mode when measuring Zn, Cu and Se, and the cell is pressurized with 99.99^{+}% ammonia gas which collides or reacts with the incoming ions to eliminate interfering ions and leave the ion of interest to be detected.

After leaving the DRC cell, the ions are focused with ion optics into a quadrupole mass analyzer with a nominal mass resolution of 0.7 amu. The quadrupole is sequentially scanned to specific mass to charge ratio of each analyte and intensity is detected with a pulse detector. Electrical signals resulting from the detection of ions are processed into digital information that is used to indicate first the intensity of the ions and then the concentration of the element. This method was originally based on the methods by Piraner and Walters [5-8] and

the DRC portions of the method are based on work published by Tanner et al. [2, 3]. The isotopes measured by this method are zinc (*m/z* 64), copper (*m/z* 65) and selenium (*m/z* 78) and the internal standard, gallium (*m/z* 71). Serum samples are diluted 1:1:28 with ≥18 MΩ·cm water and diluent containing gallium (Ga) for multi-internal standardization.

2) Limitations of method; interfering substances and conditions

a. Interferences addressed by this method

i. Correction and elimination of interferences (^{64}Ni, $^{36}Ar^{14}N_2$) on zinc (^{64}Zn).

1. Mathematical correction for nickel (^{64}Ni) interference:

The correction equation (-0.035297* ^{60}Ni) is used in the "Equations" tab of the method to correct the counts observed as *m/z* 64 to exclude counts due to ^{64}Ni.

2. Elimination of $^{36}Ar^{14}N_2$ interference using DRC: The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from $^{36}Ar^{14}N_2$ onto zinc at *m/z* 64. See Section 1.b for an explanation of this process.

ii. Elimination of interferences ($^{40}Ar^{25}$Mg, $^{36}Ar^{14}N_2{}^1$H) on copper (^{65}Cu) using DRC. The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate the interference $^{40}Ar^{25}$Mg, $^{36}Ar^{14}N_2{}^1$H on copper at *m/z* 65. See Section 1.b for an explanation of this process.

iii. Correction and elimination of interferences (^{78}Kr, $^{38}Ar^{40}$Ar, $^{38}Ar^{40}$Ca) on selenium (^{78}Se).

1. Mathematical correction for krypton (^{78}Kr) interference:

The correction equation (-0.030461*^{83}Kr) is used in the "Equations" tab of the method to correct the counts observed as *m/z* 78 to exclude counts due to ^{78}Kr.

2. Elimination of $^{38}Ar^{40}$Ar, $^{38}Ar^{40}$Ca interference using DRC: The dynamic reaction cell of the ELAN ICP-DRC-MS is used in this method to eliminate interference from $^{38}Ar^{40}$Ar, $^{38}Ar^{40}$Ca onto selenium at *m/z* 78. See Section 1.b for an explanation of this process.

b. Limitations of method

i. $^{48}Ca^{16}O^1$H interference on copper (^{65}Cu):

It has been determined that a small interference remains at *m/z* 65 when the serum matrix contains very high calcium levels. Even at extreme calcium levels, this interference has not been found to be significant (< 1%).

ii. Time between dilution of serum materials and analysis:
Selenium is not stable in the diluted sample for more than 7 hours. Diluted serum must be analyzed within 7 hours of preparation (see Appendix B, test 5 for details).

3) Procedures for collecting, storing, and handling specimens; criteria for specimen rejection; specimen accountability and tracking

a. Procedures for collecting, storing, and handling specimens:

Specimen handling conditions, special requirements, and procedures for collection and transport are discussed in the Division of Laboratory Science's (DLS) Policies and Procedures Manual [5]. In general, if more than one vacutainer of blood is to be drawn from an individual, collect the trace metals tube second or later. Draw the blood through a stainless steel needle into a pre-screened 7-mL vacutainer. Allow the blood in the stoppered vacutainer clot for 30-40 minutes, but not longer than 60 minutes. Without opening the vacutainer, centrifuge it for 10 minutes at 2400 rpm. Use a pre-screened serum separator to remove the serum from the clot. Under a laminar flow hood, pour the serum in the serum separator into pre-screened polyethylene vials.

i. No fasting or special diets are required.

ii. Use sterile, lot screened collectors for specimen acquisition.

iii. Transport serum specimens at $\leq 4^{o}C$.

iv. Once received, store at $\leq -20^{o}C$ until time for analysis. Re-freeze remaining portions $\leq -20^{o}C$ after analytical aliquots are withdrawn. Thawing and refreezing samples has not been found to compromise sample results.

v. Specimen stability for at 3 years has been demonstrated at < -70°C storage conditions.

vi. Acceptable containers for analytical aliquots include vials (like polyethylene and polypropylene) and 7-mL vacutainers determined by pre-screening to be free of significant contamination of the metals tested in this method. A 3-mL vacutainer size will not produce the optimal volume of serum for this test. Externally threaded containers are preferred because they are less prone to contamination of the specimen and to leaks (internally threaded containers can develop leaks when biological material dries within the threads, compromising resealing).

b. Criteria for specimen rejection:

Specimen characteristics that compromise test results are indicated above. Reasons for rejection of a sample for analysis include

i. Low volume: Optimal amount of serum is 1 mL, minimum is 0.8 mL. The volume of serum used for one analysis is 0.15 mL.

ii. Contamination: Improper collection procedures or collection devices can contaminate the serum by contact with dust, dirt, etc.

In all cases, request a second serum specimen.

c. Transfer or referral of specimens; procedures for specimen accountability and tracking:

Location, status, and final disposition of the specimens will be tracked and records are maintained according to the DLS Policies and Procedures Manual [5]. Use only numerical identifiers for samples within the laboratory (e.g., case ID numbers) in order to safeguard confidentiality. Only the medical supervisor (MS) or project coordinator (PC) (i.e. non CDC personnel) will have access to the personal identifiers.

4) Safety precautions

a. General safety

i. Observe all safety regulations as detailed in the Division (DLS) Safety Manual. Additional information can be found in your lab's chemical hygiene plan.

ii. Observe Universal Precautions when working with serum.

iii. Wear appropriate gloves, lab coat, and safety glasses while handling all solutions.

iv. Exercise special care when handling and dispensing concentrated nitric acid. Add acid to water. Nitric acid is a caustic chemical that is capable of causing severe eye and skin damage. ***If nitric acid comes in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.***

v. Use secondary containment for containers holding biological or corrosive liquids.

vi. The use of the foot pedal on the benchtop automatic pipette is recommended because it reduces analyst contact with work surfaces that have been in contact with serum and also keeps the analyst's hands free to hold the specimen cups and autosampler tubes and to wipe off the dispensing tip.

vii. Training will be given before operating the ICP-DRC-MS, as there are many possible hazards including ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures. This information is also detailed in the PerkinElmer ELAN® ICP-DRC-MS System Safety Manual.

viii. Place ammonia gas cylinders (either in use or in storage) in a cabinet which is well ventilated to the house exhaust. Do not place ammonia cylinders on their side while in use as the cylinder valve can become "frozen" in place as a result of the cooling capacity of expanding ammonia gas.

ix. Wipe down all work surfaces at the end of the day with disinfectant. Disinfectant may be either daily remake of diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water) or an equivalent disinfectant.

b. Waste disposal:

i. Autoclaving: All diluted biological specimens, original biological specimens being disposed, or consumables which come into contact with biological specimens

(even diluted or aerosolized). Use sharps containers or special autoclave pans for broken glass / quartz or items which are puncture hazards (e.g. pipette tips). (see the "Autoclaving" section of the CDC safety policies and practices manual located in the laboratory).

ii. Other liquid waste

1. Waste discarded down sink: Do not discard solutions at the sink having a pH lower than 5.0 or higher than 11.5 (limits defined by Dekalb County, GA). Inactivate biological compounds and cellular constituents in mixed chemical and biological waste, such as the waste carboy of the ICP-MS, by adding an approved disinfectant (e.g. household bleach at a 1:100 dilution or equivalent) prior to drain disposal. Flush the sink with copious amounts of water. Waste from the spray chamber and autosampler rinse station drain into the same carboy and are handled according to DLS 3500 standard operating procedure for handling corrosive liquid laboratory waste.

2. Waste to be picked up by hazardous waste program: Submit request for hazardous waste removal of all other liquid waste generated in the CDC laboratory for this method.

5) Instrument and material sources

a. Sources for ICP-MS instrumentation

i. ICP-MS: Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometer (ELAN® 6100 DRC^{Plus} or ELAN® DRC II) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

ii. Recirculating chiller / heat exchanger for ICP-MS: Refrigerated chiller (PolyScience 6105PE for ELAN® 6100 DRC^{Plus} instruments) or heat exchanger (PolyScience 3370 for ELAN® DRC II instruments) (PerkinElmer Norwalk, CT, www.perkinelmer.com).

iii. Autosampler: ESI SC-4 autosampler (Elemental Scientific Inc., Omaha, NE) or equivalent.

b. Sources for ICP-MS parts and consumables

NOTE: The minimum number of spares recommended before reordering (if owning one instrument) are listed as "# *Spares* =" in the descriptions below.

i. Adapter, plastic: 1/4-28 female threads on one side, 1.8mm barb adapter on the other. Connects ¼-28 nut at flanged tubing connection to 0.045" i.d. peristaltic pump tubing. Use part # B019-3342 ("Type A" adapter, PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. *# Spares = 4*.

ii. Adapter, PEEK: Securely connects 1.6mm O.D. PFA tubing to 0.03" I.D. peristaltic tubing. Composed of three PEEK parts.

1. Female nut for 1.6mm O.D. (1/16") tubing. Like part P-420 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).

2. PEEK ferrule. Like part P-260x (10pk SuperFlangeless ferrule, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).
3. Conical Adapter Body. Like part P-692 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com).

iii. Coolant, for Polyscience chiller or heat exchanger: Only PerkinElmer part # WE01-6558 (PerkinElmer Norwalk, CT, www.perkinelmer.com) is approved for use by PerkinElmer. # *Spares = 6*.

iv. Cones: Platinum or Nickel cones have been used. Platinum cones are more expensive, but will last longer, can be refurbished, and will frequently yield higher sensitivity.

1. Sampler (nickel/platinum): PerkinElmer part # WE021140/WE027802 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). # *Spares = 4*.
2. Skimmer (nickel/platinum): PerkinElmer part # WE021137/WE027803 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or cross-referenced part number manufactured by Spectron Inc. (Ventura, CA, www.spectronus.com) or Glass Expansion (Pocasset, MA, www.geicp.com). # *Spares = 4*.

v. Connector (for tubing): Use to connect 1/8" I.D. PVC tubing to 0.125" I.D peristaltic pump tubing. Use part # 3140715 (PerkinElmer Norwalk, CT, www.perkinelmer.com) or equivalent. # *Spares = 4*.

vi. Detector, electron multiplier: Like part # N8125001 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer (part # 14210, SGE Incorporated, Austin, Texas, http://www.etpsci.com) or various distributors. # *Spares = 1*.

vii. Hose, for connection to chiller: Push on hose. I.D. = ½", O.D. = ¾". Use part # PB-8 (per inch, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. Do not normally need spare hose (unless moving instrument into a new location).

viii. Hose, for exhaust of ELAN: Available as part of ELAN installation kit from Perkin Elmer (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # S-LP-10 air connector (Thermaflex, Abbeville, SC, www.thermaflex.net). Equivalent part is acceptable. # *Spares = 10 feet of 4" diameter and 10 feet of 6" diameter hose.*

ix. Injector, quartz with ball joint: I.D. = 2.0 mm. PerkinElmer part # WE023948 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-30 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

x. Injector support (for pass-through injector): PerkinElmer part # WE023951 (PerkinElmer Norwalk, CT, www.perkinelmer.com). Available direct from manufacturer as part # 400-37 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

xi. Ion Lens: PerkinElmer part # WE018034 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # Spares = 3.

xii. Nebulizer, quartz concentric: Type C, 1 mL/min nebulizer with quick disconnects for liquid and gas ports such as part # 500-70QQDAC (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). This nebulizer is designed to use quick disconnects part # 500-QD (liquid) and # 500-AC (argon).

xiii. Nebulizer connections (gas): (for nebulizer argon side-arm).

1. If *not* using quick disconnection fitting, insert nebulizer argon side-arm into the 1/8" i.d. vinyl tubing and secure the connection with a hose clamp for ¼" o.d tubing (like part # EW-06832-01, Cole Palmer Instrument Company, Vernon Hills, Illinois, www.colepalmer.com). # *Spares = 2*.
2. Quick disconnection fitting: Like part # 500-AC (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). # *Spares = 2*.

xiv. Nebulizer connections (liquid): (for nebulizer 4mm o.d. liquid sample backend). Can use quick disconnect or flangeless nut and ferrule assembly.

1. Quick disconnect: Like Part # 500-QD (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). # *Spares = 2*.
2. Flangeless nut and ferrule assembly: An assembly such as part # FIT KIT 3 (Meinhard Glass Products, Golden, CO, www.meinhard.com) or equivalent. Individual pieces of FIT KIT #3 can be purchased as follows.
   a. Nut, flangeless, 1/16", ¼-28, Delrin® (Acetal), red. Part # P202x (10pk, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). # *Spares = 10.*
   b. Ferrule, flangeless, 1/16", Tefzel® (ETFE), blue. Part # P-200x (10pk, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). # *Spares = 10.*
   c. Adapter, 1/4-28 internal to 5/16-24 internal, PEEK™. Part # P-135 (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com). # *Spares = 2.*
   d. Nut, 4mm ID PEEK. Part of fit kit 3 for concentric nebulizers. Part # S-1050 (Meinhard Glass Products, Golden, CO, www.meinhard.com). # *Spares = 2*.
   e. Ferrule, 4mm ID green Delrin. Part of fit kit 3 for concentric nebulizers. Part # S-1121 (Meinhard Glass Products, Golden, CO, www.meinhard.com). # *Spares = 2*.

xv. Nut: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Flanged, for 1/16" o.d. tubing, 1/4-28 threads. Use part # P-406x (pkg. of 10, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. Use a Teflon-coated Viton o-ring with this nut instead of the stainless steel washer that comes with part # P-406x*)*. # *Spares = 10.*

xvi. Nut: (for bottom port of autosampler rinse station) 10-32 UMC threads for 1/16" tubing. Such as part # M653x (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. # *Spares = 2.*

xvii. Nut and ferrule set, 1/8" Swagelok: Such as part # SS-200-NFSET (stainless steel) or part # B-200-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xviii. Nut and ferrule set, 1/4" Swagelok: Such as part # SS-400-NFSET (stainless steel) or part # B-400-NFSET (brass) (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. For part numbers listed here a quantity of 1 means 1 nut, 1 front ferrule, and 1 back ferrule. *Spares = 20.*

xix. Oil for roughing pumps:

1. Oil, Welch Directorr Gold: For roughing pumps. Available direct from manufacturer as part # 8995G-15 (1 gallon, Welch Rietschle Thomas, Skokie, IL, www.welchvacuum.com) or from various distributors. Equivalent oil is acceptable. # *Spares = 4.*
2. Fomblin Y14/5 fluid: PerkinElmer part # N8122265 (1 kg bottle, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares =1 per instrument.*

xx. O-ring: (for sampler cone) PerkinElmer part # N8120511 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

xxi. O-ring: (for skimmer cone) PerkinElmer part # N8120512 (pkg. of 5, PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 20 o-rings.*

xxii. O-ring: (for flanged connections of 1.59mm (1/16") o.d. PFA tubing) Teflon-coated Viton o-ring, i.d. = 1/16", thickness = 1/16", o.d. = 3/16". Such as part # V75-003 (O-rings West, Seattle, WA, www.oringswest.com) or equivalent. # *Spares = 20.*

xxiii. O-ring: (for injector support).

1. Internal o-rings: ID = ¼", OD = 3/8", thickness = 1/16". Need 2 o-rings per injector support to setup. PerkinElmer part # N8122008 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-010, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*
2. External o-rings: ID = 3/8", OD = 1/2", thickness = 1/16". Need 2 o-rings for each injector support setup. PerkinElmer part # N8122009

(PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent (such as part # V75-012, O-rings West, Seattle, WA, www.oringswest.com). # *Spares = 20.*

xxiv. O-ring: (for inside spray chamber at nebulizer port) Such as part # 120-56 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com). Additional o-rings can sometimes be obtained free of charge or at reduced price when acquired while purchasing spray chambers. # *Spares = 20.*

xxv. O-ring: (for inside of torch mount): Part # WE017284 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Do not substitute. The PerkinElmer o-ring is special metal impregnated to minimize RF leakage though the torch mount. # *Spares = 2.*

xxvi. Photon stop: PerkinElmer part # WE018278 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Alternate "snap in" lens assembly requires PerkinElmer part # W1013361 (PerkinElmer Norwalk, CT, www.perkinelmer.com). # *Spares = 1.*

xxvii. Plugs, quick change for roughing pump oil: These plugs will only work on the Varian roughing pumps which come standard on ELAN DRC II ICPMS instruments. These plugs will not fit the Leybold pumps which come standard on the ELAN DRC Plus instruments. Part # W1011013 (PerkinElmer, Shelton, CT, www.perkinelmer.com). No spares typically needed.

xxviii. Probes: (for ESI autosampler) Teflon, carbon fiber support, 0.8mm i.d., blue marker, 1/4-28 fittings. Like part number SC-5037-3751 (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com). # *Spares = 2.*

xxix. RF coil. PerkinElmer part # WE02-1816 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 2.*

xxx. Screw, for torch mount: PerkinElmer part # WE011870. (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. # *Spares = 3*.

xxxi. Spray chamber, quartz concentric: PerkinElmer part # WE025221 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-20 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or from various distributors. # *Spares = 2.*

xxxii. Torch, quartz: PerkinElmer part # N812-2006 (PerkinElmer, Shelton, CT, www.perkinelmer.com) or equivalent. Available direct from manufacturer as part # 400-10 (Precision Glass Blowing, Centennial, CO, www.precisionglassblowing.com) or various distributors. Damaged torches can often be repaired for substantially lower cost than purchasing a new one by companies such as Wilmad LabGlass (Buena, NJ, www.wilmad-labglass.com) or Precision Glass Blowing (Centennial, CO, www.precisionglassblowing.com). # *New Spares = 2.*

xxxiii. Tubing and adapter, for SC autosampler rinse station drain: Tygon tubing and adapter to attach to back of SC autosampler for draining rinse station waste (like

part # SC-0303-002, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com)

xxxiv. Tubing and adapters, for SC autosampler rinse station filling: Teflon tubing and adapters (to attach to back of SC autosampler for filling rinse stations and to attach to rinse containers). Like part # SC-0302-0500, Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com).

xxxv. Tubing, argon delivery to instrument: I.D. = 1/8", O.D. = ¼". Such as part # C-06500-02 (pkg. of 100ft, polypropylene, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent. *# Spares = 50ft*.

xxxvi. Tubing, peristaltic, 0.76 mm i.d. (sampling): Standard PVC, 2-stop (black/black) peristaltic pump tubing, i.d. = 0.76 mm. ESI part # MPP-076-F-PVC (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com) or equivalent. *# Spares = 6 packs of 12 tubes.*

xxxvii. Tubing, peristaltic, 1.3 mm i.d. (spray chamber drain): Santoprene, 2-stop (gray/gray) peristaltic pump tubing, i.d. = 1.3mm. ESI Part # MPP-130-PHR (Elemental Scientific Inc., Omaha, NE., www.elementalscientific.com) or equivalent. *# Spares = 6 packs of 12 tubes.*

xxxviii. Tubing, PFA: I.D. = 0.5mm, O.D. = 1.59mm (1/16"). Used to transfer liquid

1. possibly used between nebulizer and peristaltic pump tubing (if quick connection is not used for liquid sample delivery)

The Perfluoroalkoxy (PFA) copolymer is a form of Teflon®. Such as part # 1548 (20ft length, Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) or equivalent. *# Spares = 20ft.*

xxxix. Tubing, PVC, i.d. = 1/8", o.d. = 3/16". Used to transfer liquid

1. between spray chamber waste port and peristaltic pump

Like part # 14-169-7A (pkg. of 50ft, Fisher Scientific International, Hampton, NH, www.fishersci.com) or equivalent. *# Spares = 20ft*.

xl. Tubing, stainless steel, o.d. = 1/8", wall thickness = 0.028": Used to connect DRC gas cylinders to ELAN DRC gas ports. Also used to replace plastic tubing in the DRC gas path within the ELAN. Like part # SS-T2-S-028-20 (20ft, Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 20ft.*

xli. Tubing, Teflon, corrugated, ¼" o.d.: Connects to the auxiliary and plasma gas side-arms of the torch. Part # WE015903 (PerkinElmer, Shelton, CT, www.perkinelmer.com). *# Spares = 2.*

xlii. Tubing, Tygon, i.d. = 3/16", o.d. = 5/16": Used to transfer liquid between rinse station drain port and liquid waste jug. Like part # EW-06409-15 (50 ft, Cole Parmer, Vernon Hills, Illinois, www.coleparmer.com) or equivalent. *# Spares = 20ft.*

xliii. Tubing, vinyl (argon delivery to nebulizer): Vinyl Tubing, 1/8" ID x 1/4" OD. Like part # EW-06405-02 (Cole Parmer, Vernon Hills, Illinois, www.coleparmer.com) or equivalent. Equivalent tubing material is acceptable. # *Spares = 10ft*.

xliv. Union elbow, PTFE ¼" Swagelok: Connects argon tubing to torch auxiliary gas sidearm. Like part # T-400-9 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

xlv. Union tee, PTFE, ¼" Swagelok: Connects argon tubing to torch plasma gas sidearm and holds igniter inside torch sidearm. Like part # T-400-3 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com) or equivalent. *Spares = 2.*

c. Sources for ICP-MS maintenance equipment and supplies

i. Anemometer: Like digital wind-vane anemometer (Model 840032, SPER Scientific LTD., Scottsdale, AZ, www.sperscientific.com) or equivalent. Use to verify adequate exhaust ventilation for ICP-MS (check with hoses fully disconnected).

ii. Pan, for changing roughing pump oil: Like part # 53216 (United States Plastics Corporation, Lima, OH, www.usplastic.com) or equivalent. # *On hand = 1.*

iii. Container, to hold acid baths for glassware: Polypropylene or polyethylene containers with lids (must be large enough for torch, injector, or spray chamber submersion). Available from laboratory or home kitchen supply companies. # *On hand = 4*.

iv. Cotton swabs: Any vendor. For cleaning of cones and glassware.

v. Cutter (for 1/8" o.d. metal tubing): Terry tool with 3 replacement wheels. Like part # TT-1008 (Chrom Tech, Inc., Saint Paul, MN, www.chromtech.com) or equivalent.

vi. Getter regeneration kit: Part # WE023257 (PerkinElmer, Shelton, CT, www.perkinelmer.com). Use this as needed (at least annually) to clean the getter in the pathway of channel A DRC gas.

vii. Magnifying glass: Any 10x + pocket loupe for inspection of cones and other ICP-MS parts. Plastic body is preferred for non-corrosion characteristics. Like part # 5BC-42813 (Lab Safety Supply, Janesville, WI, www.labsafety.com).

viii. Screw driver, for ion lens removal: Screw driver with long, flexible shaft, and 2mm ball-Allen end for removal of ion lens screws, part # W1010620. Extra 2mm bits, part # W1010598 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

ix. Toothbrush: Any vendor. For cleaning ion lens and glassware.

x. Ultrasonic bath: Like ULTRAsonik™ Benchtop Cleaners (NEYTECH, Bloomfield, CT, www.neytech.com) or equivalent.

d. Sources for general laboratory consumable supplies

i. Bar code scanner: Like Xenon 1902 cordless area-imaging scanner (Honeywell International Inc., Morristown, NJ, www.honeywellaidc.com). For scanning

sample IDs during analysis setup. Any bar code scanner capable of reading Code 128 encoding at a 3 mil label density and 2D bar codes can be substituted

ii. Carboy (for preparation of serum quality control pool and waste jug for ICPMS sample introduction system): Polypropylene 10-L carboy (like catalog # 02-960-20C, Fisher Scientific, Pittsburgh, PA, www.fischersci.com) or equivalent. Carboys with spouts are not advised due to potential for leaking.

iii. Containers for diluent and rinse solution: Two liter Teflon™ containers (like catalog# 02-923-30E, Fisher Scientific, Pittsburgh, PA., www.fishersci.com) and 4L polypropylene jugs (like catalog# 02-960-10A, Fisher Scientific, Pittsburgh, PA, www.fishersci.com) have both been used. Acid rinse before use. Equivalent containers are acceptable.

iv. Flask, volumetric:

1. Four 100mL volumetric flasks (like catalog # 40000100, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.
2. One 200mL volumetric flask (like catalog # 40000200, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.
3. One 500mL volumetric flask (like catalog # 40000500, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.
4. One 1L volumetric flask (like catalog # 40001000, Thermo Scientific, Fisher Scientific, Pittsburgh, PA., www.fishersci.com). Plastic or glass is acceptable.

v. Gloves: Powder-free, low particulate nitrile (like Best CleaN-DEX™ 100% nitrile gloves, any vendor). Equivalent nitrile or latex gloves are acceptable.

vi. Paper towels: For general lab use, any low lint paper wipes such as KIMWIPES®EX-L Delicate Task Wipers or KAYDRY®EX-L Delicate Task Wipers (Kimberly-Clark Professional, Atlanta, GA, www.kcprofessional.com). For sensitive applications in cleanrooms, wipes designed for cleanroom use are available such as the Econowipe or Wetwipe (Liberty, East Berlin, CT, www.liberty-ind.com).

vii. Pipette, benchtop automatic (for preparation of serum dilutions to be analyzed): Like the Microlab 625 advanced dual syringe diluter (Hamilton, Reno, NV, http://www.hamilton.com/) equipped with a 5.0 mL left syringe, a 2.5 mL right syringe, a 12 gauge Concorde CT probe dispense tip, the Microlab cable management system and a foot pedal. PEEK valves like part # 60676-01 (left) and part # 60675-01 (right) may reduce metal background in prepared samples.

viii. Pipettes (for preparation of intermediate working standards and other reagents): Like Picus® NxT electronic, single-channel pipettes (Sartorius AG, Göttingen, Germany, www.sartorius.com). 5-120 µL (catalog # LH-745041), 10-300 µL

(catalog #LH-745061), 50-1000 µL (catalog #LH-745081), 100-5000 µL (catalog #LH-745101). Equivalent pipettes and tips can be substituted.

ix. Tubes for sample analysis (for autosampler): Like polypropylene 15-mL conical tubes, BD Falcon model #352097 (Becton Dickinson Labware, Franklin Lakes, NJ, www.bd.com). Equivalent tubes are acceptable which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Blue colored caps have also been used successfully for this method.

x. Tubes for storage of intermediate working stock standards: Like polypropylene 50-mL centrifuge tubes, Corning Incorporated #430290 (Corning, NJ, 14831. www.scienceproduct.corning.com). For use in storage of intermediate working stock standards. Equivalent tubes are acceptable which are shown by lot screening to be free of trace metal contamination. Clear plastics tend to have lowest trace metal contamination. Orange colored caps have also been used successfully for this method.

xi. Vortexer: Like MV-1 Mini Vortexer (VWR, West Chester, PA, www.vwr.com). Used for vortexing serum specimens before removing an aliquot for analysis. Equivalent item can be substituted.

xii. Water purification system: Like NANOpure DIamond Ultrapure Water System (Barnstead International, Dubuque, Iowa, www.barnstead.com), or equivalent. For ultra-pure ≥18 MΩ·cm water used in reagent and dilution preparations.

e. Sources of chemicals, gases, and regulators

i. Acid, hydrochloric acid: Veritas™ environmental grade, 30-35% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). This is referred to as "concentrated" hydrochloric acid in this method write-up. It is approximately 12 molar in concentration. For use in preparation of intermediate working stock standards. Equivalent products must meet or exceed the purity specifications of this product for trace metals content.

ii. Acid, nitric acid: Veritas™ environmental grade, 68-70% (GFS Chemicals Inc. Columbus, OH, www.gfschemicals.com). For use in diluent, rinse solution, intermediate working stock standards, and QC pool preparations. This is referred to as "concentrated" nitric acid in this method write-up. It is approximately 16 molar in concentration. Equivalent nitric acid must meet or exceed the purity specifications of this product for trace metals content.

iii. Ethyl alcohol (C_2H_5OH), USP dehydrated 200 proof (Pharmco Products, Inc.) or equivalent.

iv. Triton X-100™ ("Baker Analyzed," J.T. Baker Chemical Co. [www.jtbaker.com], or any source whose product is low in trace-metal contamination).

v. Argon gas (for plasma and nebulizer) and regulator: High purity argon (99.999^{+}% purity, Specialty Gases Southeast, Atlanta, GA, www.sgsgas.com)

for torch and nebulizer. Minimum tank source is a dewar of liquid argon (180-250L) but bulk tank for total building needs is preferred.

1. Regulator for argon (at dewar, if used): Stainless steel, single stage, specially cleaned regulator with 3,000 psig max inlet, 0-100 outlet pressure range, CGA 580 cylinder connector, and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Part number KPRAFPF415A2AG10 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. # *Spares = 1*.
2. Regulator for argon (between bulk tank and PerkinElmer filter regulator): Single Stage 316SS Regulator, with 0-300 psi Inlet Gauge, 0-200 psi Outlet Gauge, Outlet Spring Range, 0-250 psi, ¼" Swagelok Inlet Connection, ¼ turn Shut off Valve on Outlet with ¼" Swagelok Connection and Teflon Seals. Part number KPR1GRF412A20000-AR1 (Georgia Valve and Fitting, Atlanta, GA, www.swagelok.com), or equivalent. # *Spares = 1*.
3. Regulator for argon (PerkinElmer filter regulator on back of ELAN): Argon regulator filter kit. Catalog number N812-0508 (PerkinElmer, Shelton, CT, www.perkinelmer.com).

vi. Ammonia: Anhydrous ammonia (99.99^{+}%) for DRC channel A is typically purchased in cylinder size LB (2"x12") (Matheson Tri-Gas, Montgomeryville, PA, 18936. www.mathesontrigas.com).

1. Regulator for ammonia: Stainless steel, two stage, specially cleaned regulator with 3,000 psig max inlet, 2-30 outlet pressure range, cylinder connector CGA 180 or 660 (or designated by the vendor) or CGA 705 (for Airgas cylinder size 200), and needle valve shutoff on delivery side terminating in a ¼" Swagelok connector. Like part number 3813-180 or 3813-705 (Matheson Tri-Gas, Montgomeryville, PA, www.matheson-trigas.com), or equivalent. # *Spares = 1*.

vii. Disinfectant, for work surfaces: Diluted bleach (1 part household bleach containing 5.25% sodium hypochlorite + 9 parts water), remade daily, or equivalent disinfectant.

viii. Standard, Gallium: Like 1,000 mg/L, item # PLGA2-2Y. (SPEX Industries, Inc., Edison, NJ, www.spexcsp.com), or equivalent. Used as an internal standard in diluent. Standards must be traceable to the National Institute for Standards and Technology and have low trace metal contamination.

ix. Standard, multi-element stock standard: Item number SM-2107-013 (High Purity Standards, Charleston, SC, http://www.hps.net/). This is a custom mix solution (see Table 3 in Appendix C for concentrations). This solution is diluted to prepare the intermediate working standards, which are in turn diluted to prepare the working calibrators. This solution can be prepared in-house from NIST traceable single element stock solutions if necessary.

x. Triton X-100™ surfactant: Like "Baker Analyzed" TritonX-100™ (J.T. Baker Chemical Co., www.jtbaker.com), or equivalent.

6) Preparation of reagents and materials.

a. Intermediate Ga internal standard solution:

i. Purpose: Internal standards solution is prepared to be added to the sample diluent. During analysis, the internal standard will compensate for instrumental variations on the analyte signal.

ii. Preparation: To prepare 100 mL of 20 µg/mL Ga in 2% (v/v) HNO_3 solution:

1. If not previously dedicated to this purpose, acid wash a 100 mL container (PP, PMP, or Teflon™) with dilute nitric acid (e.g. 1% v/v HNO_3) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.
2. Partially fill the 100-mL volumetric flask with ≥18 MΩ·cm water.
3. Carefully add 2 mL of concentrated HNO_3. Mix into solution.
4. Add 0.2 mL of 10,000 µg/mL Ga standard. If initial Ga concentration is different adjust volume proportionally.
5. Fill to mark (100 mL) and mix thoroughly.
6. Store at ambient temperature and label appropriately. Expiration date is 1 year from preparation.

b. Intermediate Triton X-100 solution

i. Purpose: Use of the intermediate solution reduces the need to frequently dissolve pure Triton X-100 (frequently an over-night process).

ii. Preparation: To prepare 2 L of 2% Triton X-100™ in 5% (v/v) HNO_3 solution:

1. If not previously dedicated to this purpose, acid wash a 2 L PP, PMP, or Teflon™ container with dilute nitric acid (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.
2. Partially fill the pre-cleaned 2 L bottle with ≥18 MΩ·cm water (approximately 1-1.5 L).
3. Add 40 mL of Triton X-100™ and stir until completely dissolved. Use a pre-cleaned Teflon™ stir bar and stir plate if necessary.
    i. Carefully add 100 mL of concentrated HNO_3 to the partially filled 2 L bottle.
    ii. Fill to 2 L and mix thoroughly.
    iii. Store at ambient temperature and label appropriately. Expiration date is 1 year from preparation.

c. Diluent

i. Purpose: All samples (blanks, calibrators, QC, or patient samples) are combined with the diluent during the sample preparation step before analysis. This is where the internal standards are added which during the analysis will compensate for instrumental variations on the analyte signal.

ii. Preparation: To prepare 2 L of 10 µg/L Ga in 2% (v/v) HNO_3, 5% Ethyl Alcohol, and 0.01% Triton X-100™:

1. If not previously dedicated to this purpose, acid wash a 2 L container (PP, PMP, or Teflon™) with dilute nitric acid (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.
2. Partially fill (i.e. 70-80% full) the 2 L container with ≥18 MΩ·cm water.
3. Add 40 mL concentrated HNO_3 and mix.
4. Add 100 mL Ethyl Alcohol and mix.
5. Add 1 mL of 20 µg/mL Ga internal standard intermediate solution.
6. Add 10 mL of the intermediate 2% Triton X-100™ / 5% (v/v) HNO_3 solution and mix.
7. Make up to 2 L with ≥18 MΩ·cm water.
8. Store at ambient temperature and label appropriately. Expiration date is 1 year from preparation.

d. ICP-DRC-MS rinse solution

i. Purpose: Pump this solution into the sample introduction system between samples to prevent carry-over of the analytes of interest from one sample measurement to the next.

ii. Preparation: To prepare 4 L of 0.01% Triton X-100™, 2% (v/v) HNO_3, 5% ethyl alcohol and 0.5% (v/v) HCl:

1. If not previously dedicated to this purpose, acid wash a 4 L container (PP, PMP, or Teflon™) with dilute nitric acid (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.
2. Partially fill the pre-cleaned 4 L bottle with ≥18 MΩ·cm water (approximately 2-3 L).
3. Add 80 mL of concentrated HNO_3 and mix well.
4. Add 200 mL Ethyl Alcohol and mix well.
5. Add 20 mL of concentrated HCl and mix well.
6. Add 20 mL of the 2% Triton X-100™ / 5% (v/v) HNO_3 intermediate stock solution and mix well.
7. Fill to 4 L using ≥18 MΩ·cm water and mix well.

8. Store at ambient temperature and label appropriately. Expiration date is 1 year from preparation.

e. Standards, calibrators, and QC

i. Multi-element stock calibration standard

1. Purpose: All working intermediate calibrators are prepared by dilution of this stock standard which contains all 3 elements of interest for this method, per the concentrations listed in Table 3 of Appendix C.
2. Purchasing from vendors: The multi-element stock standard is typically purchased as a custom mixture (e.g. part number SM-2107-013 from High Purity Standards (Charleston, SC)). The vendor must provide documentation of traceability to the National Institute for Standards and Technology (NIST). Details of the HPS preparation of the multi-element stock standard is as follows (per statement on their literature):
3. Storage: Store the solution at ambient temperature. Expiration date is as defined by vendor or 1 year from date of opening.

ii. Diluent for intermediate working calibration standards

1. Purpose: This diluent is used to dilute stock calibration standards down to the intermediate working calibration standard concentrations.
2. Preparation: To prepare 1 L of 2% v/v HNO_3:
   a. If not previously dedicated to this purpose, acid wash a 1 L glass, PP, PMP, or Teflon™ volumetric flask with dilute nitric acid (e.g. 1% v/v) and ≥18 MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Dedicate to purpose, if possible.
   b. Partially fill the 1 L volumetric flask with ≥18 MΩ·cm water (approximately 50% to 75% full).
   c. Add 20 mL concentrated HNO_3.
   d. Fill to the mark and mix thoroughly.
   e. Store at ambient temperature and label appropriately. Expiration is 1 year from the date of preparation.

iii. Multi-element intermediate working calibration standards

1. Purpose: Use the intermediate working standard solutions 1-5 each day of analysis to prepare the final working calibrators that will be placed on the autosampler of the ELAN® ICP-DRC-MS.
2. Preparation: To prepare the volumes and concentrations of intermediate working standards per Table 4 in Appendix C:
   a. If not previously dedicated to this purpose, acid wash PP, PMP, or Teflon™ volumetric flasks with dilute nitric acid (e.g. 1% v/v) and ≥18

MΩ·cm water (at least 3 times each). Verify cleanliness through analysis of rinsate. Label and dedicate to purpose, if possible.

b. Partially fill the volumetric flasks with the 2% v/v HNO_3 diluent (approximately 50-75% full).

c. Pipette the volumes of the multi-element stock standard listed in Table 4 of Appendix C into each of the labelled volumetric flasks.

d. Dilute each volumetric flask to the mark with the 2% v/v HNO_3 diluent using a pipette for the final drops. Mix each solution thoroughly.

e. Once mixed, transfer to acid-cleaned, labeled, 50-mL containers (PP, PMP, or Teflon™) for storage.

f. Store at ambient temperature and label appropriately. Expiration is 1 year from the date of preparation. The final concentrations of the 3 elements are listed in Table 4 in Appendix C.

iv. Working multi-element calibrators

1. Purpose: The working multi-element calibrators are dilutions of the intermediate working standards. Analysis of these calibrators provides each run with a signal to concentration response curve for each analyte in the method. The concentration of an analyte in a patient serum sample dilution is determined by comparing the observed signal from the dilution of the patient serum sample to the response curve from the working multi-element calibrators.

2. Preparation: Prepare the volumes and concentrations of the matrix-matched working standards per Table 7 in Appendix C immediately prior to analysis.

v. Base serum

1. Purpose: This serum pool material will be mixed with the intermediate working calibrators just prior to analysis to matrix-match the calibration curve to the serum matrix of the unknown samples.

2. Collection of serum: A mixture of multiple human serum sources purchased from Tennessee Blood Services, 807 Poplar Ave., Memphis, TN 38105. These serum were collected from different anonymous donors are used to approximate an average serum matrix.

3. Screening serum: Screen serum sources for metal content and choose sources which reflect the low-normal population range (see Table 2 in Appendix C for maximum suggested concentrations).

4. Preparation and storage:

a. Once screened, mix the serum collections together in a larger container (i.e. acid washed polypropylene (PP), polymethylpentene

(PMP), or Teflon™) and stir for 30+ minutes on a large stir plate (acid wash large Teflon™ stir bar before use).

b. Dispense into smaller-volume, pre-screened vials for use in the lab.

c. Label appropriately and store frozen (e.g. ≤ -20° C).

vi. Internal quality control materials ("bench" QC)

1. Purpose: Internal (or "bench") quality control (QC) materials are used to evaluate the accuracy and precision of the analysis process, and to determine if the analytical system is "in control" (is producing results that are acceptably accurate and precise). They are included in the beginning and at the end of each analytical run. These pools will need to be prepared periodically, as supply indicates, by spiking base serum. Prepare new pools far enough in advance so that both old and new pools can be analytes together for a period time (preferably at least 20 runs) before switching to the new quality control materials.

2. Content: The internal (or "bench") quality control (QC) materials used in this method are pooled human serum. The serum is spiked, when necessary, with inorganic, NIST-traceable standards to achieve desired concentrations. The analyte concentrations in the "low QC" are in the low-normal concentration range. The analyte concentrations in the "high QC" are in the high-normal concentration range.

3. Preparation and storage: Quality control materials can be either prepared by and purchased from an external laboratory or prepared within the CDC laboratories. Quality control must always be traceable to the National Institute for Standards and Technology (NIST). The CDC laboratory currently prepares its own bench QC materials using the following procedures:

   a. Collection of serum: Human serum can be purchased from blood services companies such as Tennessee Blood Services, 807 Poplar Ave., Memphis, TN 38105.

   b. Screening serum: Screen different bottles for metal content before mixing together to make 2 separate base serum pools (for preparing the low and high bench QC materials).

      i. Keep serum at ≤ -20C whenever possible to minimize microbial growth.

      ii. Choose base serum with concentrations in the low-normal population range (see Table 10 in Appendix C) for low QC. Choose base serum with concentrations below the targeted high-normal population concentration for high QC.

   c. Spiking of serum

i. Analyze a sample of each serum pool. Record these results for future recovery calculations.

ii. Use these results to determine target analyte concentrations possible for the pools

iii. Calculate the volume of single element standards needed to spike each pool to the desired concentrations.

iv. While stirring the pools on large stir plates, spike each pool with calculated volumes of single element standards (all spiking standards used must be traceable to NIST).

v. Continue to stir pools for 30+ minutes after spiking, then reanalyze.

vi. Repeat steps 4 and 5 until all analytes reach target concentrations keeping track of the total volume of spiking solution added to each serum pool.

d. Dispensing and storage of serum

i. Container types: Dispense serum into lot screened containers (i.e. 2 mL polypropylene cryovials). If possible, prepare tubes of QC which have only enough volume for one typical run + 1 repeat analysis. This allows for one vial of QC to be used per day of analysis, reducing chances of contamination of QC materials due to multi-day use.

ii. Labels: Place labels on vials after dispensing and capping if the vials are originally bagged separately from the caps. This minimizes the chance for contamination during the process. Include at least the name of QC pool (text and bar code), date of preparation, and a vial number on the labels.

iii. Dispensing: Dispensing can be accomplished most easily using a benchtop automatic pipette in continuous cycling dispense mode. Carry out this process in a clean environment (e.g. a class 100 cleanroom area or hood is preferred to avoid contamination).

1. Allow serum pool to reach ambient temperature before dispensing (to prevent temperature gradients possibly causing concentration gradients across the large number of vials being dispensed and to prevent condensation problems during labeling of vials).

2. Attach tubing to the syringe of the benchtop automatic pipette with a length of clean Teflon™ tubing long enough to reach into the bottom of the carboy while it is sitting on the stir plate.

3. Check cleanliness of benchtop automatic pipette before use by analyzing 1-2% (v/v) HNO_3 which has been flushed through

the pipette with a portion of the same solution which has not been through the pipette.

4. Approximately one hour before dispensing begins,
   a. With the large stir plate close to the left side of the pipette, begin stirring the serum pool to be dispensed.
   b. Also during this time, flush the pipette syringe(s) with serum from the pool to be dispensed. Place the ends of the tubing attached to both the sample and dispensing syringes into the carboy of serum so that serum won't be used up during this process. Secure both ends of tubing in the carboy with Parafilm so they will not come out during the flushing process.
5. After dispensing the serum into the vials, cap the vials and label them. Placing labels on vials after capping minimizes the chance for contamination during the process.

iv. Homogeneity testing: After dispensing, check homogeneity of analyte concentrations in pool aliquots by analysis of every Nth sample dispensed (where N ~20 - 50 depending on the pool size). Sample more heavily from the beginning and the ending portions of the tubes dispensed (these are the regions where most homogeneity problems occur). Keep samples pulled for homogeneity analysis in the sequence that they were dispensed for the purpose of looking for trends in concentrations. Once dispensed and homogeneity has been shown to be good throughout the tubes of a pool, store tubes at ≤ -20°C and pull tubes out as needed for analysis.

v. Storage: Store serum pools long term at ≤ -20°C. Short term storage (up to several days) is permitted at refrigerated temperatures (~2-8°C).

f. Optimization solutions

i. DRC optimization:

1. Purpose: For periodic testing of the DRC cell parameters. Procedure requires at a minimum a blank (i), an analyte solution (ii), a blank with interference (iii), and an analyte and interference containing solution (iv).
   a. Solutions for testing elimination of $^{36}Ar^{14}N_2$ plasma interference on ^{64}Zn.
      i. Base serum in diluent (1+29)
      ii. Base serum in diluent (1+29) + 90 µg/dL Zn
   b. Solutions for testing elimination of $^{40}Ar^{25}Mg$ interference on ^{65}Cu:

i. Base serum in diluent (1+29)

ii. Base serum in diluent (1+29) + 90 µg/dL Cu

iii. Base serum in diluent + 3 mg/L Mg

iv. Base serum in diluent + 90 µg/dL Cu + 3 mg/L Mg

c. Solutions for testing elimination of $^{38}Ar^{40}Ca$ interference on ^{78}Se:

i. Base serum in diluent (1+29)

ii. Base serum in diluent (1+29) + 90 µg/L Se

iii. Base serum in diluent (1+29) + 100 mg/L Ca

iv. Base serum in diluent (1+29) + 90 µg/L Se + 100 mg/L Ca

2. Preparation: To prepare these DRC optimization solutions, use the 10 µg/L Ga, 2% (v/v) HNO_3, 5% Ethyl Alcohol, 0.01% Trion X-100™ diluent as described in section 6 (same as used to prepare serum samples for analysis). Prepare different volumes by adding proportionally larger or smaller volumes of solution constituents. Interference concentrations can be prepared higher as needed by adjusting the volume of this spike. Keep interference spike volume small (<0.3 mL) using a high concentration stock solution (i.e. 1000 mg/L). The Ca spike has to be 0.5 mL because a stock solution at a concentration higher than 10,000 mg/L is not available. In this case, 0.5 mL of ≥18 MΩ·cm water is added to the non-spike solutions as well. Analyte concentrations can be made higher if needed for sensitivity reasons by preparing a higher concentration calibrator.

a. Solutions testing elimination of $^{36}Ar^{14}N_2$ plasma interference on ^{64}Zn.

i. Base serum in diluent (1 + 29)

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 of Appendix C (multiply volumes by 11).

ii. Base serum in diluent (1+29) + 90 µg/dL Zn

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 of Appendix C (multiply volumes by 11).

Store at ambient temperature and label appropriately. Expiration is 8 hours from preparation.

b. Solutions for testing elimination of $^{40}Ar^{25}Mg$ interference on ^{65}Cu:

i. Base serum in diluent (1 + 29)

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 of Appendix C (multiply volumes by 11).

ii. Base serum in diluent (1+29) + 90 µg/dL Cu

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 of Appendix C (multiply volumes by 11).

iii. Base serum in diluent + 3 mg/L Mg

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.15 mL of 1000 mg/L Mg standard

iv. Base serum in diluent + 90 µg/dL Cu + 3 mg/L Mg

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.15 mL of 1000 mg/L Mg standard

Store at ambient temperature and label appropriately. Expiration is 1 year from the date of preparation.

c. Solutions for testing elimination of $^{38}Ar^{40}Ca$ interference on ^{78}Se:

i. Base serum in diluent (1+29)

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.5 mL of ≥18 MΩ·cm water.

ii. Base serum in diluent (1+29) + 90 µg/L Se

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.5 mL of ≥18 MΩ·cm water.

iii. Base serum in diluent (1+29) + 100 mg/L Ca

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 0 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.5 mL of 10,000 mg/L Ca

iv. Base serum in diluent (1+29) + 90 µg/L Se + 100 mg/L Ca

1. In a 50 mL lot screened or acid-washed polypropylene tube, prepare a 49.5 mL portion of working calibrator 3 as described in Table 7 of Appendix C (multiply volumes by 11).
2. Add 0.5 mL of 10,000 mg/L Ca

Store at ambient temperature and label appropriately. Expiration is 1 year from the date of preparation.

7) Analytical instrumentation and parameters

(see Section 5 for details on hardware used, including sources)

a. Instrumentation and equipment setup:

i. Configuration for liquid handling

See Figure 1 in Appendix C for an example setup.

1. Tubing for liquid sample uptake:

a. Probe-to-peristaltic pump tubing: Use of a 'peristaltic to Teflon tubing adapter' is recommended to prevent damage to small i.d. tubing when making connections.

b. Nebulizer-to-peristaltic pump tubing: It is recommended to use quick connection fittings on either end of the PFA tubing: a plug which pushes inside the liquid port of the nebulizer and a 'peristaltic to Teflon tubing adapter' to prevent damage to small i.d. tubing when making connections.

2. Spray chamber waste removal

Use of a 'peristaltic to Teflon tubing adapter' is recommended to prevent damage to small i.d. tubing when making connections.

a. Between spray chamber and peristaltic tubing:

i. Spray chambers with threaded connection: Use vendor-supplied threaded connector on base of chamber, connecting tubing directly to peristaltic pump tubing through a PEEK adapter or directly.

ii. Spray chambers without threaded connection: Use of specialized push-on connectors available from various vendors (like UFT-075 from Glass Expansion, Pocasset, MA) are preferred for safety reasons to direct connection of PVC tubing (e.g. 1/8" i.d. x ¼" o.d.).

b. Between peristaltic pump tubing and waste container: Connect 1/8" i.d. x ¼" o.d. PVC tubing to the white/black peristaltic pump tubing using a tubing connector (PerkinElmer item # B3140715). Connect the free end of the PVC tubing to the lid of the waste jug. The waste jug must be in a deep secondary containment tray in case of overflow (large enough to hold 110% of waste container volume).

3. Rinse solution for autosampler:
    a. Rinse solution jug: Leave one of the caps on the top of the rinse jug loose to allow air venting into the jug as liquid is removed. Otherwise the jug will collapse on itself as the liquid is removed and a vacuum is created inside. Use secondary containment tray.
    b. Rinse solution uptake to autosampler rinse station: Use tubing of different lengths and inner diameters between the rinse solution container and the autosampler rinse station to control uptake rate of rinse solution. These can be obtained from the autosampler manufacturer, their distributors, or custom built in the lab. Optimize these factors along with fill time in the software so that waste of rinse solution is minimized and rinse station does not go empty.
    c. Autosampler rinse station waste removal: Gravity drain of waste to the waste container is sufficient. Use minimum drain tubing to make this connection. If this tube is too long, the rinse station will not drain properly.

ii. Gas delivery and regulation

1. ICP-MS modifications:
    a. Plastic tubing between mass flow controllers and dynamic reaction cell have been replaced with stainless steel. Stainless steel tubing is preferred between the reaction gas cylinder/regulator and the back of the ICP-MS instrument.
2. Argon gas: Used for various ICP-MS functions including plasma and nebulizer.
    a. Regulator for argon source (if a dewar): Set delivery pressure of this regulator at least 10 psi higher than the delivery pressure of the step-down regulator to allow for pressure drop across tubing that stretches to the instrument.
    b. Step down regulator (if source of argon is a bulk tank): Place this single stage regulator in the lab so that incoming argon pressure can be monitored and adjusted. Set delivery pressure to 10 psig above the delivery pressure of the filter regulator on the ICP-MS.
    c. Filter Regulator at ICP-MS: Single stage “argon regulator filter kit” supplied with the ICP-DRC-MS. Set the delivery pressure depending on the instrument setup:
        i. ELAN with a 0-60psi gauge on the filter regulator: 52±1 psi when plasma is running (need 0-150 psi regulator if using a PolyPro or PFA nebulizer made by Elemental Scientific Inc).
        ii. ELAN with a 0-150psi gauge on the filter regulator: 90-100 psi when plasma is running.

iii. Chiller / heat exchanger: Refrigerated chiller (for ELAN® 6100 DRC^{Plus} instruments) or heat exchanger (for ELAN® DRC II instruments). For refrigerated chiller, set temperature control to 18°C.

b. Parameters for instrument and method:

See Tables and Figures in Appendix C for a complete listing of the instrument and method parameters and software screen shots.

8) The run: quality, execution, evaluation, and reporting

a. Bench QC, reference materials, and calibration verification:

i. Bench "QC": Analysis of bench QC permits assessment of methodological imprecision, determination of whether the analytical system is 'in control' during the run, and assessment of time-associated trends. Before QC materials can be used in the QC process, they must be characterized by at least twenty (20) analytical runs to determine appropriate QC parameters.

Bench QC pool analyte concentrations in this method span the analyte concentration range of the calibrators including "low-normal" ('Low QC') and "high-normal" ('High QC') concentrations.

In each analytical run the analyst will test each of the two bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibrators but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 20-30 patient samples total when analyzing for all elements in the method) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples. For example, the schemes shown in Table 5 in Appendix C are both acceptable ways to analyze multiple consecutive "runs".

ii. Reference materials: Use standard reference material (SRM, e.g. SRM 1598A) from the National Institute of Standards and Technology (NIST) to verify method accuracy. Use previously characterized samples from proficiency testing program or commercially-produced reference materials when NIST SRMs are unavailable.

iii. Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibrators. These calibrators, along with the QCs and blanks, are used to verify that the test system is performing properly.

b. Perform, evaluate, and report a run

i. Starting the equipment for a run

1. Power on the computer, printer, autosampler, and instrument computer controller.
2. Peristaltic pump: Set proper tension on peristaltic pump tubing.
3. Software: Start software for the ICP-MS and autosampler control.
4. Daily pre-ignition maintenance checks: Perform and document daily maintenance checks (e.g., Ar supply pressure, interface components cleanliness and positioning, interface pump oil condition, vacuum pressure, etc.).
5. Place probe in adequate volume of rinse solution: Send the autosampler probe to a rinse solution (e.g. autosampler rinse station).
6. Start the plasma
7. Start the peristaltic pump: *S*tart the pump running slowly, making sure that the rotational direction is correct for the way the tubing is set up.
8. Warm-up time: Allow warm-up time suggested by the manufacturer for the ICP-MS (e.g. RF generator) after igniting the plasma. There will be another warm-up time (or "stability time") for the DRC later in this procedure.
9. Daily performance check: Perform and document a daily performance check and any optimizations necessary.

   Save new parameters to the "default.tun" and "default.dac" files.
10. Readying the instrument for quick-start analysis: Leave the plasma running to eliminate the need for an initial instrument warm-up period and/or a DRC stabilization period as long as appropriate planning is made for sufficient solution supply and waste collection. Analysis of conditioning samples (diluted serum matrix) can also be scheduled to occur at roughly a predetermined time. Accomplish this by setting up multiple sample analyses with extended rinse times (e.g. one analysis with a 1600s rinse time will take approximately 30 minutes to complete). Initial samples would be non-matrix, while final samples would be diluted matrix for conditioning. If running a DRC-only method during these scheduled analyses, the ICP-MS will remain in DRC-mode for approximately 45 minutes without depressurizing the cell. Prepare working dilutions of serum materials close in time to analysis so that they are not more than 7 hours old when analyzed (see Appendix B, ruggedness test 5).
11. Software setup for analysis:
    a. Workspace (files and folders): Verify and set up the correct files and data directories for your analysis (See Table 1 in Appendix C for defaults).

b. Samples / batch window: Update the software to reflect the current sample set. Use a bar code scanner to input data whenever possible. See Table 1 in Appendix C for times and speeds.

1. Serum vs. aqueous method files:

a. The difference: There are two method files for this one method (see Table 1 in Appendix C). It is necessary to use both to accomplish each run because the current PerkinElmer software will not allow for more than one blank per method file. The ONLY DIFFERENCE between these two files is on the Sampling tab where one lists the autosampler positions of the serum blank and serum calibrators (the "sblk" method file) and the other lists the autosampler position of the aqueous blank (the "aqblk" method file).

b. Use: The ONLY TIME when it matters which of these files is used is when the measurement action *includes* "Run blank" or "Run standards". When the measurement action is only 'run sample', it does not matter whether the "sblk" or "aqblk" method file is used. Analysts typically follow the pattern below, however, for the sake of consistency and as a reminder of which blank must be used for which type of sample. See Table 6 in Appendix C.

i. The *"sblk" method file:* *U*se to analyze the initial serum blank (blank for the calibration curve), the serum calibrators, and the serum blank checks at the very beginning of the run. The serum blank method defines the autosampler location of the serum blank and the serum calibrators.

ii. The "*aqblk*" method file must be used to analyze all QC materials and patient samples. The aqueous blank method defines the aqueous blank in autosampler location.

ii. Preparation of samples for analysis (See Table 7 in Appendix C)

1. Thaw serum samples; allow them to reach ambient temperature.

2. If instrument stability in DRC mode requires it, prepare 50mL^{+} of a junk serum sample to be analyzed repeatedly before the beginning of the run to achieve a stable analyte-to-internal standard ratio. Time to reach stability is instrument-specific but 1-1.5 hours is typical (~18 measurements of the 3 element serum method can be made in 1 hour). See Table 6 in Appendix C for example of setup in the Samples/Batch window.

NOTE: Selenium is not stable in the diluted sample for more than 7 hours. Diluted serum must be analyzed within 7 hours of preparation (see ruggedness parameter test 5 in Appendix B for details)

3. Prepare the following solutions into pre-labeled containers using the benchtop automatic pipette. See Table 7 of Appendix C for a summary of sample preparation.

   Prepare samples in the cleanest environment available to prevent trace element contamination and an environment which provides personnel protection (e.g. Class II, Type A/B3 biological safety cabinet).

   a. *Aqueous blank*: Prepare at least two aqueous blanks. One will be the actual reagent blank for patient and QC samples and the other will be a backup ("Aqueous Blank Check") in case the original aqueous blank is unusable.

   b. *Calibrators*: Prepare the working calibrators (S0-S5). Prepare at least three separate tubes of S0. One of these S0 preparations will be the zero standard (serum blank) for the calibrators; the other two will be analyzed after the last calibrator to verify washout.

   c. *Patient and QC samples*: Before taking an aliquot for analysis, homogenize the sample (e.g. vortex for 3-5 seconds).

   After preparation, mix and cover the diluted samples. Place prepared dilutions on the autosampler of the ICP-MS in the order corresponding to the sequence setup in the ICP-MS software.

   Original serum samples are not compromised by staying at ambient temperature during the work day. However, store long-term at ≤ -20 °C.

iii. Start the analysis using the ICP-MS software.

iv. Monitor the analysis in real-time as much as possible. If necessary, leave the run to complete itself unattended as long as appropriate planning is made for either overnight operation or Auto Stop (see below).

Monitor the analysis for the following:

1. *Verify proper operation of the instrument (sample reaching nebulizer in correct timing, autosampler arm moving properly, etc…).*
2. Verify that background signal from instrument and reagents are low. Helpful checks when diagnosing high background problems include:
   a. ≥18 MΩ·cm water to be used in Aq Blank Checks and dilutions.
   b. Diluent before and after being flushed through the benchtop automatic pipette.

   If contamination is observed from the pipette, flush the pipette with ≥500 mL of nitric acid solution (≤ 5% v/v HNO_3) and retest.

c. Comparison with other instruments.

3. *Verify analyte / internal standard ratio stability (esp. DRC measurements)*

   The net intensity (analyte / internal standard ratio) of the measurements made while stabilizing the DRC can be evaluated to determine the readiness of the system to begin analysis. Continual trending in this ratio indicates that unwanted instrument drift will occur within the run.

4. *Verify calibration curves* meet R^2 requirements (minimum of 0.98, typically 0.99 to 1.000).

5. *Verify bench QC results within the acceptable limits*.

   If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then the following steps are recommended:

   a. Evaluate the blank results.

   b. Evaluate the reproducibility of the 3 replicates within the measurements.

   c. Evaluate the consistency of the internal standard across the measurements (especially the calibrators).

   d. Evaluate calibration curves. If a particular calibrator is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte between or including S2 and S4 can be removed from the curve. Follow-up repeated problems with calibrators with appropriate corrective actions (e.g. re-preparation of intermediate working calibration standards or troubleshooting instrument parameters).

   e. Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.

   f. Prepare a fresh dilution of the failing QC material (unused vial) and analyze it to see if the QC vial had become compromised.

   g. Prepare and analyze new working calibrators.

   h. Test a different preparation of intermediate working calibration standards.

   If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions.

6. *Verify good precision among replicates of each measurement*.

7. *Verify consistent measured intensities of the internal standards*.

   Some sample-to-sample variations are to be expected, however, intensities drifting continuously in one direction resulting in failing results for ending

QC indicate the instrument needs additional pre-conditioning before the run or environmental conditions are changing too much around the instrument.

8. *Verify elevated patient results.*

   Refer to Figure 14 in Appendix C for flowchart.

   a. Confirming an elevated concentration: Repeat for confirmation any sample having a concentration greater than the 1UB threshold (see Table 9 in Appendix C).

   b. Dilution of a sample to within the calibration range: Repeat in duplicate with extra dilution any sample having a concentration greater than the highest calibrator to bring the observed result within the concentration range of the calibrators (see Table 8 in Appendix C).

   c. Confirming proper washout after an elevated sample: When monitoring the analysis in real-time, if a sample concentration is greater than standard 5 + 10% (see Table 4 in Appendix C), do the following to verify that the run is still in control for low concentration samples before proceeding with analysis.

      i. Stop run following elevated sample

      ii. Verify that the run is still in control for lower concentration samples before proceeding with analysis. Analyze 2 serum blank checks followed by a low bench QC washout check. If the low bench QC wash check is not in control (within ± 3SD limits), repeat these 3 check samples until washout is verified before proceeding with analysis.

      Example:
      3006 sblkchk Wash1
      3006 sblkchk Wash2
      LSXXXXX Wash

      iii. If the run is not verified in-control for low concentration samples before the next samples are analyzed, see Section 8.b.viii.2. for directions.

v. Instrument cleaning between consecutive runs: In between consecutive runs, aspirate ≥18 MΩ·cm water through the sample introduction system for approximately 30 minutes at peristaltic pump speed similar to that used in the analysis. This assists cleaning out the sample introduction system to prevent clogging.

vi. Overnight operation or using auto stop: Ensure sufficient solution supply and waste collection during unattended operation. Turn on the AutoStop feature of the ICP-MS software. Delay the shutdown at least 10 minutes (use peristaltic pump speed approximately that of the method wash) to rinse the sample introduction system of serum matrix before turning off the plasma. It will be

necessary to replace the sample peristaltic pump tubing the next day since it will have been clamped shut overnight.

vii. Records of results: Run results will be documented after each run in both electronic and paper form.

1. Electronic records: Transfer data electronically to the laboratory information system. When keyboard entry must be used, proofread transcribed data after entry.

   a. Export data from the ICP-MS software using “original conditions” or files and folders used during the analysis. Use descriptive report filenames (e.g. 2005-0714a_group55.txt). In the ICP-MS software under “Report Format” (METHOD window, REPORT tab) choose the “Use Separator” option, and under the “File Write” Section choose “Append.”

   b. Move the generated .TXT data file to the appropriate subdirectory on the network drive where exported data are stored prior to import to the laboratory information management system.

   c. Import the instrument file into the laboratory information system with appropriate documentation (e.g. instrument ID, analyst, calibrator lot number, and run or sample specific comments).

2. Paper records: Printed run sheets must be documented with

   i. Analyst initials

   ii. Instrument ID

   iii. Date of analysis and run # for the day

viii. Analyst evaluation of run results:

1. Bench quality control: After completing a run, and importing the results into the laboratory information system, evaluate the run bench QC according to laboratory QC rules. The QC limits are based on the average and standard deviation of the beginning and ending analyses of each of the bench QC pools, so it will not be possible to know if the run is in control until statistically reviewed.

   a. Quality Control Rules: The SAS program applies the division QC rules to the data as follows:

      i. If both QC run means (low and high bench QC) are within 2Sm limits and individual results are within 2Si limits, then accept the run.

      ii. If 1 of the 2 QC run means is outside a 2Sm limit - reject run if:

         1. Extreme Outlier – Run mean is beyond the characterization mean +/- 4Sm

2. 1 3S Rule – Run mean is outside a 3Sm limit
3. 2 2S Rule – Both run means are outside the same 2Sm limit
4. 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean

iii. If one of the 4 QC individual results is outside a 2Si limit - reject run if:

1. R 4S Rule – Within-run ranges for all pools in the same run exceed 4Sw (i.e., 95% range limit)

Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.

Abbreviations:

Si = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements).

Sm = Standard deviation of the run means (the limits are shown on the chart).

Sw = Within-run standard deviation (the limits are not shown on the chart).

b. Implications of QC Failures: If the division SAS program declares the run out of control" for any analyte, use the following to determine the implications on usability of the data from the run.

i. If only one analyte of the three fails bench QC, then report results for the other two which passed bench QC.

ii. If two analytes of the three fail bench QC, then no results are reportable from the run. Investigate the cause of QC failures and repeat the run with the appropriate corrective action.

2. Patient results:

a. Concentrations outside of the normal range (refer to Figure 14 in Appendix C for flowchart on handling elevated concentration samples):

i. Boundaries requiring confirmatory measurement:

1. Results outside of the first (1LB or 1UB) or second (2LB or 2UB) boundaries.

The concentrations assigned to 2LB, 1LB, 1UB, and 2UB for an element is determined by study protocol but default concentrations are in Table 9 in Appendix C.

a. Results lower than the first lower boundary or greater than the first upper boundary (1UB): Confirm by repeat analysis

of a new sample preparation any concentration observed lower than the 1LB or greater than the 1UB. Report the first analytically valid result, as long as the confirmation is within 10% or 3SD of the characterized bench low QC. Continue repeat analysis until a concentration can be confirmed.

b. Analyst reporting of results outside of the normal range: Report any patient results confirmed to be less than the second lower boundary (2LB) as an "unusually low result" or greater than the second upper boundary (2UB) as an "elevated result".

2. Results greater than highest calibrator: Samples that exceed the high calibrator must be prepared with minimum extra dilution in duplicate to bring the observed result within the calibration range (≤ S5). Report the first analytically valid result (i.e. the first one within the calibration range), as long as the confirmation is within 10%. Continue repeat analysis until a concentration can be confirmed.

ii. Concentrations requiring verification of washout: after a result is observed that is greater than the highest concentration validated for washout, do the following:

1. If the run was verified to be in control for lower concentration samples before subsequent sample analysis was performed, no further action is required.

2. If the run was not verified to be in control for lower concentration samples before subsequent sample analysis was performed, confirm by re-analysis the results for the 2 samples immediately following the elevated sample. Report the results if they confirm the initial results within ±10% or ±3SD of the low bench QC, whichever is greater.

b. Unacceptable measurement reproducibility: If the range of the three replicate readings (maximum replicate concentration value - minimum replicate concentration value) for a single sample analysis is greater than the range maximum criteria listed in Table 9 of Appendix C ***and*** the range of the three replicate readings is greater than 10% of the observed concentration, do not use the measurement for reporting. Repeat the analysis of the sample.

ix. Submitting final work for review: All analyses must undergo quality control and quality assurance review. After appropriately documenting the run in the laboratory information system (e.g. sample and run QC, and run and sample comments), inform the first level reviewer of the completed work and submit any printed documentation.

9) Routine equipment maintenance and data backups

Maintenance activities will be documented in the instrument logbook.

a. Equipment maintenance:

Analysts are expected to regularly evaluate the need for, and when necessary, perform cleaning, replacement, or re-positioning of components in ICP-MS the sample introduction system, interface, ion optics region, and equipment required resources (e.g. autosampler, exhaust, compressed gases, and coolant). Frequency of equipment maintenance will be dependent on instrument throughput.

b. Parameter optimizations:

Analysts are expected to optimize instrument parameters.

DRC optimizations: DRC conditions (cell gas flow rate and RPq value) can be verified by analyzing the DRC optimization solutions (see Section 6.f.i) as needed to ensure proper reduction of potential ICP-MS interferences.

c. Data backup:

Data on the instrument computer will be backed up via two backup routines. Files used and produced by the ICP-MS in analyzing samples will be backed up and kept a minimum of two years after analysis.

i. Daily backups to secondary hard drive: Program automatic backups of the relevant computer files to occur each night onto a secondary hard drive to prevent loss of data from failure of primary hard drive.

ii. Weekly backup: Backup relevant computer files weekly either to secondary hard drive which is remote to the laboratory or to removable media which will be placed remote to the laboratory for retrieval in the case of catastrophic data loss elsewhere.

10) Reporting thresholds

a. Reportable range:

Serum element concentrations are reportable in the range between the method LOD and the high calibrator times the maximum permitted extra dilution (see Table 8 of Appendix C).

Serum multi-element values are reportable in the range between the method LOD and the highest calibrator (see 'calibrator concentrations' in Table 1 of Appendix C) times the maximum validated extra dilution (see Table 7 of Appendix C). Above the highest calibrator, extra dilutions are made of the serum sample to bring the observed concentration within the calibration range.

b. Reference ranges (normal values):

In this method the 95% reference ranges (see Table 10 in Appendix C) for these elements in serum fall within the range of the calibrators.

c. Action levels:

There is no routine notification for levels of every analyte determined with this method. The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol.

11) Method calculations

a. Method limit of detection (LOD):

The method detection limits for elements in serum specimens are defined as 3 times s_0, where s_0 is the estimate of the standard deviation at zero analyte concentration. S_0 is taken as the y-intercept of a linear or 2nd order polynomial regression of standard deviation versus concentration (4 concentration levels of the analytes in serum each measured 60 times across at least a 2-month timeframe). Method LODs are re-evaluated periodically.

b. Method limit of quantitation (LOQ):

The Division of Laboratory Sciences does not currently utilize limits of quantitation in regards to reporting limits [5].

c. QC limits:

Quality control limits are calculated based on concentration results obtained in at least 20 separate runs. It is preferable to perform separate analyses on separate days and using multiple calibrator lot numbers, instruments, and analysts to best mimic real life variability. The statistical calculations are performed using the SAS program developed for the Division of Laboratory Sciences).

12) Alternate methods for performing test and storing specimens if test system fails

If the analytical system fails, setup analysis on other ICP-MS instrument, if available. If no other instrument is available, store the specimens at ≤ -20 °C until the analytical system can be restored to functionality.

13) Method performance documentation

Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method**.

Appendix A. Method performance documentation

a. Accuracy

i. Copper

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/dL
Reference material: **NIST SRM 1598a 10x, NIST SRM 1598a 2x, NIST SRM 1598a**
Analyte: **copper**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	15.8	19	17	15	16	12	15.13	2.56	16.95	-4.2
	2		18	17	13	13	12				
Level 2	1	79	80	75	79	85	71	77.24	5.38	6.96	-2.2
	2		78	76	78	84	67				
Level 3	1	158	168	161	167	182	157	164.28	8.88	5.40	4.0
	2		165	155	167	170	150				

ii. Selenium

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/L
Reference material: **NIST SRM 1598a 10x, NIST SRM 1598a 2x, NIST SRM 1598a**
Analyte: **selenium**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	13.4	13	18	10	11	10	12.01	3.06	25.46	-10.4
	2		13	17	10	10	10				
Level 2	1	67.2	89	67	66	65	66	70.06	8.72	12.44	4.2
	2		83	65	66	65	66				
Level 3	1	134.4	215	128	132	127	132	144.32	31.09	21.54	7.4
	2		189	129	132	127	133				

Appendix A. Method performance documentation

a. Accuracy

i. Copper

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/dL
Reference material: **NIST SRM 1598a 10x, NIST SRM 1598a 2x, NIST SRM 1598a**
Analyte: **copper**

Reference material	Replicate	Nominal value	Measured concentration Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	Difference from nominal value (%)
Level 1		15.8	19	17	15	16	12	15.13	2.56	16.95	-4.2
			18	17	13	13	12				
Level 2		79	80	75	79	85	71	77.24	5.38	6.96	-2.2
			78	76	78	84	67				
Level 3		158	168	161	167	182	157	164.28	8.88	5.40	4.0
			165	155	167	170	150				

ii. Selenium

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/L
Reference material: **NIST SRM 1598a 10x, NIST SRM 1598a 2x, NIST SRM 1598a**
Analyte: **selenium**

Reference material	Replicate	Nominal value	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	Difference from nominal value (%)
Level 1	1	13.4	13	18	10	11	10	12.01	3.06	25.46	-10.4
	2		13	17	10	10	10				
Level 2	1	67.2	89	67	66	65	66	70.06	8.72	12.44	4.2
	2		83	65	66	65	66				
Level 3	1	134.4	215	128	132	127	132	144.32	31.09	21.54	7.4
	2		189	129	132	127	133				

iii. Zinc

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/dL
Reference material: **NIST SRM 1598a 10x, NIST SRM 1598a 2x, NIST SRM 1598a**
Analyte: **zinc**

Reference material	Replicate	Nominal value	Measured concentration								Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	
Level 1	1	8.8	15	15	6.4	8.7	11	9.90	3.48	35.14	12.5
	2		8.0	13	8.5	6.4	6.5				
Level 2	1	44	43	44	39	48	46	43.50	3.62	8.33	-1.1
	2		41	45	38	48	43				
Level 3	1	88	93	102	84	105	96	93.69	6.79	7.24	6.5
	2		87	90	87	98	94				

b. Precision

i. Copper

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/dL
Analyte:	**copper**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	100	107	103.10	12.4541939	12.4541939	21257.17867
2	92	93	92.31	0.103458722	0.103458723	17043.28763
3	108	107	107.69	0.25170289	0.25170289	23194.0999
4	102	102	101.94	0.046893902	0.046893903	20781.71271
5	101	98	99.74	3.01890625	3.01890625	19895.53676
6	99	94	96.10	6.332520602	6.332520603	18469.01697
7	101	107	104.19	10.91972025	10.91972025	21712.69592
8	106	99	102.76	12.97368361	12.97368361	21119.85176
9	104	101	102.62	1.70380809	1.70380809	21061.27727
10	107	103	104.92	2.765735302	2.765735302	22014.3774

Grand sum 2030.7143 **Grand mean** 101.535715

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	101.141247	10.1141247	3.180271168	3.13
Between Run	359.0065728	39.8896192	3.858464364	3.80
Total	460.1478199		5.000187192	**4.92**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	251	271	261.03	92.5838462	92.5838462	136275.0446
2	241	242	241.32	0.621495723	0.621495722	116467.5477
3	239	236	237.54	1.529797923	1.529797923	112852.071
4	278	275	276.63	3.293317563	3.293317563	153052.3526
5	247	240	243.62	10.91575521	10.91575521	118705.5017
6	240	243	241.84	1.825336102	1.825336103	116975.0576
7	236	250	242.97	50.38728256	50.38728256	118064.1768
8	240	244	241.66	4.638208323	4.638208323	116795.0997
9	260	245	252.48	49.60807489	49.60807489	127491.6948
10	241	239	240.21	1.50749284	1.50749284	115396.8841

Grand sum 4958.5906 **Grand mean** 247.92953

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	433.8212147	43.38212147	6.586510568	2.66
Between Run	2694.393548	299.3770609	11.31359668	4.56
Total	3128.214763		13.09120282	**5.28**

ii. Selenium

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/L
Analyte:	**selenium**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	131	132	131.71	0.54745	0.54745	34695.15357
2	125	128	126.51	2.70997	2.70997	32009.25658
3	123	122	122.44	0.48275	0.48275	29982.47052
4	131	131	131.20	0.00853	0.00853	34426.69632
5	129	127	128.36	1.29743	1.29743	32951.06457
6	129	127	127.92	0.35295	0.35295	32729.40657
7	123	123	122.73	0.01943	0.01943	30123.24397
8	126	122	124.24	5.01469	5.01469	30870.98126
9	125	117	120.62	16.35838	16.35838	29096.46304
10	125	122	123.49	3.12883	3.12883	30499.78248

Grand sum 2518.4231 **Grand mean** 125.921155

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	59.84085507	5.984085507	2.446239053	1.94
Between Run	261.7733426	29.08592696	3.39866455	2.70
Total	321.6141977		4.187482087	**3.33**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	259	261	259.59	1.06069401	1.06069401	134770.7173
2	258	267	262.12	21.03827556	21.03827556	137409.3328
3	259	255	256.82	4.902460222	4.902460223	131911.7407
4	243	239	241.26	4.700440802	4.700440802	116416.7802
5	282	277	279.48	5.53049289	5.53049289	156214.8988
6	252	251	251.61	0.63059481	0.63059481	126620.1158
7	240	242	241.32	1.252049102	1.252049103	116466.9685
8	251	241	246.08	22.6437981	22.6437981	121108.715
9	241	233	236.90	17.0730108	17.0730108	112245.5416
10	229	226	227.85	2.23293249	2.23293249	103830.1513

Grand sum 5006.0458 **Grand mean** 250.30229

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	162.1294976	16.21294976	4.026530735	1.61
Between Run	3970.23443	441.1371589	14.57607988	5.82
Total	4132.363927		15.12200563	**6.04**

iii. Zinc

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/dL
Analyte:	**zinc**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	67	66	66.26	0.19084	0.19084	8779.54281
2	62	62	61.79	0.03644	0.03644	7636.15650
3	72	72	72.06	0.10775	0.10775	10385.84928
4	69	71	70.06	0.81072	0.81072	9817.78806
5	71	68	69.18	2.10787	2.10787	9570.92849
6	68	61	64.38	10.21346	10.21346	8288.57738
7	69	75	72.34	9.96318	9.96318	10466.28141
8	73	68	70.57	7.23368	7.23368	9959.38886
9	68	68	68.19	0.04933	0.04933	9298.79756
10	72	69	70.47	2.83956	2.83956	9932.80289

Grand sum	1370.5824	**Grand mean**	68.52912

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	67.10564165	6.710564165	2.590475664	3.78
Between Run	211.3074877	23.47860975	2.895517707	4.23
Total	278.4131294		3.88517528	**5.67**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	253	247	250.05	9.87907761	9.87907761	125047.7046
2	243	245	243.93	0.892930502	0.892930503	118999.3479
3	238	235	236.55	2.01696804	2.01696804	111914.6436
4	278	279	278.83	0.28643904	0.28643904	155492.3378
5	258	250	253.71	16.02641089	16.02641089	128733.1644
6	245	249	246.64	4.240098722	4.240098723	121661.642
7	233	255	244.08	129.8391881	129.8391881	119148.5307
8	241	257	248.84	62.87934912	62.87934912	123842.94
9	262	245	253.21	73.04693556	73.04693556	128234.4064
10	240	237	238.59	3.514687563	3.514687563	113850.5194

Grand sum	4988.8471	**Grand mean**	249.442355

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	605.2441703	60.52441703	7.779744021	3.12
Between Run	2495.467355	277.2741506	10.41032501	4.17
Total	3100.711526		12.99612572	**5.21**

c. Stability

i. Copper

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -20°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples in cryovial stored at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples from 2 characterized pools stored at -70°C for 3 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/dL
Analyte:	**copper**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	96	101	100	100	98	102	96	96
Replicate 2	100	97	99	101	96	99	100	100
Replicate 3	98	98	99	102	97	102	102	98
Mean	97.72366667	98.592	99.50733333	100.9	96.90933333	101.257	99.40943333	97.7
% difference from initial measurement	--	**0.9**	--	**1.4**	--	**4.5**	--	**-1.7**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	254	244	262	243	245	245	229	254
Replicate 2	258	248	265	245	252	255	243	258
Replicate 3	271	252	250	259	239	251	278	271
Mean	260.9496667	247.9396667	258.9463333	249.1	245.182	250.188	250.3471667	260.9
% difference from initial measurement	--	**-5.0**	--	**-3.8**	--	**2.0**	--	**4.2**

ii. Selenium

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -20°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples in cryovial stored at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples from 2 characterized pools stored at -70°C for 3 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Multi-Elements in Serum by ICP-DRC-MS
Method #: 3006
Matrix: Serum
Units: µg/L
Analyte: **selenium**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	127	129	128	126	128	130	126	127
Replicate 2	128	124	128	125	127	129	125	128
Replicate 3	126	125	129	126	127	129	125	126
Mean	126.96	125.7446667	128.465	125.7	127.419	129.396	125.4663	127.0
% difference from initial measurement	--	**-1.0**	--	**-2.1**	--	**1.6**	--	**1.2**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	260	247	271	253	259	257	251	260
Replicate 2	266	249	278	254	263	257	241	266
Replicate 3	280	258	266	259	255	260	229	280
Mean	268.5686667	251.046	271.722	255.4	259.2036667	257.9403333	240.4046667	268.6
% difference from initial measurement	--	**-6.5**	--	**-6.0**	--	**-0.5**	--	**11.7**

iii. Zinc

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Three times frozen at -20°C and then thawed (3 freeze-thaw cycles) to room temperature.
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Original samples in cryovial stored at room temperature for 1 day prior to preparation and analysis.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Processed samples (ready for instrument analysis) stored at room temperature for 1 day prior to analysis.
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Samples from 2 characterized pools stored at -70°C for 3 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/dL
Analyte:	**zinc**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	65	68	68	71	66	69	73	65
Replicate 2	68	66	68	72	65	68	68	68
Replicate 3	66	65	67	72	66	69	72	66
Mean	66.389	66.57566667	67.988	71.9	65.76233333	68.527	71.2743	66.4
% difference from initial measurement	--	**0.3**	--	**5.7**	--	**4.2**	--	**-6.9**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measuremen**	**Bench-top stability**	**Initial measuremen**	**Processed sample**	**Initial measuremen**	**Long-term**
Replicate 1	257	242	265	247	247	245	241	257
Replicate 2	262	261	262	249	254	256	262	262
Replicate 3	274	253	271	247	241	252	240	274
Mean	264.2793333	252.086	265.802	247.9	247.354	250.9143333	247.712	264.3
% difference from initial measurement	--	**-4.6**	--	**-6.7**	--	**1.4**	--	**6.7**

d. Analytical Sensitivity and Specificity

LOD, specificity and fit for intended use

Method name:	Multi-Elements in Serum by ICP-DRC-MS
Method #:	3006
Matrix:	Serum
Units:	µg/dL (selenium in µg/L)

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
copper (SCU)	2.5	Yes	Yes
selenium (SSE)	4.5	Yes	Yes
zinc (SZN)	2.9	Yes	Yes

Appendix B. Ruggedness testing results.

a. Ruggedness parameter test #1

Evaluate the impact on analysis results if the set RF power is increased to 1600W (instrument maximum) or decreased to 1150W (by 20%).

i. Test details: Three different PF power settings were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the RF power was changed. Each run had 20 “Junk serum” samples analyzed between the beginning and ending QC. All other method parameters were kept per method.

1. Run #1: Method default with RF power 1450W.
2. Run #2: Decreased RF power by 20% to 1150W.
3. Run #3: Increased RF power to instrument maximum, 1600W.
4. Run #4: Increased RF power to 1525W.

ii. Results: See Ruggedness Table 1.

iii. Conclusion: Results are not compromised by changes in RF power within the range of 1150W to 1600W.

Ruggedness Table 1. Impact of changing RF power on observed analyte concentrations.

QC pool ID	RF power tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	1150w (reduced)	52.5	63.1	75.6
	1450w (per method)	49.0	62.7	70.1
	1525w (increased)	43.3	63.4	75.7
	1600w (increased)	54.1	63.9	75.6
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	1150w (reduced)	178	197	145
	1450w (per method)	168	196	146
	1525w (increased)	157	201	145
	1600w (increased)	178	199	149

Appendix B. Ruggedness testing results (continued).

b. Ruggedness parameter test #2

Evaluate the impact on analysis results if the Cell Gas Flow Rate is increased or decreased by 20% for the analytical run.

i. Test details: Three different Cell Gas Flow Rates were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

1. Run #1: Method default = 0.5mL/min.
2. Run #2: Decreased Cell Gas Flow Rate by 20% to 0.4mL/min.
3. Run #3: Increased Cell Gas Flow Rate by 20% to 0.6mL/min.

ii. Results: See Ruggedness Table 2.

iii. Conclusion: Results are not compromised by changes in cell gas flow rate within the range tested (0.40-0.60 mL/min).

Ruggedness Table 2. Impact of changing DRC mode cell gas flow rate on observed analyte concentrations

QC pool ID	cell gas flow rate tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	0.40 ml/min (reduced)	49.5	65.3	80.7
	0.50 ml/min (per method)	49.5	67.6	73.8
	0.60 ml/min (increased)	47.8	63.4	75.6
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	0.40 ml/min (reduced)	167	204	152
	0.50 ml/min (per method)	169	205	146
	0.60 ml/min (increased)	171	203	147

Appendix B. Ruggedness testing results (continued).

c. Ruggedness parameter test #3

Evaluate the impact on analysis results if the RPq is increased or decreased by 20% for the analytical run.

i. Test details: Three different RPq settings were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

1. Run #1: Method default DRC RPq: 0.56
2. Run #2: Decreased DRC RPq 20%: 0.70
3. Run #3: Increased DRC RPq 20%: 0.84

ii. Results: See Ruggedness Table 3

iii. Conclusion: Results are not compromised by changes in RPq within the range of 0.56 – 0.84.

Ruggedness Table 3. Impact of changing RPq value on observed analyte concentrations.

QC pool ID	RPq tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	75.0 66.7 – 83.3
	DRC RPq:0.56 (reduced by 20%)	52.5	63.1	70.8
	DRC RPq:0.70 (per method)	49.0	62.7	70.1
	DRC RPq:0.84 (increased by 20%)	54.1	63.9	75.9
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	DRC RPq:0.56 (reduced by 20%)	178	197	129
	DRC RPq:0.70 (per method)	168	196	131
	DRC RPq:0.84 (increased by 20%)	178	199	145

Appendix B. Ruggedness testing results (continued).

d. Ruggedness parameter test #4

Evaluate the impact on analysis results if the axial field voltage (AFV) is increased or decreased by 20% for the analytical run.

i. Test details: Three different DRC AFV were tested in separately prepared, consecutive runs on the instrument without turning off the plasma. At least 15 minutes stabilization time was allowed between each run after the axial field voltage was changed. "Junk serum" samples (20) were analyzed between the beginning and ending QC of each run. All other method parameters were kept per method.

1. Run #1: Method default DRC AFV = 450
2. Decreased DRC AFV to 360
3. Increased DRC AFV to 500

Results: See

ii. Ruggedness Table 4.

iii. Conclusion: Results are not compromised by changes in the axial field voltage within the range of 360 to 500V.

Ruggedness Table 4. Impact of changing axial field voltage (AFV) on observed analyte concentrations.

QC pool ID	axial field voltage tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range	50.7 41.9 - 59.5	64.9 61.9 – 67.9	74.9 66.7 – 83.3
	AFV-360 (reduced)	46.9	61.5	72.8
	AFV-360 (per method)	47.2	64.0	75.0
	AFV-500 (increased)	48.6	63.5	74.1
HS-03601b	characterized mean 2SD range	175 142 – 209	203 191 – 215	144 130 – 157
	AFV-360 (reduced)	163	195	142
	AFV-360 (per method)	170	205	147
	AFV-500 (increased)	168	200	146

Appendix B. Ruggedness testing results (continued).

e. Ruggedness parameter test #5

Method descriptions and SOP assume preparation and analysis on same day. Evaluate the impact on analysis results if the analytical run is prepared to analyze but circumstances do not allow for analysis to occur until 24 or 48 hours later.

i. Test details (Part 1: 24-hour increments): All runs had 20 "Junk serum" samples analyzed between the beginning and ending QC of each run, making each a normal length run. All other method parameters were kept per method.

1. Day 1: Prepare samples for analysis in triplicate (calibrators, blanks, QC and reference materials in three separate sets of tubes). Set '#1 was analyzed immediately. Sets #2 and #3 were capped and stored at ambient temperature.
2. Day 2: Prepare a new run (set #4). Analyze set #4 immediately, then set #2.
3. Day 3: Prepare a new run (set #5). Analyze set #5 immediately, then set #3.

ii. Test details (Part 2 within 24 hrs.): Due to the observations in test #1 for selenium, a shorter time frame was examined in part two of this test.

1. Sample Preparation: Seven preparations of the low bench QC serum material were made at the beginning of the experiment. Each of these seven preparations were 4x the normal preparation volume (4 preparations into each vial).
2. Sample Analysis: Four consecutive runs of the serum method were then carried out. Each run included blanks, calibrators, and run judge QC (beginning and ending) prepared immediately prior to the beginning of each run and seven preparations of the low bench QC prepared immediately prior to the beginning of each run. The seven preparations of the low bench QC pool prepared before the first run were measured within each run alternated with the freshly prepared low bench QC sequentially throughout the run.

iii. Results: See results in Ruggedness Table 5 and Ruggedness Table 6.

iv. Conclusions:

1. Test part 1: The serum ICP-MS method is rugged for Zn and Cu to delays in analysis of samples after preparation for up to 48 hours and not rugged for Se to delay in analysis of samples after preparation for even 24 hours. The serum ICP-MS method is rugged for Zn and Cu to delays in analysis of samples after preparation for up to 48 hrs (see part 1).

2. Test part 2: The method is only rugged to delays in analysis for selenium for up to approximately 7 hours (one 90 patient sample run, or two 40

patient sample runs). Suggested maximum amount of time from sample prep to end of the run is 450 min, which consists of 3 analytical runs.

Ruggedness Table 5. Stability of sample preparations part 1 (24-hour increments).

QC pool ID	time from preparation	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	*characterized mean* *±2SD range* *±3SD range*	50.7 41.9 – 59.5 37.5 – 63.9	64.9 61.9 – 67.9 60.4 – 69.4	74.9 66.7 – 83.3 52.5 – 87.4
	fresh preparation	47.2	65.7	74.7
	after 24 hours	51.9	67.1	***102*** ***(150, 54.5)***
	after 48 hours	51.0	66.2	***57.1*** ***(123, -8.8)***
HS-03602b	*characterized mean* *±2SD range* *±3SD range*	175 142 – 209 126 - 225	203 191 – 215 185 - 221	144 130 – 157 124 – 164
	fresh preparation	160	203	143
	after 24 hours	167	203	145
	after 48 hours	174	207	***130*** ***(98.2, 161)***

Ruggedness Table 6. Stability of sample preparations part 2 (within 24 hours).

QC pool ID	axial field voltage tested	Zn (µg/dL)	Cu (µg/dL)	Se (µg/L)
LS-03601b	characterized mean 2SD range 3SD range	50.7 41.9 – 59.5 37.5 – 63.9	64.9 61.9 – 67.9 60.4 – 69.4	74.9 66.7 – 83.3 52.5 – 87.4
	Run 1 (up to 139 min elapsed)	41.8	58.3	72.5
	Run 2 (up to 303 min elapsed)	43.1	60.0	71.3
	Run 3 (up to 427 min elapsed)	51.4	65.9	71.0
	Run 4 ***(up to 576 min elapsed)***	43.5	59.1	***54.7***

Appendix B. Ruggedness testing results (continued).

f. Ruggedness parameter test #6

Evaluate the impact on observed concentration if an extra dilution is performed on the sample relative to the calibration standards.

i. Test details: A large serum sample, spiked to elevated analyte concentrations, was prepared for analysis each day for 4 days.

1. Day 1: A large serum sample was spiked to elevated concentrations using single element standards and mixed well. The spiked sample was then prepared for analysis to dilution levels of 2x, 5x, 10x, and 20x using ≥18 MΩ·cm water as the makeup liquid.
2. Day 2: The spiked sample prepared on Day 1 was prepared for analysis to dilution levels of 2x, 5x, 10x, and 20x using ≥18 MΩ·cm water as the makeup liquid.
3. Day 3: The spiked sample prepared on Day 1 was prepared for analysis to dilution levels of 2x, 5x, 10x, and 20x using ≥18 MΩ·cm water as the makeup liquid.
4. Day 4: The spiked sample prepared on Day 1 was prepared for analysis to dilution levels of 2x, 5x, 10x, and 20x using ≥18 MΩ·cm water as the makeup liquid.

ii. Results: See Ruggedness Table 7, Ruggedness Table 8, and Ruggedness Table 9.

iii. Conclusion: SCU results are not unacceptably affected by the matrix difference in the dilution with ≥18 MΩ·cm water when performing a 2x, 5x or 10x dilution. However, the average observation of the 20x dilution is affected more than is acceptable (≥10% bias with noticeable impact on reproducibility).

SSE results are not unacceptably affected by the matrix difference in the dilution with ≥18 MΩ·cm water when performing a 2x or 5x dilution. However, the average observation of the 10x and 20x dilutions are affected more than is acceptable (≥10% bias with noticeable impact on reproducibility).

SZN results are not unacceptably affected by the matrix difference in the dilution with ≥18 MΩ·cm water when performing a 2x, 5x or 10x dilution. However, the average observation of the 20x dilution is affected more than is acceptable ≥10% bias with noticeable impact on reproducibility).

In summary, an extra dilution up to 10x with ≥18 MΩ·cm water was successful for SCU and SZN with less than a 10% impact on the observed concentration. However only up to a 5x extra dilution with ≥18 MΩ·cm water was acceptable for SSE.

Appendix B. Ruggedness testing results (continued).

Ruggedness Table 7. Impact of extra dilutions on observed concentrations of serum copper (SCU) in µg/dL.

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SCU Average	SCU STDEV	SCU Normalized Average and Relative STDEV
No Extra	353	348	347	350	350	3	1.00 ± 0.01
2x dilution	363	355	396	354	367	20	1.05 ± 0.05
5x dilution	414	342	343	366	366	34	1.05 ± 0.09
10x dilution	378	345	342	347	353	17	1.01 ± 0.05
20x dilution	334	313	269	337	313	31	0.90 ± 0.10

Ruggedness Table 8. Impact of extra dilutions on observed concentrations of serum selenium (SSE) in µg/L.

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SSE Average	SSE STDEV	SSE Normalized Average and Relative STDEV
No Extra	367	340	347	343	349	12	1.00 ± 0.03
2x dilution	361	343	387	331	355	24	1.02 ± 0.07
5x dilution	392	326	337	316	343	34	0.98 ± 0.10
10x dilution	347	316	340	262	316	38	0.91 ± 0.12
20x dilution	283	272	268	191	253	42	0.72 ± 0.17

Ruggedness Table 9. Impact of extra dilutions on observed concentrations of serum zinc (SZN) in µg/dL.

Dilution level	4/20/12 b	5/8/12 b	5/9/12 b	6/6/12 b	SZN Average	SZN STDEV	SZN Normalized Average and Relative STDEV
No Extra	343	335	334	336	337	4	1.00 ± 0.01
2x dilution	348	340	382	338	352	20	1.05 ± 0.06
5x dilution	390	326	330	351	349	29	1.04 ± 0.08
10x dilution	344	320	329	340	333	11	0.99 ± 0.03
20x dilution	293	283	260	340	294	34	0.87 ± 0.12

Appendix C. Tables and figures.

Table 1. Instrument and method parameters.

Instrument: PerkinElmer ELAN DRC^{Plus} or DRC II ICP-MS ESI SC4 autosampler	
Optimization window parameters	
RF power	1.45 KW
plasma gas flow (Ar)	15 L/min
auxiliary gas flow (Ar)	1.2 L/min
nebulizer gas flow (Ar)	0.80 – 1.0 L/min (optimized as needed for sensitivity)
ion lens voltage(s)	AutoLens (optimized as needed for sensitivity)
QRO, CRO, CPV, AFV,Discriminator Threshold	Optimized per instrument by service engineer, or advanced user.
Parameters of x-y alignment, nebulizer gas flow, AutoLens voltages, mass calibration, and detector voltages are optimized regularly. Optimization file name = default.dac.	
Configurations window parameters	
cell gas changes pause times	pressurize delay (From Standard to DRC mode) = 30 exhaust delay (From DRC to Standard mode) = 30 flow delay (Gas changes while in DRC mode) = 25 channel delay (Gas channel change in DRC mode) = 25
File names and directories	
method file names	Serum multi panel_DLS3006.8_sblk.mth Serum multi panel_ DLS3006.8_aqblk.mth
dataset	Create a new dataset subfolder each day. Name as "2006-0718" for all work done on July 18, 2006
sample file	Create for each day's work
report file name	See Figure 7 of Appendix C. *For sample results printouts* cdc_quant comprehensive.rop *For calibration curve information* CDC_Quant Comprehensive (calib curve info).rop
tuning	Default.tun
optimization	Default.dac
calibration	N/A
polyatomic	elan.ply
report options template (transferring results to the database)	CDC_Database Output.rop *Report Format Options: select only "Use Separator"* *File Write Option: Append* *Report File name: include date, instrument, and group being analyzed in file name (i.e. 20060724a_DRCC_HM-0364.txt)*
Method parameters	

Method parameters: timing page (see Figure 2 in Appendix C)	
sweeps/reading	90
readings/replicate	1
replicates	3
enable qc checking	on
isotopes monitored and internal standard associations (exact mass)	*use ^{71}Ga as an internal standard* ^{64}Zn (63.9291), ^{65}Cu (64.9278), ^{71}Ga (70.9247), ^{78}Se (77.9173)
dwell times	30 ms for ^{64}Zn, ^{65}Cu, ^{71}Ga (70.9247), ^{78}Se (77.9173)
scan mode	Peak Hopping for all isotopes (1 MCA channel)
drc channel a gas flow rate	Ammonia (5-7 psig delivery pressure) 0.5 L/min * (*Optimized per instrument)
RPa	0 for all isotopes
RPq	0.7 for all isotopes
Method Parameters: processing page (see Figure 3 in Appendix C)	
detector mode	pulse (see Section 6.f.ii)
process spectral peak	N/A
autolens	On
isotope ratio mode	Off
enable short settling time	Off
blank subtraction	after internal standard
measurement units	Cps
process signal profile	N/A
Method Parameters: equations page (see Figure 4 in Appendix C)	
equations	On ^{64}Zn, use "-0.035297 * Ni60" On ^{78}Se, use "-0.030461 * Kr83"
Method Parameters: calibration page (see Figure 5 in Appendix C)	
calibration type	external std.
curve type	simple linear
sample units	µg/L
calibration standard concentrations	Zn (µg/dL): 3, 9, 30, 90, 300 Cu (µg/dL): 3, 9, 30, 90, 300 Se (µg/L): 3, 9, 30, 90, 300

Method Parameters: sampling page (see Figure 6 in Appendix C)	
"peristaltic pump under computer control"	On
sample flush	~40s at typically -10.8 rpm (optimize time so that solution reaches nebulizer before Read Delay begins)
read delay	45s at typically -8.1 rpm (optimize time so that signal is stable before analysis begins)
wash	60s at typically -10.8 rpm (optimize time as needed for effective washout of unusually elevated samples)
extended wash (via ICP-MS software QC checking)	For sample concentrations greater than these, setup the ICP-MS software's 'QC checking' feature to "Wash for X and continue." See Figure 8 in Appendix C. **Analyte** / **Extended Rinse Trigger Conc.*** / **Extended Rinse Time** Cu >330 µg/dL 120 s Se >330 µg/L 120 s Zn >330 µg/dL 120 s
autosampler locations of blanks and standards	*For calibration curve (points to serum blank)* Serum multi panel_DLS3006.8_sblk.mth Serum Blank and Calibration Stds 1 – 5 in autosampler positions 101 – 106. *For QC and patient sample analysis (points to aqueous blank)* Serum multi panel_DLS3006.8_aqblk.mth Aqueous Blank in autosampler position 109. See figures 9 through 13 in Appendix C for other default autosampler settings.

Appendix C. Tables and figures (continued).

Table 2. Suggested maximum analyte concentrations for base serum.

Analyte	Concentration (µg/L)
Zn	800 (80 µg/dL)
Cu	1100 (110 µg/dL)
Se	130

Table 3. Stock calibration standard concentrations.

Analyte	*stock standard concentrations (mg/L)* High Purity Standards Item # SM-2107-013 (2% HNO_3)
Cu	300
Zn	300
Se	30

Table 4. Preparation of multi-element intermediate working calibration standards.

Standard #	Units	1	2	3	4	5
Vol of flask (mL)		500	200	100	100	100
Vol spike of int. stock std. (mL)		0.050	0.060	0.10	0.30	1.0
	Concentrations					
Zn	µg/L µg/dL*	30 3	90 9	300 30	900 90	3,000 300
Cu	µg/L µg/dL*	30 3	90 9	300 30	900 90	3,000 300
Se	µg/L	3	9	30	900	300

* Use µg/dL units for Zn and Cu in the ELAN software and for reporting.

Appendix C. Tables and figures (continued).

Table 5. Acceptable ways to perform two consecutive analytical runs, bracketing with bench quality control samples.

Setup 1*	**Setup 2** (typical)*
Run #1 calibration standards low bench QC high bench QC patient samples low bench QC high bench QC *Run #2* low bench QC high bench QC patient samples low bench QC high bench QC * Use ≥18 MΩ·cm water to rinse the system for ~30 min. between the two runs.	*Run #1* calibration standards low bench QC high bench QC patient samples low bench QC high bench QC *Run #2* calibration standards low bench QC high bench QC patient samples low bench QC high bench QC * Use ≥18 MΩ·cm water to rinse the system for ~30 min. between the two runs.

Appendix C. Tables and figures (continued).

Table 6. A typical SAMPLE/BATCH window.

AS Location*	Sample ID	Measurements Action	Method
5	DRCstability1	Run sample	. . ._sblk.mth
5	DRCstability2	Run sample	. . ._sblk.mth
5	DRCstability3	Run sample	. . ._sblk.mth
5	DRCstability4	Run sample	. . ._sblk.mth
Continue DRC stability samples . . .			
5	DRCstability9	Run sample	. . ._sblk.mth
5	DRCstability10	Run sample	. . ._sblk.mth
100	Sblkchk1	Run blank, standards, and sample **	. . ._sblk.mth
101	Sblkchk2	Run sample	. . ._sblk.mth
127	Aq Blk Check	Run blank and sample ¥	. . ._aqblk.mth
138	L Bench QC	Run sample	. . ._aqblk.mth
134	H Bench QC	Run sample	. . ._aqblk.mth
146	Sample 1	Run sample	. . ._aqblk.mth
147	Sample 2	Run sample	. . ._aqblk.mth
148	Sample 3	Run sample	. . ._aqblk.mth
.	.	.	.
.	.	.	.
.	.	.	.
139	L Bench QC	Run sample	. . ._aqblk.mth
135	H Bench QC	Run sample	. . ._aqblk.mth

* The exact autosampler positions of QCs and patient samples do not have to be those shown above.

** When executing this row, the ELAN will first analyze the serum blank at AS position 101, then standards 1-5 at autosampler positions 102-106, then the “sblkchk1” sample at A/S position 100. The sampling information about AS positions 101-106 are stored in the “sblk” method file.

¥ When executing this row, the ELAN will first analyze the aqueous blank at AS position 109, then the “Aq Blk Check” at AS position 20. The sampling information about AS positions 109 is stored in the “aqblk” method file.

Appendix C. Tables and figures (continued).

Table 7. Preparation of samples, working calibrators, and QC materials for analysis.

Dilution ID	Water (µL)	Base Serum (µL)	AQ Intermediate Working Calibration Standard (µL)	Patient or QC Serum sample (µL)	Diluent * (µL)	Total volume (µL)
Working Calibrators (S0-S5) And SBlkchk (S0)	-	150 x 1	150 x 1	-	4,200 (2,100 x 2)	4,500
AQ Blank	300 (150 x 2)	-	-	-	4,200 (2,100 x 2)	4,500
Patient Serum or Serum-Based QC	150 x 1	-	-	150 x 1	4,200 (2,100 x 2)	4,500
Patient Serum *2x Extra Dilution* [H]	225 (225 x 1)	-	-	75 x 1	4,200 (1,400 x 3)	4,500
Patient Serum *3x Extra Dilution* [H]	250 (250 x 1)	-	-	50 x 1	4,200 (2,100 x 2)	4,500
Patient Serum *5x Extra Dilution* [H]	540 (150 x 3, 90 x 1)			60 x 1	8,400 (1,680 x 5)	9,000
Patient Serum *10x Extra Dilution* [H]	570 (150 x 3, 120 x 1)	-	-	30 x 1	8,400 (1,680 x 5)	9,000

If a different total volume is prepared, adjust the volumes for each component proportionally.

These directions are written with the expectation of a 5,000 µL syringe on the left side and a 250 µL syringe on the right side of the benchtop automatic pipettor.

* By splitting the dispense step of diluent into two or more portions, liquids pulled up into the right pipette tip are flushed out more completely. For example, when preparing a working serum blank (S0) above, do the preparation in 2 steps: in step 1, dispense 150 µL intermediate working S0 + 2100 µL diluent; in step 2, dispense 150 µL base serum + 2100 µL diluent.

[H] Extra dilution is performed on serum samples whose concentration is greater than the concentration of the highest working calibrator listed in Table 8 of Appendix C.

Any extra dilution within these limits can be prepared as long as the 14:15 ratio of diluent to total dilution volume is maintained. Use of the lowest extra dilution level is preferred to minimize differences between the calibrators and the samples (i.e. 2x dilution is preferred over 10x if 2x is sufficient to dilute analyte into the documented linearity range).

Appendix C. Tables and figures (continued).

Table 8. Reportable range concentrations.

Analyte (units)	Limit of Detection (LOD)*	High Calibrator	Maximum Extra Dilution**	Reportable Range Upper Boundary
Zn (µg/dL)	2.9	300	10	3,000
Cu (µg/dL)	2.5	300	10	3,000
Se (µg/L)	4.5	300	5	1,500

*Re-evaluated periodically (2+ years) or at significant method changes. LODs shown were calculated 1/26/2011.
**See ruggedness test 6 in Appendix B for supporting validation data.

Table 9. Boundary concentrations and replicate range maximums for serum.

Analyte (units)	Lower Boundaries		Upper Boundaries		Range Maximum ("Rep Delta Limit")†	Highest Concentration Validated for Washout
	2LB*	1LB*	1UB*	2UB*		
Zn (µg/dL)	35	35	120	240	17 for values < 170 10% for values ≥170	330
Cu (µg/dL)	50	50	300	600	20 for values < 200 10% for values ≥ 200	330
Se (µg/L)	45	45	165	330	20 for values < 200 10% for values ≥ 200	330

* The concentrations assigned to these boundaries is determined by study protocol but default concentrations are listed in this table.

† Range maximum is the range of the three replicate readings for a single sample analysis. This value is also called "Rep Delta Limit" in the LIMS.

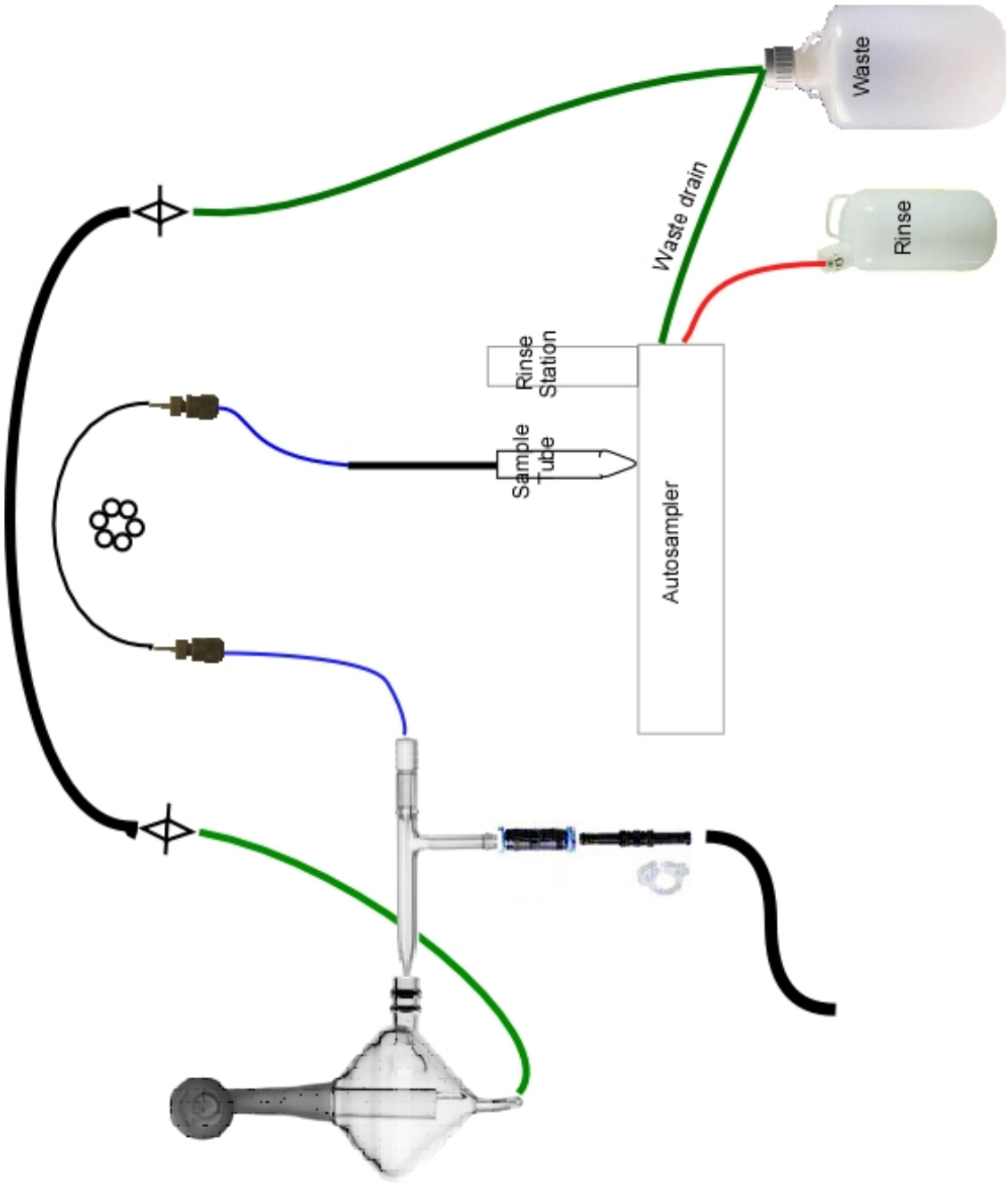
Appendix C. Tables and figures (continued).

Table 10. Reference ranges for concentrations of zinc, copper, and selenium in serum [6]

analyte (units)	survey years	geometric mean	50th	75th	95th	N
Zn (µg/dL)	11-12	81.9 (80.9 - 82.9)	81.8 (80.8 - 82.9)	91.5 (89.8 - 93.1)	109 (105-112)	2329
	13-14	80.4 (78.2-82.6)	80.7 (78.4-82.9)	90.5 (88.0-93.1)	109 (104-113)	2519
Cu (µg/dL)	11-12	114 (111 – 116)	112 (109 – 114)	130 (127 - 134)	169 (161-183)	2329
	13-14	115 (112-118)	114 (110-117)	132 (129-135)	171 (165-180)	2520
Se (µg/L)	11-12	127 (125 - 130)	127 (124-130)	139 (136 - 142)	161 (156-164)	2329
	13-14	128 (126-130)	127 (126-130)	139 (137-142)	159 (156-161)	2519

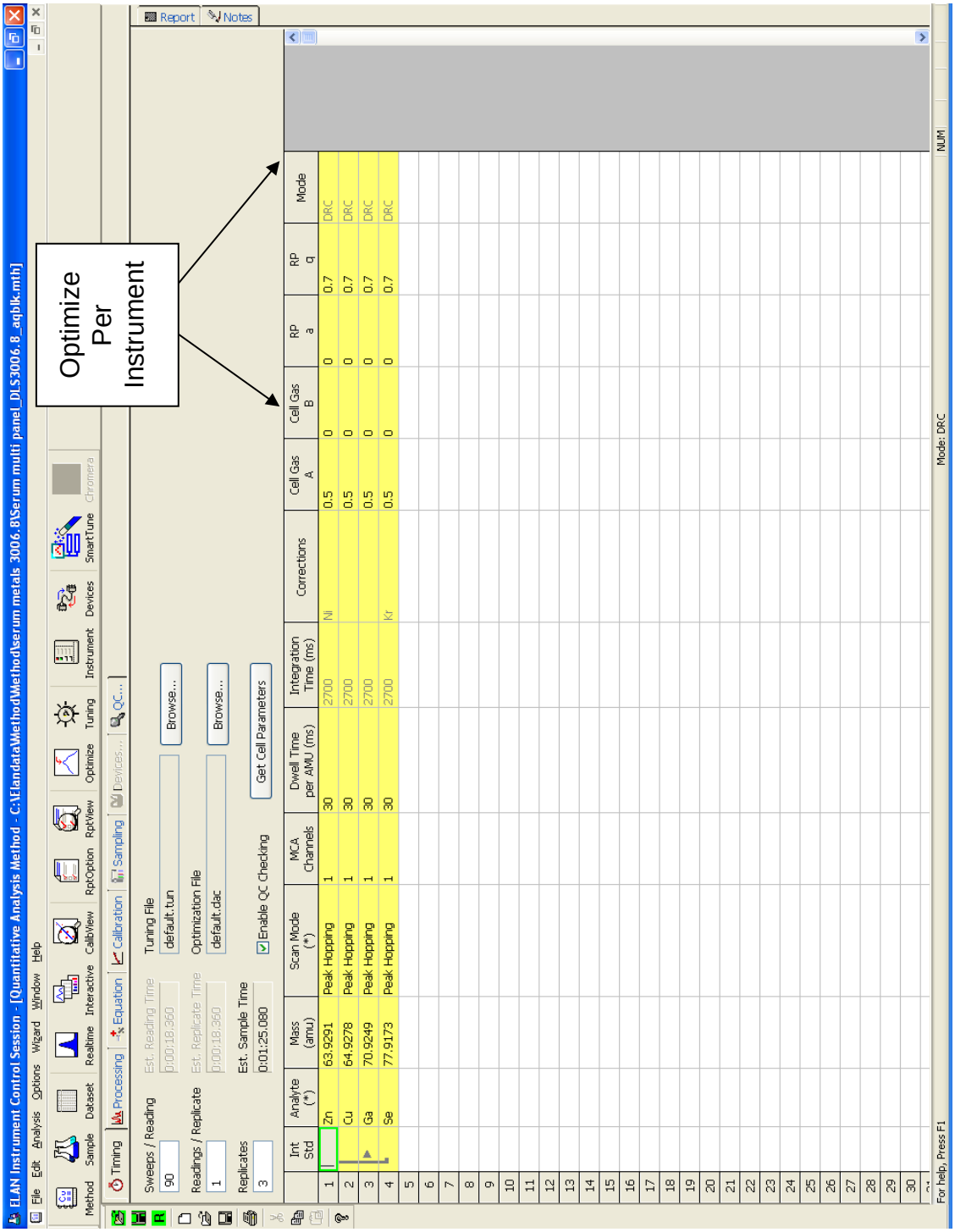
Appendix C. Tables and figures (continued).

Figure 1. Example configuration of tubing and devices for liquid handling.



Appendix C. Tables and figures (continued).

Figure 2. ELAN ICP-DRC-MS method screen shots (timing page).

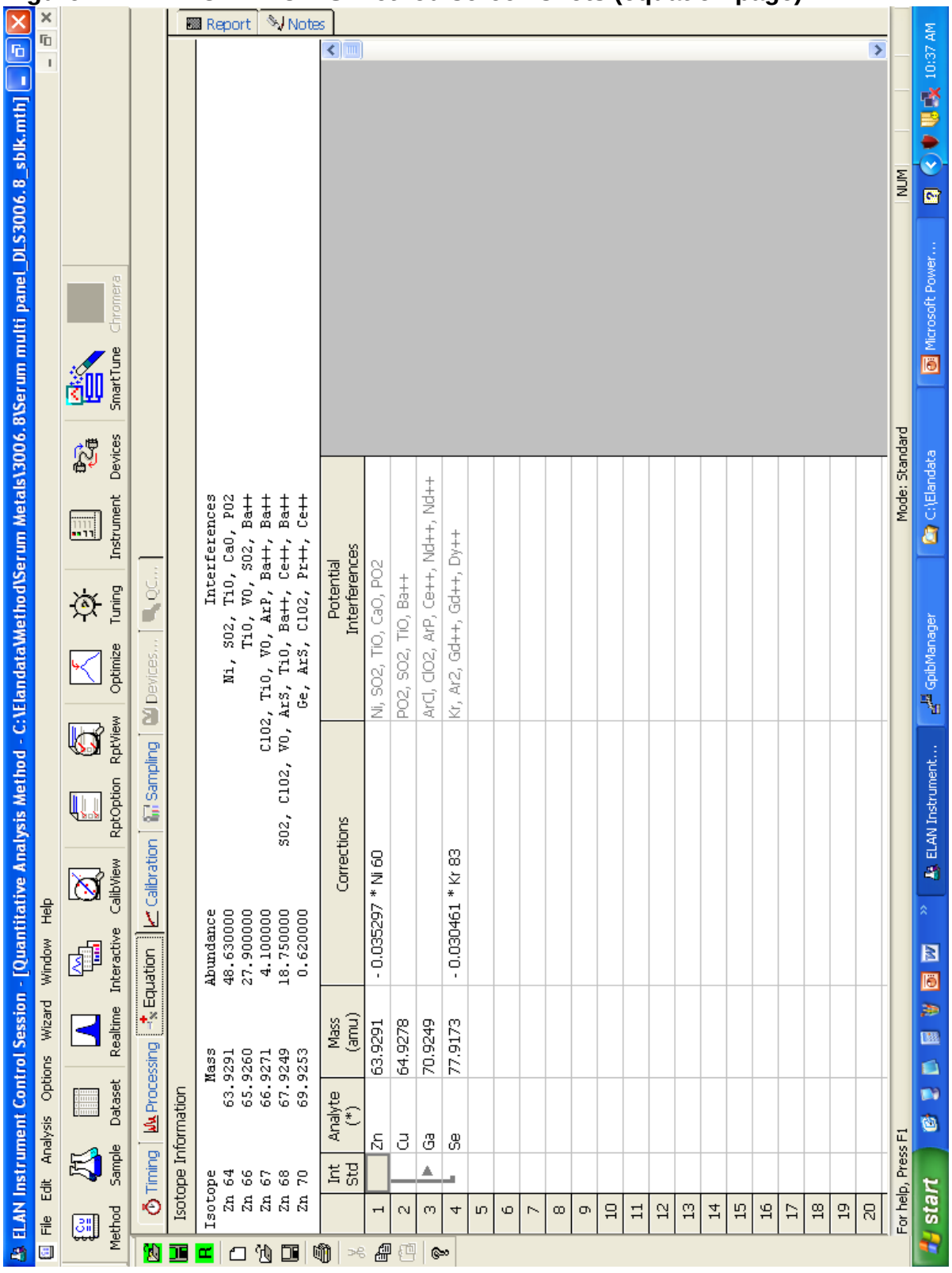


Appendix C. Tables and figures (continued).

Figure 3. ELAN ICP-DRC-MS method screen shots (processing page).

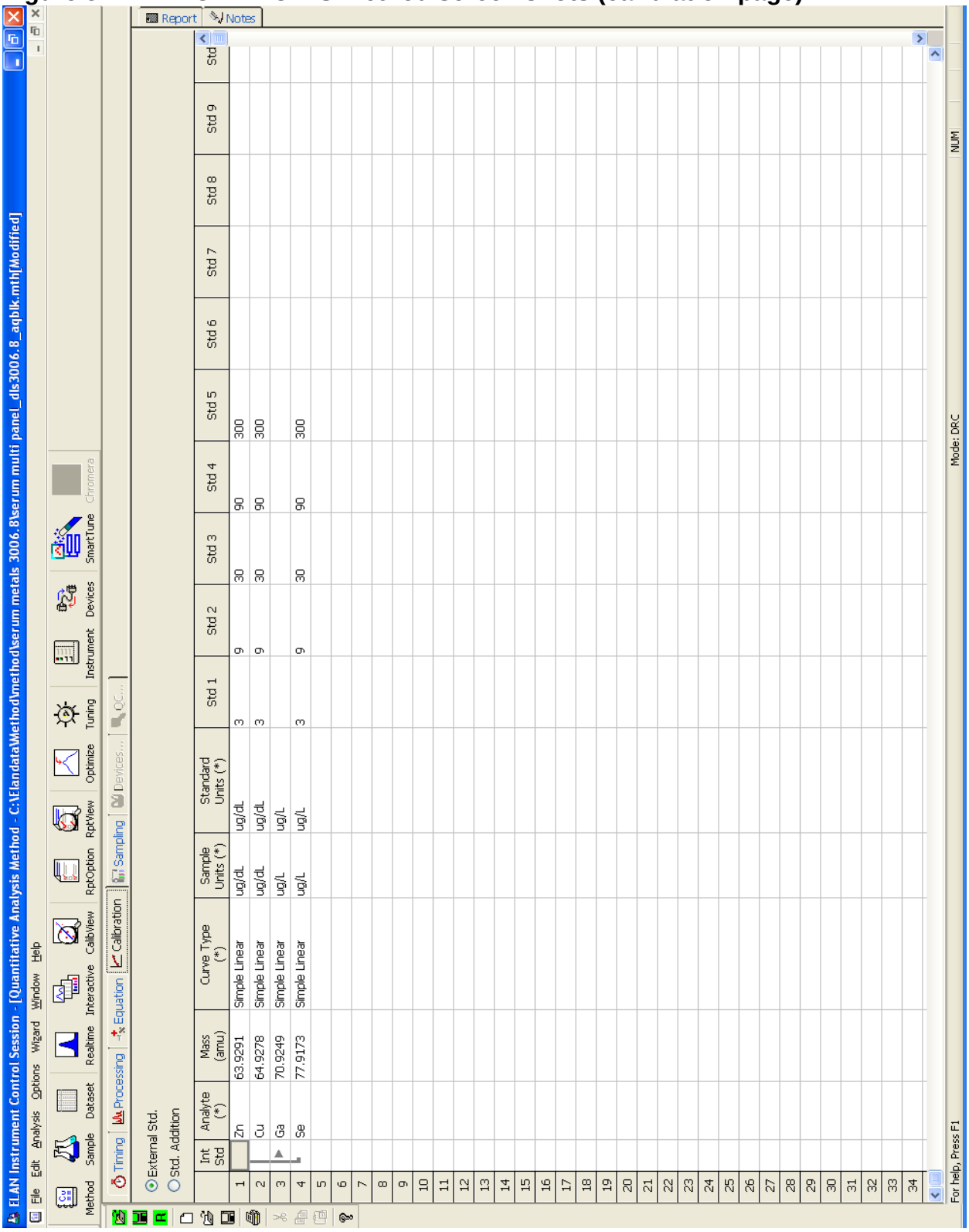
Appendix C. Tables and figures (continued).

Figure 4. ELAN ICP-DRC-MS method screen shots (equation page).



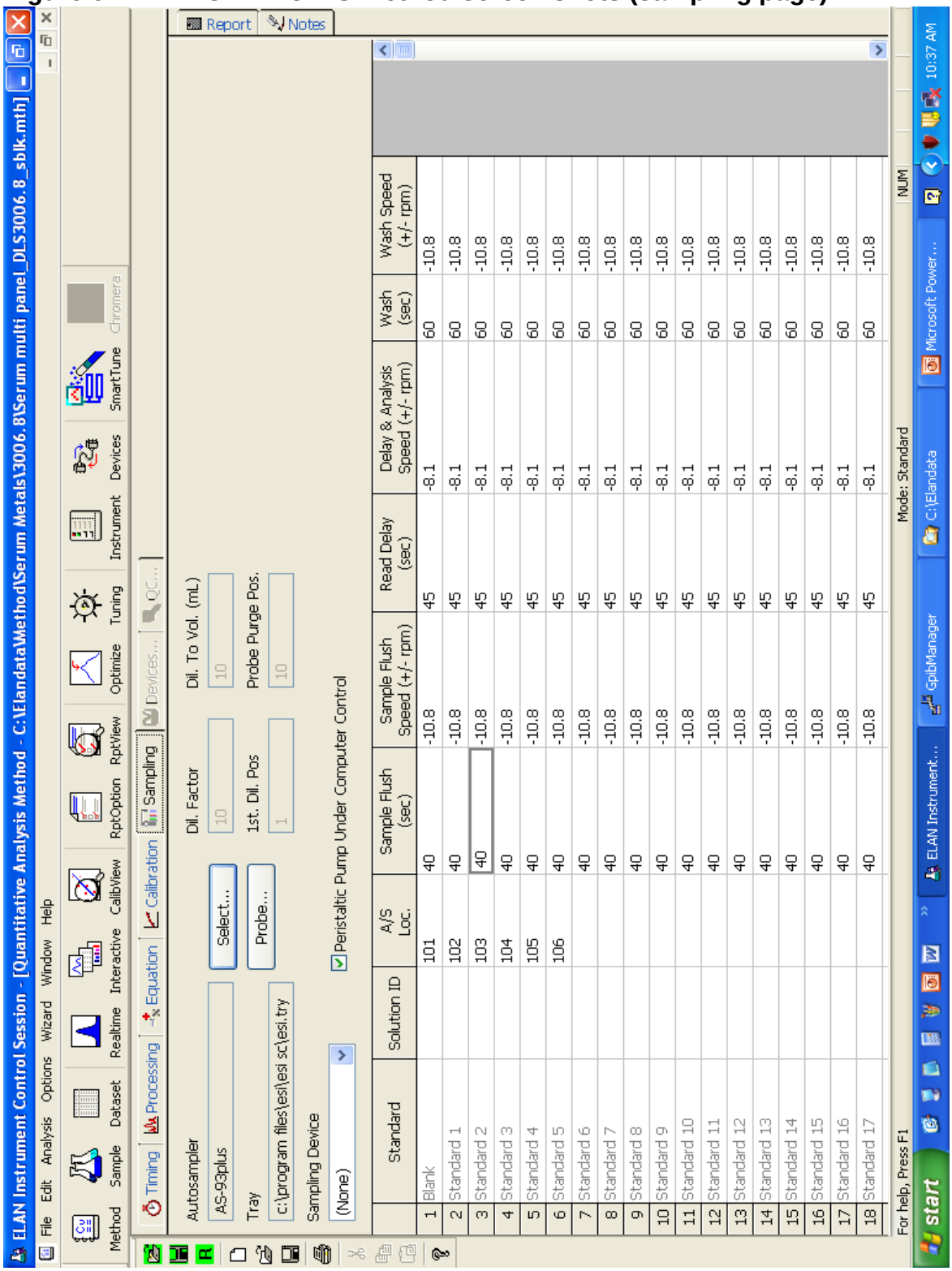
Appendix C. Tables and figures (continued).

Figure 5. ELAN ICP-DRC-MS method screen shots (calibration page).



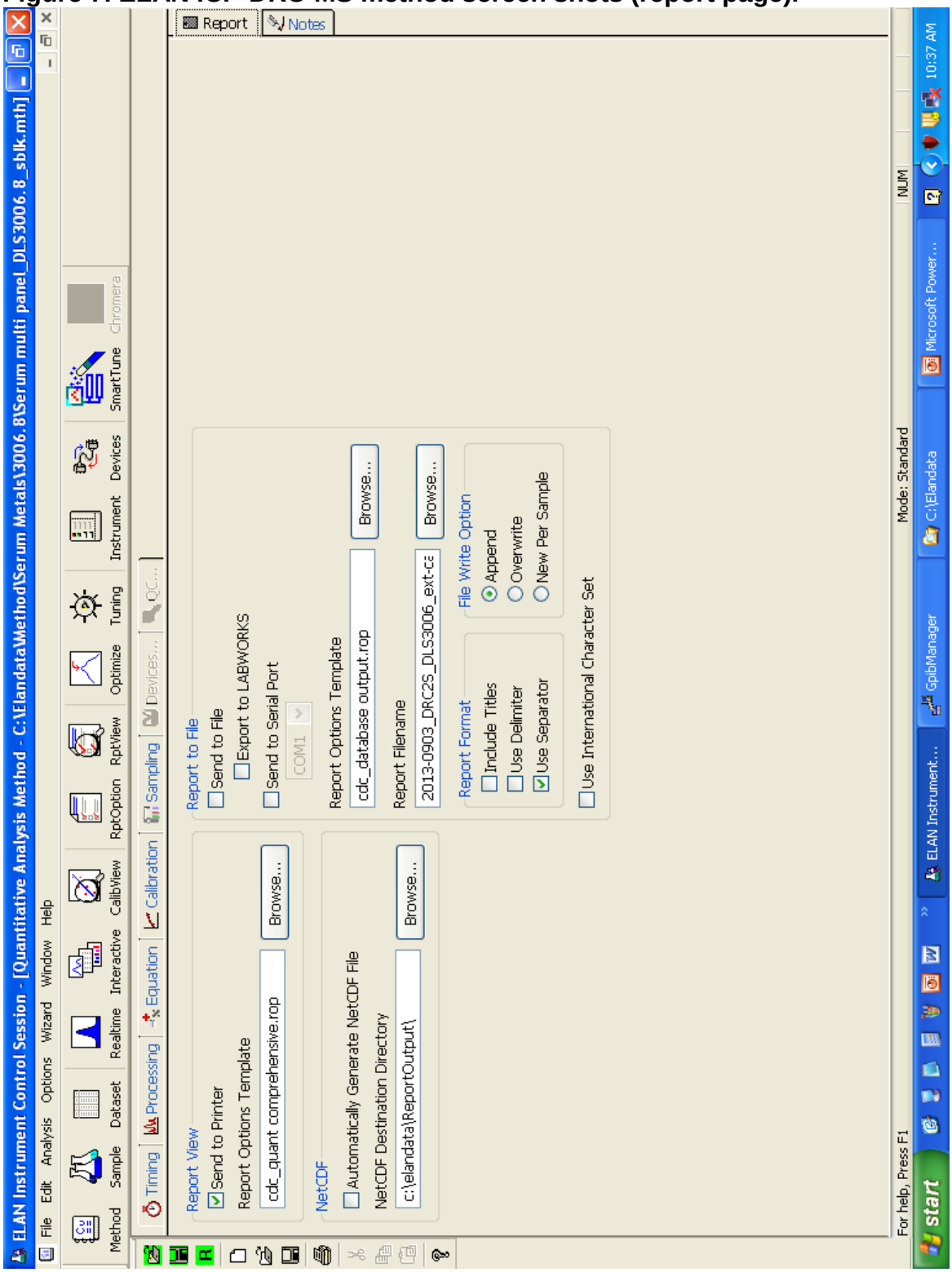
Appendix C. Tables and figures (continued).

Figure 6. ELAN ICP-DRC-MS method screen shots (sampling page).



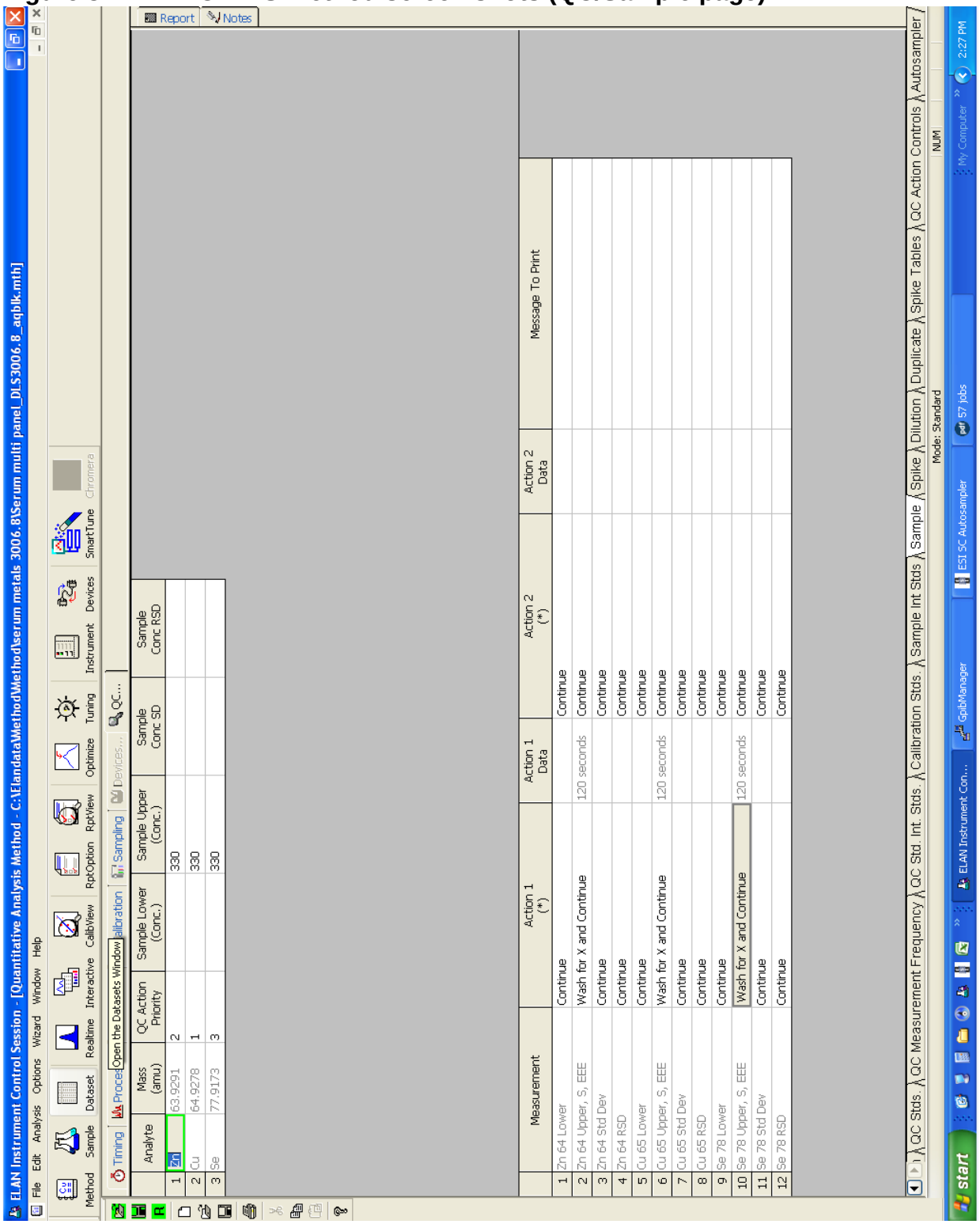
Appendix C. Tables and figures (continued).

Figure 7. ELAN ICP-DRC-MS method screen shots (report page).



Appendix C. Tables and figures (continued).

Figure 8. ELAN ICP-MS method screen shots (QC/Sample page).

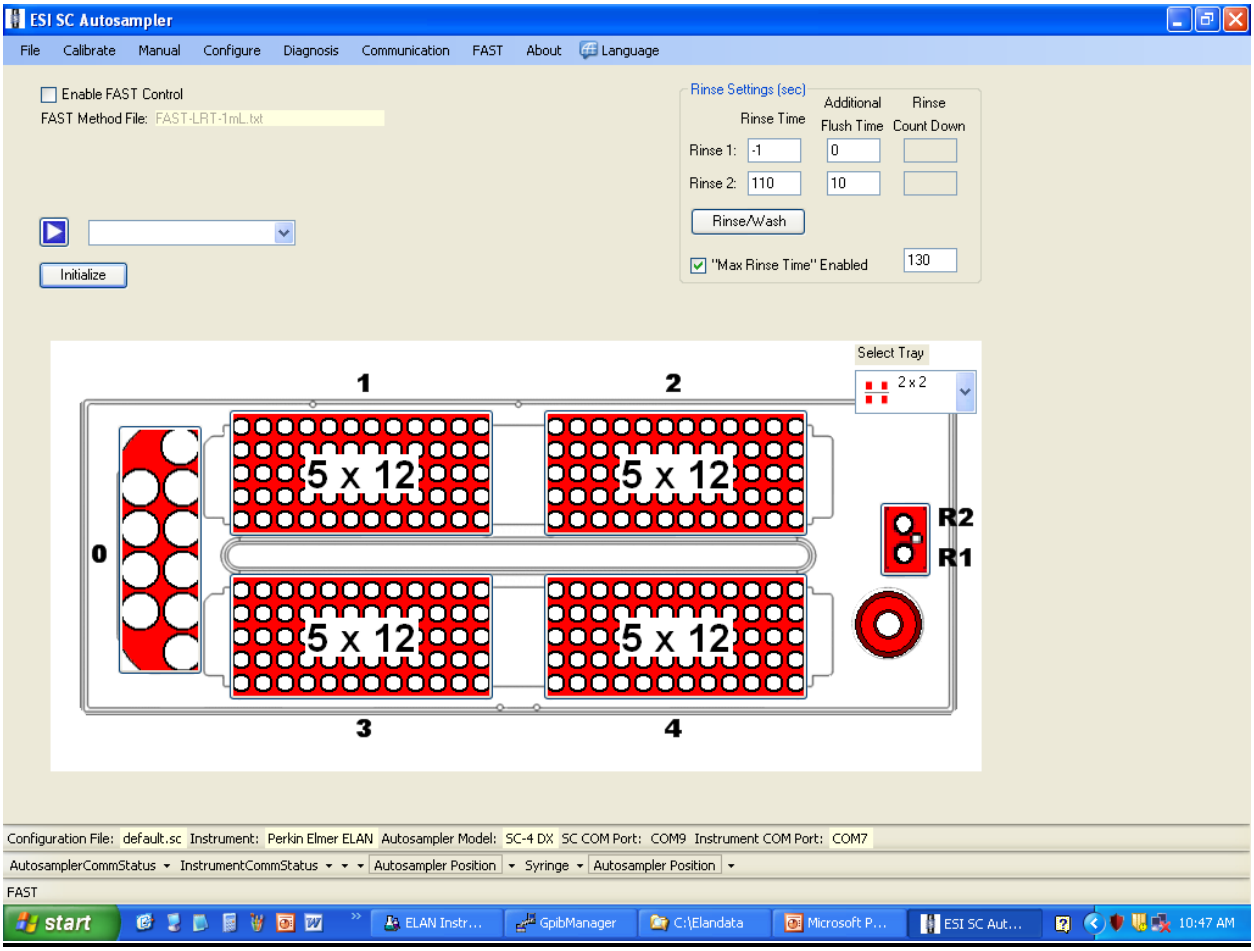


Appendix C. Tables and figures (continued).

Figure 9. ESI SC4 autosampler screen shots used (main page).
Additional flush times and “Max Rinse Time” are default, but can be optimized for best reduction of elemental carry-over between samples. Tray types can be changed to allow for different volumes of diluted sample digests. Do not enable ‘FAST control’. Rinse and additional flush times for eliminating carry-over from one sample to the next while using the minimum amount of rinse solution.

A rinse time of -1 causes the rinse station to be skipped.
A rinse time of 0 causes the probe to only dip into the station, but spends no time there.

Additional flush times can be optimized to keep the rinse station full while not using too much rinse solution. The inner diameter size of the tubing providing the rinse solution to the rinse station determines how quickly the station will fill. Various sizes are available for purchase or can be made in the laboratory.



Appendix C. Tables and figures (continued).

Figure 10. ESI SC4 autosampler screen shots used (configuration page).
"High Speed" option is to only be used for 'High Speed' models of the SC4 (look for "HS" in serial number). Speeds and accel/decel values can be optimized per analyst preference and to minimize droplet splatter off of probe.

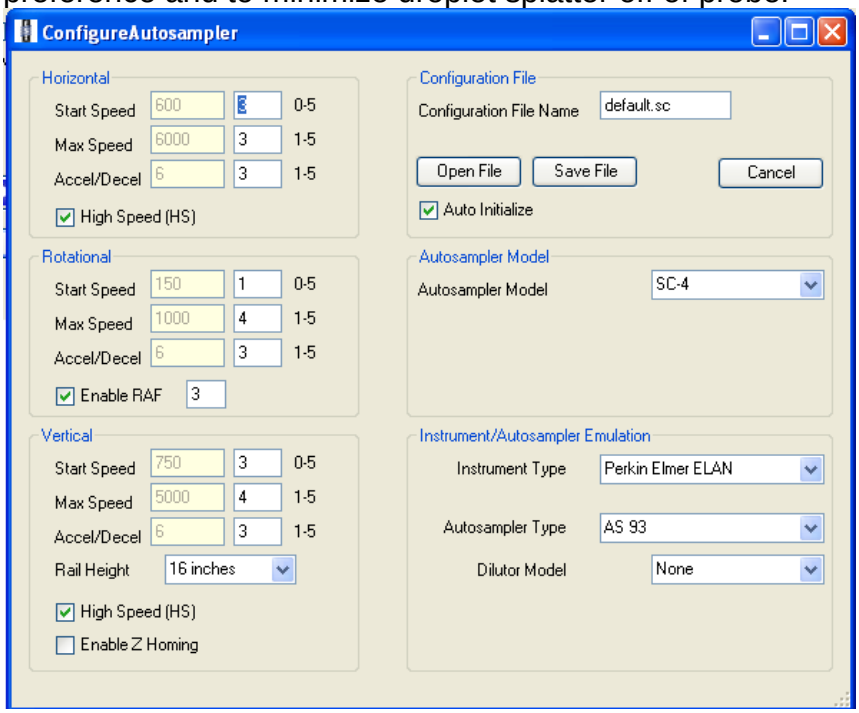
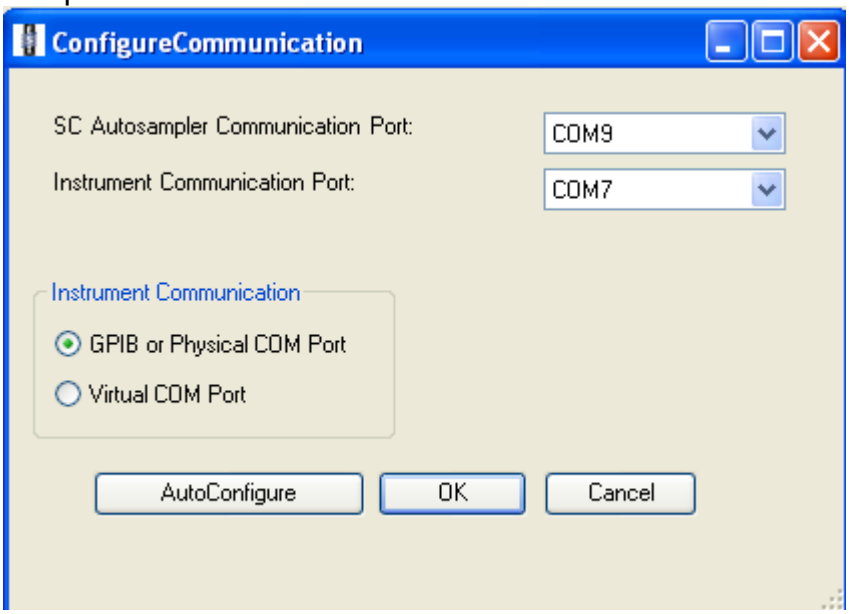


Figure 11. ESI SC4 autosampler screen shots used (communication page).
Communication ports will differ depending on available ports on instrument control computer.



Appendix C. Tables and figures (continued).

Figure 12. ESI SC4 autosampler screen shots (5x12 rack setup window). Settings are approximate. To be sure the loop is filled, setup the probe to go down close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.

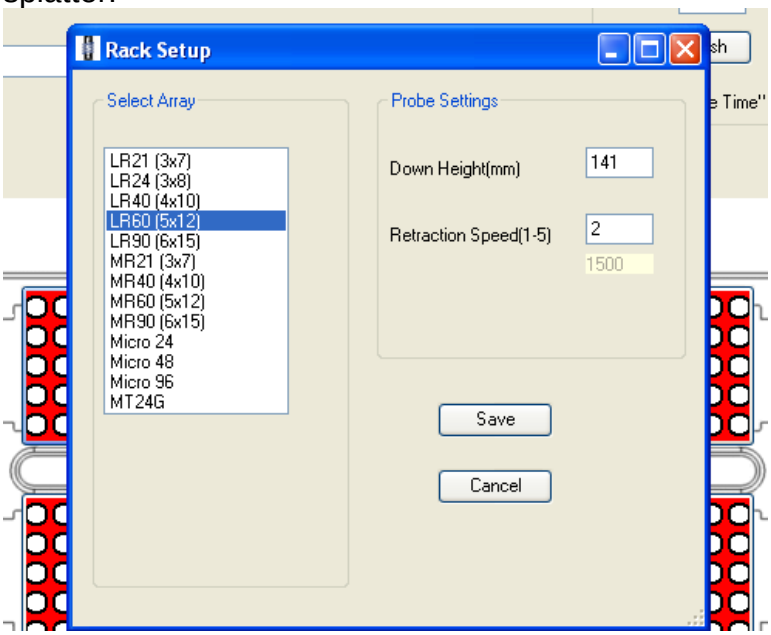
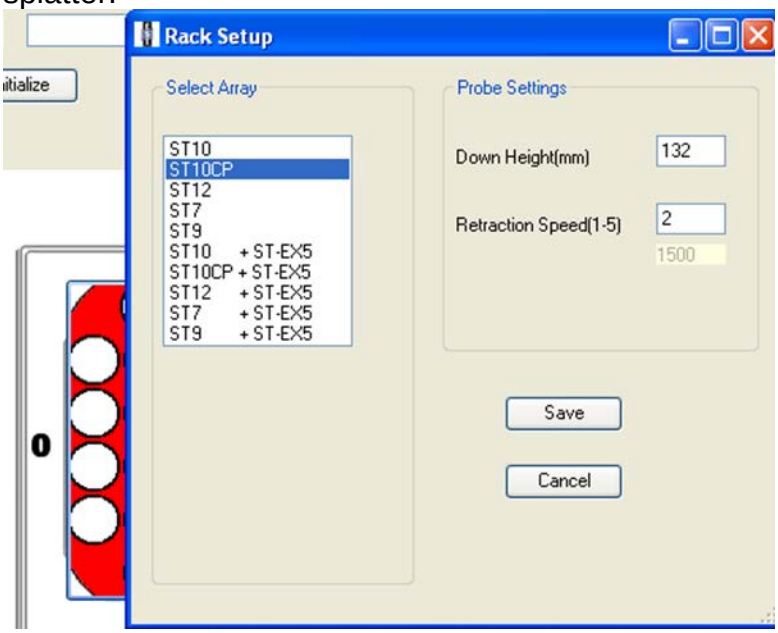
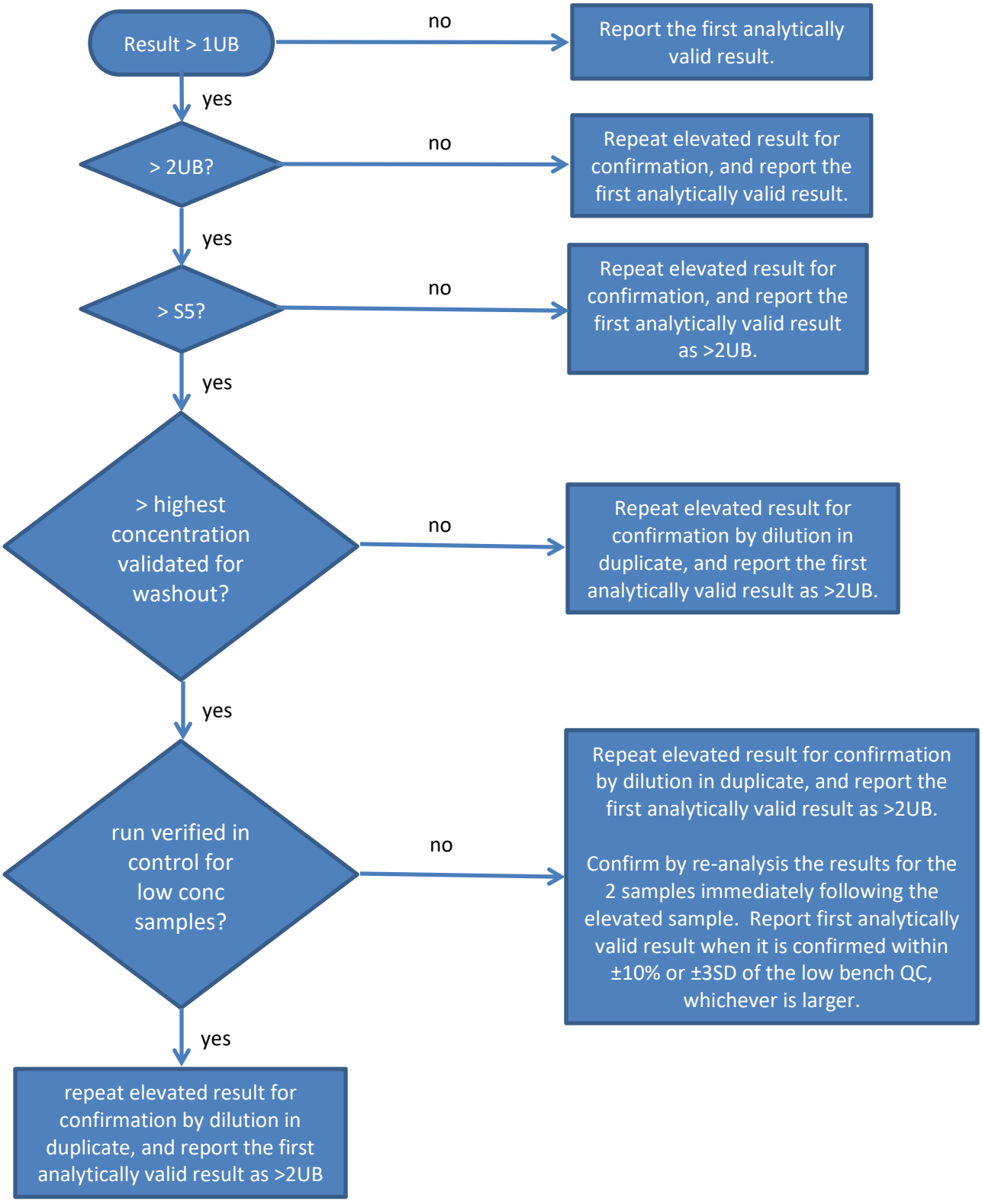


Figure 13. ESI SC4 autosampler screen shots (50mL tube rack setup window). Settings are approximate. To be sure the loop is filled, setup the probe to go down close to the bottom of the cup, but not touch. Optimize retraction speed for least droplet splatter.



Appendix C. Tables and figures (continued).

Figure 14. Flow chart for handling an elevated result.



Appendix D. Help sheets

Reagent Preparation (page 1 of 2)

NOTE:
mg/L = ppm
µg/L = ppb
µg/mL = ppm

Rinse solution
(0.01% Triton X-100, 5% ethyl alcohol, 2% (v/v) HNO_3, 0.5% (v/v) HCl)

1. Partially fill the pre-cleaned 4 L bottle with ≥18 MΩ·cm water (approximately 2-3 L).
2. Add 80 mL of concentrated HNO_3 and mix well.
3. Add 200 mL ethyl alcohol and mix well.
4. Add 20 mL of concentrated HCl and mix well.
5. Add 20 mL of the 2% Triton X-100 and mix well.
6. Fill to 4 L using ≥18 MΩ·cm water and mix well.

Sample diluent
(10 ug/L Ga, 0.01% Triton X-100, 5% ethyl alcohol, 2% (v/v) HNO_3)

1. Partially fill (i.e. 70-80% full) the 2 L container with ≥18 MΩ·cm water.
2. Add 40 mL concentrated HNO_3 and mix.
3. Add 100 mL ethyl alcohol and mix.
4. Add 1 mL of 20 µg/mL Ga internal standard intermediate solution.
5. Add 10 mL of the intermediate 2% Triton X-100 solution and mix.
6. Fill to 2 L with ≥18 MΩ·cm water and mix well.

5% (v/v) HNO_3

1. Partially fill a 2 L bottle with ≥18 MΩ·cm water.
2. Add 100 mL of concentrated HNO_3.
3. Fill to 2 L using ≥18 MΩ·cm water

Appendix D. Help sheets (continued).

Reagent Preparation (page 2 of 2)

2% Triton X-100 in 5% (v/v) HNO_3

1. Partially fill a 2 L bottle with ≥18 MΩ·cm water.
2. Add 40 mL of Triton X-100.
3. Add 100 mL of concentrated HNO_3.
4. Fill to 2 L using ≥18 MΩ·cm water.
5. Allow to dissolve overnight (or add a Teflon magnetic stirring bar and stir on stirrer until dissolved).
6. Mix well by gently inverting several times.

20 µg/ml Ga internal standard solution

1. Partially fill the 100-mL volumetric flask with ≥18 MΩ·cm water.
2. Carefully add 2 mL of concentrated HNO_3. Mix into solution.
3. Add 0.2 mL of 10 µg/mL Ga standard. lf initial Ga concentration is different, adjust volume proportionally.
4. Fill to mark (100 mL) and mix thoroughly.

Daily solution (1 µg/L) in 2% (v/v) HNO_3

1. Partially fill a 1 L volumetric flask with ≥18 MΩ·cm water.
2. Add 1 mL of High Purity Standard SM-2107-018 (or current lot #)
3. Add 20 mL of concentrated HNO_3.
4. Fill to 1 L using ≥18 MΩ·cm water.
5. Mix well by gently inverting several times.

DRC Stability Solution (Junk Serum)

1. Add 33 mL of diluent into a plastic bottle
2. Add 1.2 mL of ≥18 MΩ·cm water
3. Add 1.2 mL of junk serum
4. Repeat until you've reached your desired volume.

Appendix D. Help sheets (continued).

Day-to-Day Operations (page 1 of 2)

Readying ICP-MS and materials

1. Remove “Junk Serum”, QC materials and patient samples from the -70C freezer and place into a biological safety cabinet (BSC) to warm up to ambient temperature.
2. Check the peristaltic pump for proper tension on the tubing
3. Perform daily maintenance checks
    a. Ar supply pressure, interface components etc.
4. Start the plasma
5. Place autosampler probe into freshly poured ≥18 MΩ·cm water
    a. Allow for warm-up time (≈30 minutes)

Optimization of the ICP-MS

1. Perform daily performance checks
2. Record the daily into the Daily Logbook
3. Prepare materials for DRC stability time

Preparing and analyzing the curve

1. Prepare the calibrators while the DRC stability is running,
2. Evaluate the calibration curve
    a. The minimum acceptable R^2 value for each curve is 0.98.
    b. Check that the blank is not over-subtracting from the standards (i.e. each successive standard has a net intensity greater than the previous standard). Ensure that net intensity for all standards is positive.

Preparing and monitoring the run

1. Preparing the Run
    a. Thoroughly wash the benchtop automatic pipette probe in-between samples
    b. Ensure the prepared samples are homogenized (e.g. vortex for 3-5 seconds, or invert 5-10 times) before placing them in the autosampler.

Appendix D. Help sheets (continued).

1. Day-to-Day Operations (page 2 of 2)

2. Monitoring the Run
    a. Ensure proper operation of the instrument (sample reaching nebulizer in correct timing, autosampler arm moving properly, etc…).
    b. Ensure DRC stability (analyte / internal standard ratio stability) before starting the run.
    c. Verify that bench QC results are within the acceptable limits.
        i. If an analyte result for the beginning QC material(s) falls outside of the ± 3SD limits, then follow the steps listed on page 37.
        ii. If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions.
    d. Verify good precision among replicates
    e. Verify consistent measured intensities of the internal standards.
    f. Confirm elevated patient results.
        i. Repeat for confirmation any sample having a concentration greater than the 1UB.
        ii. Repeat with extra dilution (in duplicate) any sample having a concentration greater than the highest calibration standard.
3. After analysis,
    a. flush the ICP-MS sample introduction system with ≥18 MΩ·cm water
    b. turn off the plasma
    c. flush the benchtop automatic pipette
        i. 10% Ethyl Alcohol
        ii. ≥18 MΩ·cm water
    d. Leave the benchtop automatic pipette syringes with ≥18 MΩ·cm water in the lines and turn off the power

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2. Tanner, S.D., Baranov, Vladimir I, *Theory, Design, and Operation of a Dynamic Reaction Cell for ICP-MS.* Atomic Spectroscopy, 1999. **20**(2): p. 45-52.
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4. PerkinElmer SCIEX Instruments, *ELAN DRC II Hardware Guide*. 2001, Canada.
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Appendix G
Video Activity Data

Table G-1. The Micro-activities (Events/Hour) of Child Athletes Playing Soccer, Field Hockey, and Football from Publicly-available Videotapes (Phase 1)

Child Micro-activity	Statistic	Soccer (n = 10)[a]	Field Hockey (n = 10)	Football (n = 10)	Total (n = 30)
Hand-to-mouth	Mean	14	15	58	29
	SD	9.9	13	75	47
	Minimum	0.0	0.0	0.0	0.0
	25th	8.0	4.0	9.0	6.0
	50th	16	15	30	18
	75th	22	26	69	28
	Maximum	28	32	250	250
Object-to-mouth	Mean	0.0	3.6	17	7.0
	SD	0.0	5.1	14	11
	Minimum	0.0	0.0	0.0	0.0
	25th	0.0	0.0	7.5	0.0
	50th	0.0	0.0	15	0.0
	75th	0.0	7.0	29	12
	Maximum	0.0	12	36	36
Hand-to-turf	Mean	12	5.8	83	33
	SD	26	7.5	51	48
	Minimum	0.0	0.0	6.0	0.0
	25th	0.0	0.0	57	0.0
	50th	2.0	3.0	75	8.0
	75th	8.0	10	110	63
	Maximum	84	20	190	190
Body-to-turf	Mean	6.4	2.8	52	21
	SD	12	5.0	25	28
	Minimum	0.0	0.0	12	0.0
	25th	1.0	0.0	44	0.0
	50th	4.0	0.0	48	4.0
	75th	4.0	4.0	68	42
	Maximum	40	16	90	90

[a] Number of athletes

Table G-2. The Micro-activities (Events/Hour) of Adult Athletes Playing Soccer, Field Hockey, and Football from Publicly-available Videotapes (Phase 1)

Adult Micro-activity	Statistic	Soccer (n = 10)[a]	Field Hockey (n = 10)	Football (n = 10)	Total (n = 30)
Hand-to-mouth	Mean	4.2	11	74	30
	SD	6.5	14	99	65
	Minimum	0.0	0.0	36	0.0
	25th	0.0	0.0	15	0.0
	50th	0.0	6.0	39	7.0
	75th	5.5	17	99	30
	Maximum	16	0.0	320	320
Object-to-mouth	Mean	4.0	5.2	25	10
	SD	1.3	9.1	33	22
	Minimum	0.0	0.0	0.0	0.0
	25th	0.0	0.0	0.0	0.0
	50th	0.0	0.0	6.0	0.0
	75th	0.0	7.0	47	7.0
	Maximum	4.0	28.	90	90
Hand-to-turf	Mean	14	2.4	110	42
	SD	17	6.3	150	99
	Minimum	0.0	0.0	18	0.0
	25th	0.0	0.0	26	0.0
	50th	8.0	0.0	60	12
	75th	22	0.0	92	35
	Maximum	48	20	530	530
Body-to-turf	Mean	11	1.2	49	21
	SD	11	3.8	54	37
	Minimum	0.0	0.0	6.0	0.0
	25th	2.0	0.0	24	0.0
	50th	8.0	0.0	36	8.0
	75th	16	0.0	47	24
	Maximum	32	12	190	190

[a] Number of athletes

Table G-3. Guide for Activity Levels of Athletes[a]

Activity Level	Activity
Resting	Kneeling
Resting	Sitting
Resting	Standing
Low	Catching/kicking/throwing (stationary)
Low	Shuffling
Low	Stretching
Low	Walking
High	Jogging
High	Jumping
High	Jumping jacks
High	Knee lifting
High	Lunging
High	Planks/push-ups/pull ups
High	Running
High	Scissors/grapevines/crossovers
High	Sit-ups
High	Skipping
High	Sprinting
High	Squatting
High	Tackling
High	Tire-drills

[a] Adapted from CDC activity levels (https://www.cdc.gov/nccdphp/dnpa/physical/pdf/PA_Intensity_table_2_1.pdf)

Table G-4. The Micro-activities (Events/Hour) of Child Athletes Playing Soccer and Football on Videotape (Phase 2)

Children	Statistic	Soccer (n = 9)[a]	Football (n = 5)	Total (n = 14)
Hand-to-mouth	Mean	19	10	16
	SD	13	5.2	12
	Minimum	6.0	2.0	2.0
	25th	9.0	8.0	8.3
	50th	17	12	13
	75th	19	--[b]	18
	Maximum	44	15	44
Object-to-mouth	Mean	3.8	22	10
	SD	3.6	17	13
	Minimum	0.0	2.0	0.0
	25th	1.0	11	1.4
	50th	1.6	18	5.5
	75th	6.0	--	11
	Maximum	10	43	43
Hand-to-turf	Mean	16	21	18
	SD	28	10	23
	Minimum	0.0	6.0	0.0
	25th	0.0	15	1.0
	50th	1.0	25	9.4
	75th	11	--	27
	Maximum	81	31	81
Body-to-turf	Mean	3.6	6.8	4.7
	SD	5.7	2.6	4.9
	Minimum	0.0	3.0	0.0
	25th	0.0	6.0	1.0
	50th	1.0	7.0	3.0
	75th	3.0	--	7.8
	Maximum	16	10	16

[a] Number of athletes

[b] The 75th percentile was not reported when the sample size was less than 9 athletes

Table G-5. The Micro-activities (Events/Hour) of Adult Athletes Playing Soccer on Videotape (Phase 2)[a]

Adults	Statistic	Soccer (n = 3)[b]
Hand-to-mouth	Mean	7.3
	SD	4.0
	Minimum	3.0
	Maximum	11
Object-to-mouth	Mean	10
	SD	13
	Minimum	1.0
	Maximum	25
Hand-to-turf	Mean	26
	SD	28
	Minimum	4.0
	Maximum	57
Body-to-turf	Mean	2.0
	SD	3.5
	Minimum	0.0
	Maximum	6.0

Appendix H

Feasibility Assessment for Silicone Wristband Passive Samplers at Synthetic Turf Fields

Final Report

for

Feasibility Assessment for Silicone Wristband Passive Samplers at Synthetic Turf Fields

Solicitation RFQ-SAB-17-00056

In conjunction with

US EPA
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2) ***Appendix 2:*** **OPAHs by GC-MS**
3) ***Appendix 3:*** **Multiple Analyte Screen by GC-MS and deconvolution software (DRS)**
4) ***Appendix 4:*** **Volatile Organic Compounds (VOCs) by thermal desorption**

Abbreviations:
PAHs – Polycyclic aromatic hydrocarbons
OPAHs – Oxygenated polycyclic aromatic hydrocarbons
VOCs – Volatile organic compounds
DRS – Deconvolution Reporting Software
CV – Calibration verification
IB – Instrument blank
MC – Microchamber blank
GT – Glass tube blank
PD – Post-deployment blank
LOD – Limit of Detection

Deliverables checklist– From Statement of Work

1) Brief description of the sample collection procedures and parameters.
    a. Wristband deployment information
        i. Sample ID numbers - *table one*
        ii. Collection dates and times – *table one*
        iii. Sampling locations – *table one, figure one and two*
        iv. Sampling height sections - *2a and 2b*
        v. Sampling duration – *table one*
        vi. Other relevant information for interpreting results - *sections 2a and 2b*
    b. Facility information associated and linkable to the samples
        i. Facility type (outdoor or indoor) *sections 2a and 2b*
        ii. Facility uses - general *sections 2a and 2b*
        iii. Facility uses – during sampler deployment *sections 2a and 2b*
        iv. Infill type *sections 2a and 2b*
        v. Field installation date *sections 2a and 2b*
        vi. Most recent date of infill additions (if available) *sections 2a and 2b*

2) Brief description of laboratory method used for analysis of wristbands.
    a. Extraction methods and materials – *section 3c*
    b. Analytical system and materials (manufacturer and model/product numbers) – *section 4*
    c. Analytical conditions – *section 4 and previously supplied SOPs*

3) Brief descriptions of the methods used for data analysis/chemical identification in wristband analysis.
    a. Software (including version), procedures, and databases used for identification/confirmation *previously supplied SOPs*
    b. Chemicals/standards included in target database *previously supplied SOPs*
    c. Method used to provide concentration estimates *previously supplied SOPs*

4) List of all identified chemicals in sampler extracts and for relevant sampling methods, the amounts intensity (e.g., peak abundance) or concentration estimates for each compound identified. - *In appendices one through four*

5) Relevant quality assurance/quality control data (e.g., accuracy, precision, uncertainty) to support compound identification and concentration estimates. - *Summarized sections 5a –5d*

A. Electronic data file with measurement results
An electronic file version of the sample measurement results (sample ID, chemical names, intensities and/or estimated concentrations) in a readily usable format (e.g. Excel). - *Excel versions of appendices attached*

Section 1 - Executive Summary

In October of 2017, the Food Safety and Environmental Stewardship (FSES) program at Oregon State University (OSU) was contracted by US EPA to evaluate the application of FSES silicone wristband sampling technologies in a pilot project examining potential exposures related to the use of crumb-rubber infilled synthetic turf athletic fields. Two sampling sites were identified, one indoor and one outdoor (described below). Conditioned wristbands installed in FSES standard air cages were deployed for seven days at each site, approximately one meter above or directly adjacent to the surface of the synthetic turf, at locations indicated on the associated maps. Additional samplers were deployed at sampling sites away from the synthetic turf surface. Using duplicate wristband samplers at each site allowed one to be used for liquid extraction and one for thermal desorption. Additionally, two samplers were held in their transport bags for the duration of the sampling campaign. These are designated as trip blanks one and two and their results reported with the field-deployed samplers. Date of deployment was November 10th through 17th, 2017.

After recovery, one set of field samples and trip blanks were liquid extracted and analyzed for polycyclic aromatic hydrocarbons (PAHs) and their oxidized derivatives (OPAHs) as well as a broad analyte presence/absence screen for 1528 chemicals. The replicate set was thermally desorbed and analyzed for volatile organic compounds (VOCs). Appropriate quality assurance samples were included in every analytical batch. This report will provide summary statistics of the analytical findings and summaries of the quality control/quality assurance data produced in support of these results. Four appendices summarizing the complete analytical findings are appended to this report. Sampling sites are identified in table one below and fully described in sections 2a and 2b:

Sample name	Site description	deployment time	retrieval time
site 1	NW corner of indoor practice facility	7:15AM	8:04AM
site 1 dup	NW corner of indoor practice facility	7:15AM	8:08AM
site 2	SE corner of indoor practice facility	7:25AM	8:18AM
site 3	Sampler mounted on fence in atrium of indoor facility	7:35AM	8:31AM
site 4	Northern perimeter of outdoor field	7:35AM	8:55AM
site 5	Northern perimeter of outdoor field	7:55AM	8:58AM
site 6	Western perimeter of outdoor field	8:30AM	8:45AM
site 7	Sampler mounted to lamp post ~ 25M due West of turf field	8:40AM	8:50AM
trip blank 1	N/A	N/A	N/A
trip blank 2	N/A	N/A	N/A

Table 1. Sampler identification

Section 2 - Sampling

2a - Indoor site

The indoor sampling site is an indoor athletic practice facility with a wall-to-wall synthetic turf field approximately 125M long and 62M wide (see figure one). The synthetic turf in this facility is fifteen months old. Crumb rubber is stored on site and added to the field on a continual as-needed basis. This field is very well maintained and heavily used by a rotation of different teams who utilize the indoor facility. Two collocated samplers were deployed in the NW corner (site 1) of the facility. Because of the difficulty of attaching air cages without disrupting ongoing activities, these samplers were collocated within a single cage. These samplers are identified as *site 1* and *site 1 dup* (duplicate). A sampler was also placed at the SE corner (site 2) of the facility. These wristbands were designated *site 2*. In both site 1 and 2 samplers were directly above the turf bed. Two additional samplers were deployed at sample site 3, an adjacent atrium with a concrete floor, separated from the turf field by a 3' door and a secondary partial wall. These samplers are designated *site 3*. Site 3 was approximately 12 meters from the turf. In normal use, the door is expected to be opened whenever teams are practicing, which is the majority of the day. Mean temperature during deployment was 14.6°C ± 1.6°C, with a range from 11.4°C to 22.7 °C. Figure 1 drawing is not to scale.

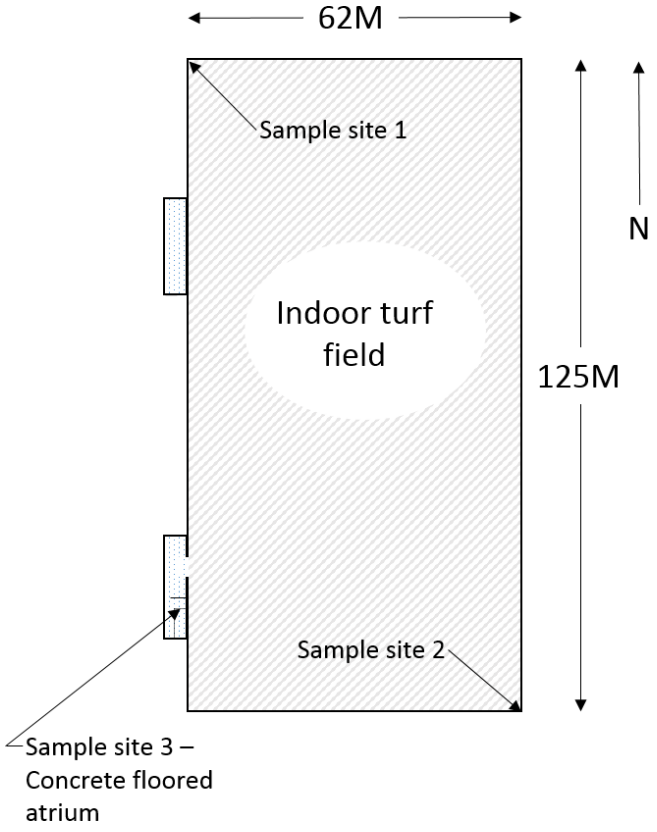

Figure 1. Indoor sampling site map.

2b - Outdoor site

The outdoor site has a synthetic turf field approximately 115M long and 100M wide (see Figure two). The synthetic turf in this facility is approximately five years old. Crumb rubber is available to the caretakers and added to the field on an as-needed basis. This field is moderately maintained and heavily used by a rotation of different activities ranging from organized classes to recreational organizations. In discussions with facility staff, fresh crumb rubber is added approximately every six months to a year. Collocated air cages were attached to the perimeter fence on the Northern edge of the field. These samples are designated as *site 4* and *site 5*. A single air cage was attached to the western perimeter fence and these samples are designated as *site 6*. These cages are directly adjacent to the turf and 1 meter above the ground. Because these samplers are adjacent to the field, their theoretical sampling perimeter will include non-turfed ground. Sample *site 7* was on a lamppost adjacent to a concrete walkway and natural grass area, separated from the turf field by approximately 18 meters. Typical fall/winter wind patterns are from the SW to the NE, but vary widely dependent on weather (see table 2). There was rain recorded on six of the seven days of deployment, averaging 0.94 cm per day. Mean temperature during deployment was 9.7°C ± 3.2°C, with a range from 3.1°C to 22.7 °C. By contrast, summer surface temperatures on this field have been measured as high as 65° C. Adjacent roadways are paved and will see significant traffic during working hours. Drawing is not to scale.

Date	Rain (cm)	Wind (kph)	wind max (kph)	max gust (kph)
11/10/2017	1.39	14.4 SSE	22.4	NA
11/11/2017	0	14.4 SSE	24	NA
11/12/2017	0.53	8 SSE	28.8	NA
11/13/2017	1.45	20.8 S	35.2	54.4
11/14/2017	0.05	16.0 S	36.8	46.4
11/15/2017	2.97	12.8 S	25.6	NA
11/16/2017	2.41	14.4 S	22.4	NA
11/17/2017	0.43	6.4 SSW	25.6	NA

Table 2. Outdoor site weather parameters

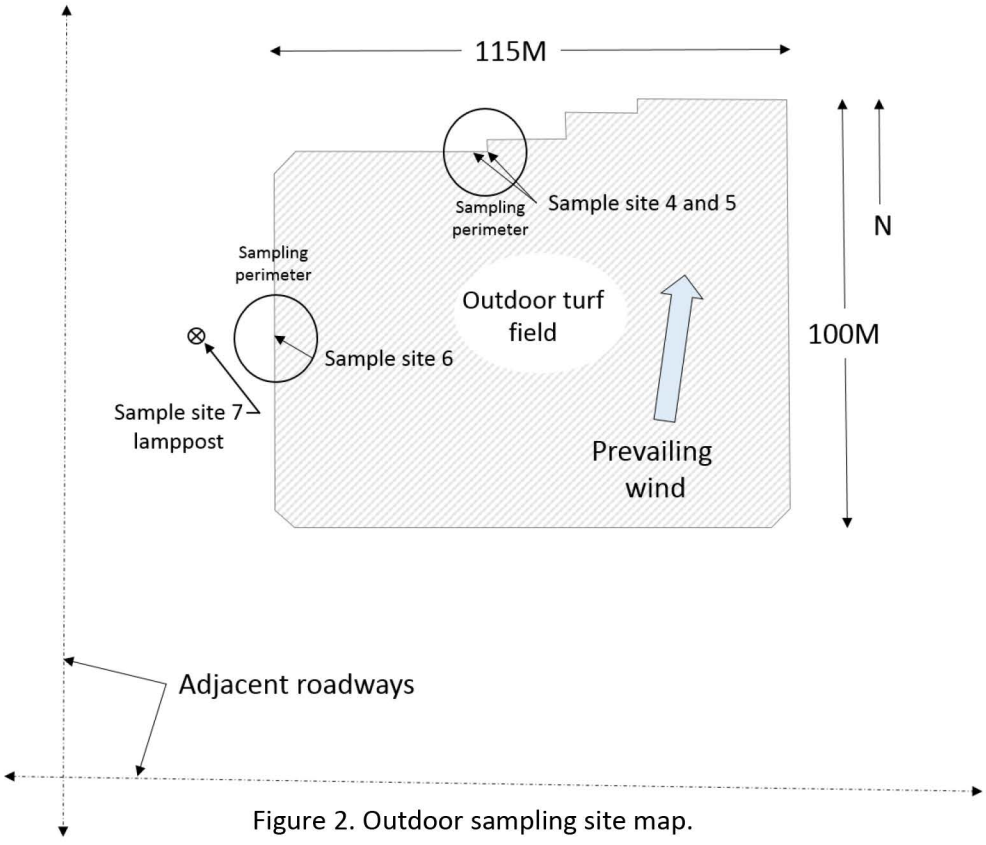

Figure 2. Outdoor sampling site map.

2c - Site pictures

Indoor site

Site 1

Site 2

Site 3

Outdoor site

Sites 4 & 5

Site 6

Site 7

Section 3 – Deployment, Recovery and Processing

3a - Deployment

Air cages were temporarily mounted to facility walls or fences using available support structures. These cages are designed to allow unrestricted air flow and have been used in multiple successful environmental sampling campaigns. Conditioned wristbands were transported to the site within individual PTFE (Teflon) bags. Once sampling cages were installed, conditioned wristbands were removed from their bags and affixed within the cages using zip ties. Deployment times are provided in Table 1.

3b - Recovery

After a seven-day deployment, FSES air cages were carefully opened and each wristband removed and placed in a pre-labeled, air-tight PTFE bag for storage and transport to the FSES laboratory. Air cages were removed from support structures. Samplers were transported to the FSES laboratory at Oregon State University where they were logged in and stored as appropriate, described further in SOP 2003.03 *Sample Inspection upon Receipt*.

3c - Processing and extraction

All samplers were post-deployment cleaned as per SOP 110.00 *Cleaning Field Deployed Silicone Passive Sampling Devices*. Samples destined for PAH, OPAH and DRS analysis were extracted as per SOP 419.00 *Extraction of Organic Compounds from Silicone Passive Sampling Devices*. Wristbands for VOC analysis were thermally desorbed as per SOP 422.0 *Determination of Volatile Organic Compounds (VOCs) using thermal desorption purge and trap interfaced with EI GC/MS.*

Section 4 – Analytical Determinations

Aliquots from each ethyl-acetate extract were injected on triple-quadrapole (PAHs; Agilent 7890/7000C GC/MS/MS) or single quadrapole (OPAHs and DRS; Agilent 6890/5975 GC/MS) gas chromatograph mass spectrometers as per SOP 418.00 *Determination of Parent and Alkyl Substituted PAHs by GC-MS/MS*, SOP 414.00 *Determination of Oxygenated Polycyclic Aromatic Hydrocarbons (OPAHs) using GC/MS* or SOP 423.00 *Multiple Analyte Screen by GC-MS and deconvolution software* as appropriate.

Aliquots from the thermal tubes were analyzed by SOP 422.00 *Determination of Volatile Organic Compounds (VOCs) using thermal desorption purge and trap interfaced with EI GC/MS* (Agilent 6890/5975 GC/MS). Each analytical run was individually quantified and all peak values manually confirmed. Additional quality control samples were run with each analytical batch. The results of these quality control analyses will be presented with each analytical method summary below. *Additional method parameters including oven profiles, flow rates and instrument specifics are provided within the previously supplied SOPs (approved 12/4/17).*

Analytical effort associated with this project generated over 1,100 quantified chemical data points and over 15,000 chemical presence/absence determinations. Additionally, over 2,100 quality control data points were rigorously quantified and reviewed.

All field samples were analyzed on all methods with QC that met applicable DQOs defined in each SOP.

Section 5 - Results

Four appendices are associated with this document. They are provided in PDF and Excel formats. The appendices correspond to individual client reports for:

1) PAHs by GC-MS-MS
2) OPAHs by GC-MS
3) Multiple Analyte Screen by GC-MS and deconvolution software (DRS)
4) Volatile Organic Compounds (VOCs) by thermal desorption

Continuing calibration (CV) quality control samples were analyzed within each analytical batch as defined in our SOPs. All CVs meet the data quality objectives outlined in each of our SOPs unless otherwise indicated. FSES generated instrument blanks (IB), field blanks (FB) and lab-process blanks (LB) meet DQOs defined in our SOP. Background subtraction using values derived from LBs are designated with a "B" flag in the client report. Method-specific summations are provided by tabulating the QC performed for each method and designated ***Quality Control Summary***.

Reported concentration units are ng/g wristband for all methods except DRS, which is reported as presence/absence.

As further detailed in our Quality Assurance Program Plan, all samples and quality control samples were reviewed by the Senior Chemist and reviewed and approved by the Program Director.

Section 5a - PAHs

Quality Control Summary –

In addition to the two trip blanks reportable as part of this study, seventeen additional quality control/ quality assurance samples were run along with the field wristband samplers, representing 170% QC. Quality assurance samples included four CVs, seven IBs, and trip, cleaning, construction, and multiple reagent blanks. All CVs showed greater than 80 % of their analytes within 20 % of true values, passing DQOs.

ΣPAHs from indoor samples (mean 80 ± 16.5 ng/g). ΣPAHs from outdoor samples (mean 31.7 ± 1.7 ng/g). Trip blank wristbands one and two show no PAHs above LOD.

The complete data report for PAHs is attached as Appendix 1.

Location	Σ63 PAHs ng/g wristband	Number of detections
S ite 1	**71.8**	15
S ite 1 - d u p	**60.9**	14
S ite 2	**105**	17
S ite 3	**81.9**	17
S ite 4	**28.9**	14
S ite 5	**33.9**	14
S ite 6	**33.4**	14
S ite 7	**31.9**	13
T rip b lan k 1	**0**	0
T rip b lan k 2	**0**	0

Table 3. PAH Summary stats

Section 5b - OPAHs

Quality Control Summary –

In addition to the two trip blanks reportable as part of this study, seventeen additional quality control/ quality assurance samples were run along with the field wristband samplers, representing 170% QC. Quality assurance samples included five CVs, seven IBs, and trip, cleaning, construction, and multiple reagent blanks. All CVs showed greater than 85 % of their analytes within 50 % of true values, passing DQOs.

The complete data report for OPAHs is attached as appendix 2.

Section 5c - Multiple Analyte Screen with DRS

Quality Control Summary –

Fourteen quality control/ quality assurance samples were run in addition to the ten wristband samplers, representing 140% QC. Quality assurance samples included five CVs, five IBs, and trip, cleaning, construction, and reagent blanks. All CVs passed DQOs. The reagent blank showed a positive detection of bis(2-ethyhexyl)phthalate. All additional QC samples showed no analytes above the limits of detection.

Table 4 indicates the chemicals detected in this study. Note "J" and "*" superscript flags denote compounds near the limit of detection or shared isomers respectively.

Appendix 4 describes these results in full and includes a brief description of the chemical classes represented by these detections.

** Indicates a compound that shares an isomer, see table 3 for more information.*
j indicates a compound that was observed near the limit of detection.

Sample Name | Number	Compounds Found		
Trip blank 1 | A171396	Dicyclohexyl phthalate		
Trip blank 2 | A171397	naphthalene		
Site 1 | A170976	2,6-dimethylnaphthalene^{j*}	acenaphthene^{j}	Benzothiazole
	Bis(2-ethylhexyl)phthalate	Butylated hydroxyanisole	Dibenzofuran
	fluorene	naphthalene^{j}	
Site 1 - dup | A170978	1,5-dimethylnaphthalene*	1-methylnaphthalene*	Benzothiazole
	Butylated hydroxyanisole	Dicyclohexyl phthalate^{j}	Phthalimide
Site 2 | A170980	1,6-dimethylnaphthalene*	1-methylnaphthalene*	Benzothiazole
	Bis(2-ethylhexyl)phthalate	Butylated hydroxyanisole	fluorene
	naphthalene	Phthalimide	
Site 3 | A170982	1-methylnaphthalene*	2-ethylnaphthalene^{j*}	Benzothiazole
	Bis(2-ethylhexyl)phthalate	d-Limonene	Dibenzofuran
	Triethyl phosphate		
Site 4 | A170984	Benzothiazole	Bis(2-ethylhexyl)phthalate	naphthalene^{j}
Site 5 | A170986	1-methylnaphthalene^{j*}	Benzothiazole	Bis(2-ethylhexyl)phthalate
	Di-n-butyl phthalate		
Site 6 | A170988	1-methylnaphthalene^{j*}	Bis(2-ethylhexyl)phthalate^{j}	
Site 7 | A170990	1-methylnaphthalene^{j*}	Bis(2-ethylhexyl)phthalate	naphthalene^{j}

Table 4. DRS detections

Compund	Alternative Isomers
1,5-dimethylnaphthalene	2-Ethylnaphthalene,2,6-dimethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,8-dimethylnaphthalene
1,6-dimethylnaphthalene	2-Ethylnaphthalene,2,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene
1-methylnaphthalene	2-methylnaphthalene
2,6-dimethylnaphthalene	2-Ethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene
2-ethylnaphthalene	2,6-dimethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene

Table 5. DRS isomers

Section 5d - VOCs

Quality Control Summary –

Twenty-one quality control/ quality assurance samples were run in addition to the ten wristband samplers, representing 210% QC. Quality assurance samples included three CVs, fourteen GT, one MC, on PD and two cleaning blanks. MC levels were assigned as background. All CVs passed DQOs. All additional QC samples showed no analytes above the limits of detection.

Sample Name	Σ29 VOCs ng/g wristband	Number of detections
Site 1	87.29	6
Site 1 - dup	23.19	3
Site 2	68.78	6
Site 3	7.14	4
Site 4	2.13	2
Site 5	38.51	7
Site 6	89.68	9
Site 7	19.99	8
Trip blank 1	0.34	1
Trip blank 2	14.86	3

Table 5. VOC Summary stats

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Certificate of Analysis

Client Report For: EPA - José L. Zambrana, Jr., PhD
National Exposure Research Laboratory
US EPA Office of Research and Development

zambrana.jose@epa.gov

Project Name: EPA turf (wristbands) - Appendix 4
Project Number: F17-20
Report Date: February 23 2018

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QC Review **Date**

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FSES Director Approval: Kim A. Anderson **Date**

Methodology:

SOP 418.00: Determination of Parent and Alkyl Substituted PAHs by Gas Chromatography-Tandem Mass Spectrometry

Unit Conversions:
ppb = parts per billion
ppm = parts per million
ppt = parts per trillion
ng/g = ppb
ng/L = ppt
ng/mL = ppb
ng/µL = ppm
ng/g(Wristband) = ppb
pg/µL = ppb
µg/mL = ppm

Abbreviations:
J flag: Indicates lower precision in quantitation due to values near limits of detection or matrix effects.
B flag: The sample was background corrected.
< 123.45 U: Detection limit, indicates value was below limit of detection.

COA Notes:
Concentrations reported in ng/g wristband

Client Sample Name:	Site 1	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170976		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	4.83	benzo[c]fluorene, 205-12-9	0.435
1,4-dimethylnaphthalene, 571-58-4	1.04 JB	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	2.23	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	6.59 B	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	< 0.215 U	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	4.71 B	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.26	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	10.3 B	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	2.17	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	2.21
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	1.97
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	16.4
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	7.51	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	15.6
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	< 0.101 U
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	< 0.203 U
benzo[b]fluorene, 243-17-4	1.06 J	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 1 - dup	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170978		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	4.35	benzo[c]fluorene, 205-12-9	0.447
1,4-dimethylnaphthalene, 571-58-4	0.944 JB	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	1.91	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	7.42 B	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	< 0.215 U	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	4.44 B	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	< 0.114 U	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	11.8 B	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	1.57	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	1.81
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	1.81
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	13.1
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	7.29	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	11.0
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	< 0.101 U
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	< 0.203 U
benzo[b]fluorene, 243-17-4	1.04 J	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 2	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170980		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	4.88	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	1.09 JB	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	2.26	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	7.15 B	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	1.62	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	14.3 B	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	4.78 B	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.84	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	11.6 B	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	2.92	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	2.73
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.70
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	19.0
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	9.11	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	22.0
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	1.91
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	2.00
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 3	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170982		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	3.91	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	0.638 JB	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	1.71	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	5.65 B	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	1.21	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	10.2 B	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	3.72 B	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.01	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	9.06 B	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	1.42	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	1.95
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.66
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	16.1
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	7.54	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	16.2
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	1.10
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	1.52
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 4	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170984		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	2.00	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	0.534 J	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	< 0.0676 U	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	0.942 J	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	1.04 JB	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	0.409 JB	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.41	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	< 0.169 U	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	< 0.0942 U	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.49
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	3.48
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	< 0.258 U	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	6.86
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	1.50
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	3.62
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 5	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170986		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	2.13	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	0.580 J	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	< 0.0676 U	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	1.23 B	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	0.462 JB	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.66	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	< 0.169 U	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	1.42	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.58
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	4.18
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	< 0.258 U	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	8.79
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	1.65
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	4.23
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 6	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170988		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	2.04	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	0.633 J	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	0.169 JB	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	1.45 B	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	0.540 JB	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.57	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	< 0.169 U	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	1.31	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.44
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	4.83
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	1.42	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	7.56
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	1.66
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	3.94
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Site 7	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A170990		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	2.20	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	0.698 J	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	0.942 B	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	1.84 B	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	0.702 JB	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	1.40	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	1.35 B	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	1.29	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	2.33
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	3.82
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	2.06	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	7.12
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	< 0.101 U
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	3.70
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Trip blank 1	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A171396		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	< 0.227 U	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	< 0.287 U	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	< 0.0676 U	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	< 0.215 U	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	< 0.234 U	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	< 0.114 U	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	< 0.169 U	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	< 0.0942 U	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	< 0.130 U
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	< 0.191 U
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	< 0.258 U	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	< 0.111 U
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	< 0.101 U
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	< 0.203 U
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

Client Sample Name:	Trip blank 2	**Test Method:**	Parent and Alkyl Substituted PAHs by GC-MS/MS
FSES Sample ID:	A171397		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-dimethylnaphthalene, 573-98-8	< 0.227 U	benzo[c]fluorene, 205-12-9	< 0.0725 U
1,4-dimethylnaphthalene, 571-58-4	< 0.300 U	benzo[e]pyrene, 192-97-2	< 0.171 U
1,5-dimethylnaphthalene, 571-61-9	< 0.287 U	benzo[ghi]perylene, 191-24-2	< 0.0821 U
1,8-dimethylnaphthalene, 569-41-5	< 0.200 U	benzo[j]fluoranthene, 205-82-3	< 0.135 U
1-methylnaphthalene, 90-12-0	< 0.0676 U	benzo[k]fluoranthene, 207-08-9	< 0.128 U
1-methylphenanthrene, 832-69-9	< 0.256 U	chrysene, 218-01-9	< 0.121 U
1-methylpyrene, 2381-21-7	< 0.0918 U	coronene, 191-07-1	< 0.169 U
2,3-dimethylanthracene, 613-06-9	< 0.0821 U	cyclopenta[cd]pyrene, 27208-37-3	< 0.128 U
2,6-diethylnaphthalene, 59919-41-4	< 0.196 U	dibenzo[a,e]fluoranthene, 5385-75-1	< 0.114 U
2,6-dimethylnaphthalene, 581-42-0	< 0.215 U	dibenzo[a,e]pyrene, 192-65-4	< 1.56 U
2-ethylnaphthalene, 939-27-5	< 0.234 U	dibenzo[a,h]anthracene, 53-70-3	< 0.246 U
2-methylanthracene, 613-12-7	< 0.114 U	dibenzo[a,h]pyrene, 189-64-0	< 0.126 U
2-methylnaphthalene, 91-57-6	< 0.169 U	dibenzo[a,i]pyrene, 189-55-9	< 0.343 U
2-methylphenanthrene, 2531-84-2	< 0.0942 U	dibenzo[a,l]pyrene, 191-30-0	< 0.116 U
3,6-dimethylphenanthrene, 1576-67-6	< 0.101 U	dibenzo[e,l]pyrene, 192-51-8	< 0.403 U
5-methylchrysene, 3697-24-3	< 0.403 U	dibenzothiophene, 132-65-0	< 0.0580 U
6-methylchrysene, 1705-85-7	< 0.215 U	fluoranthene, 206-44-0	< 0.130 U
7,12-dimethylbenz[a]anthracene, 57-97-6	< 0.227 U	fluorene, 86-73-7	< 0.191 U
9,10-dimethylanthracene, 781-43-1	< 0.205 U	indeno[1,2,3-cd]pyrene, 193-39-5	< 0.0628 U
9-methylanthracene, 779-02-2	< 0.210 U	naphthalene, 91-20-3	< 0.251 U
acenaphthene, 83-32-9	< 0.258 U	naphtho[1,2-b]fluoranthene, 111189-32-3	< 0.403 U
acenaphthylene, 208-96-8	< 0.563 U	naphtho[2,3-a]pyrene, 196-42-9	< 0.403 U
anthanthrene, 191-26-4	< 0.0797 U	naphtho[2,3-e]pyrene, 193-09-9	< 0.403 U
anthracene, 120-12-7	< 0.254 U	naphtho[2,3-j]fluoranthene, 205-83-4	< 0.403 U
benz[a]anthracene, 56-55-3	< 0.181 U	naphtho[2,3-k]fluoranthene, 207-18-1	< 0.403 U
benzo[a]chrysene, 213-46-7	< 0.179 U	perylene, 77392-71-3	< 0.242 U
benzo[a]fluorene, 238-84-6	< 0.403 U	phenanthrene, 85-01-8	< 0.111 U
benzo[a]pyrene, 50-32-8	< 0.285 U	pyrene, 129-00-0	< 0.101 U
benzo[b]fluoranthene, 205-99-2	< 0.0894 U	retene, 483-65-8	< 0.203 U
benzo[b]fluorene, 243-17-4	< 0.403 U	triphenylene, 217-59-4	< 0.0990 U
benzo[b]perylene, 197-70-6	< 0.403 U		

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Certificate of Analysis

Client Report For: EPA - José L. Zambrana, Jr., PhD
National Exposure Research Laboratory
US EPA Office of Research and Development

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Project Name: EPA turf (wristbands) - Appendix 2
Project Number: F17-20
Report Date: February 23 2018

QC Review **Date**

FSES Director Approval: Kim A. Anderson **Date**

Methodology:

SOP 414.00: Determination of Oxygenated Polycyclic Aromatic Hydrocarbons by Gas Chromatography-Mass Spectrometry

Unit Conversions:
ppb = parts per billion
ppm = parts per million
ppt = parts per trillion
ng/g = ppb
ng/L = ppt
ng/mL = ppb
ng/µL = ppm
ng/g(Wristband) = ppb
pg/µL = ppb
µg/mL = ppm

Abbreviations:
J flag: Indicates lower precision in quantitation due to values near limits of detection or matrix effects.
B flag: The sample was background corrected.
< 123.45 U: Detection limit, indicates value was below limit of detection.

COA Notes:
Concentrations are reported in ng/g wristband

Client Sample Name:	Site 1	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170976		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 1 - dup	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170978		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 2	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170980		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 3	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170982		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 4	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170984		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 5	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170986		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	2.13	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 6	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170988		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	6.86
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	0.838 J
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Site 7	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A170990		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

Client Sample Name:	Trip blank 1	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A171396		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

COA Report

Client Sample Name:	Trip blank 2	**Test Method:**	OPAHs by GC-MS
FSES Sample ID:	A171397		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2-Naphthoquinone, 524-42-5	< 604 U	Acenaphthenequinone, 82-86-0	< 2.66 U
1,4-Anthraquinone, 635-12-1	< 1.35 U	Benz[a]anthracene-7,12-dione, 2498-66-0	< 0.205 U
1,4-Benzoquinone, 106-51-4	< 0.118 U	Benzanthrone, 82-05-3	< 0.188 U
1,4-Naphthoquinone, 130-15-4	< 0.109 U	Benzo(c)phenanthrene(1,4)quinone, 109699-80-1	< 0.411 U
2-Ethylanthraquinone, 84-51-5	< 0.085 U	Benzo(cd)pyrenone,	< 0.266 U
4H-cyclopenta[def]phenanthren-4-one, 5737-13-3	< 0.051 U	Benzofluorenone, 76723-60-9	< 0.109 U
5,12-Naphthacene-quinone, 1090-13-7	< 0.314 U	Chromone, 491-38-3	< 0.215 U
9,10-Anthraquinone, 84-65-1	< 0.362 U	Perinaphthenone, 548-39-0	< 0.215 U
9,10-Phenanthrenequinone, 84-11-7	< 60.4 U	Phenanthrene-1,4-dione, 569-15-3	< 0.208 U
9-Fluorenone, 486-25-9	< 0.048 U	Pyrene-4,5-dione, 6217-22-7	< 604 U
Aceanthracenequinone,	< 604 U	Xanthone, 90-47-1	< 0.092 U

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Certificate of Analysis

Client Report For: EPA - José L. Zambrana, Jr., PhD
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US EPA Office of Research and Development

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Project Name: EPA turf (wristbands) - Appendix 3
Project Number: F17-20
Report Date: February 23 2018

QC Review **Date**

FSES Director Approval: Kim A. Anderson **Date**

COA Report - Appendix 3

Project#: F17-20: EPA turf (wristbands)

Table 1: Sample detections

** Indicates a compound that shares an isomer, see table 3 for more information.*
[j] indicates a compound that was observed near the limit of detection.

Sample Name | Number	Compounds Found		
Trip blank 1 | A171396	Dicyclohexyl phthalate		
Trip blank 2 | A171397	naphthalene		
Site 1 | A170976	2,6-dimethylnaphthalene[j]*	acenaphthene[j]	Benzothiazole
	Bis(2-ethylhexyl)phthalate	Butylated hydroxyanisole	Dibenzofuran
	fluorene	naphthalene[j]	
Site 1 - dup | A170978	1,5-dimethylnaphthalene*	1-methylnaphthalene*	Benzothiazole
	Butylated hydroxyanisole	Dicyclohexyl phthalate[j]	Phthalimide
Site 2 | A170980	1,6-dimethylnaphthalene*	1-methylnaphthalene*	Benzothiazole
	Bis(2-ethylhexyl)phthalate	Butylated hydroxyanisole	fluorene
	naphthalene	Phthalimide	
Site 3 | A170982	1-methylnaphthalene*	2-ethylnaphthalene[j]*	Benzothiazole
	Bis(2-ethylhexyl)phthalate	d-Limonene	Dibenzofuran
	Triethyl phosphate		
Site 4 | A170984	Benzothiazole	Bis(2-ethylhexyl)phthalate	naphthalene[j]
Site 5 | A170986	1-methylnaphthalene[j]*	Benzothiazole	Bis(2-ethylhexyl)phthalate
	Di-n-butyl phthalate		
Site 6 | A170988	1-methylnaphthalene[j]*	Bis(2-ethylhexyl)phthalate[j]	
Site 7 | A170990	1-methylnaphthalene[j]*	Bis(2-ethylhexyl)phthalate	naphthalene[j]

Table 2: Compound Isomers

Compund	Alternative Isomers
1,5-dimethylnaphthalene	2-Ethylnaphthalene,2,6-dimethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,8-dimethylnaphthalene
1,6-dimethylnaphthalene	2-Ethylnaphthalene,2,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene
1-methylnaphthalene	2-methylnaphthalene
2,6-dimethylnaphthalene	2-Ethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene
2-ethylnaphthalene	2,6-dimethylnaphthalene,1,6-dimethylnaphthalene,1,4-dimethylnaphthalene,1,2-dimethylnaphthalene,1,5-dimethylnaphthalene

Table 3: Compound classification summary

Classification	Number of samples with detections	Compounds
Dioxins and Furans	2	Dibenzofuran
Endocrine Disruptors	10	Bis(2-ethylhexyl)phthala, Butylated hydroxyanisole, Di-n-butyl phthalate, Dicyclohexyl phthalate, fluorene, naphthalene
Flame Retardant	1	Triethyl phosphate
Industrial	9	1-methylnaphthalene, Benzothiazole, Bis(2-ethylhexyl)phthala, Di-n-butyl phthalate, Dicyclohexyl phthalate, Phthalimide, Triethyl phosphate
OPAH	0	N/A
PAH	9	1,5-dimethylnaphthalene, 1,6-dimethylnaphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, 2-ethylnaphthalene, acenaphthene, fluorene, naphthalene
PBB	0	N/A
PBDE	0	N/A
PCB	0	N/A
Personal Care	5	Butylated hydroxyanisole, d-Limonene, Di-n-butyl phthalate
Pesticides	8	Bis(2-ethylhexyl)phthala, Di-n-butyl phthalate, Phthalimide, Triethyl phosphate
Pharmacological	3	Butylated hydroxyanisole
Uncategorized	0	N/A

Appendix A: All compounds and their classifications

* Data taken from World Health Organization (WHO) International Agency for Research on Cancer (IARC) *Agents Classified by the IARC Monographs, Volumes 1–118*, last updated 3/1/2017

Groups are indicated by the Group and year classified, e.g. 2B(1999), for more info visit http://fses.oregonstate.edu/who-iarc

- Group 1: Carcinogenic to humans
- Group 2A: Probably carcinogenic to humans
- Group 2B: Probably carcinogenic to humans
- Group 3: Not classifiable as to its carcinogenicity to humans
- Group 4: Probably not carcinogenic to humans

** Data taken from the United States Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS), last updated on 5/1/2017,
for more info visit http://fses.oregonstate.edu/epa-iris

*** Data taken from State of California Environmental Protection Agency (Cal/EPA) *Chemicals Known to the State to Cause Cancer or Reproductive Toxicity*, last updated 3/1/2017,
for more info visit http://fses.oregonstate.edu/cal-epa

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
(2,3-Dibromopropyl) (2,4,6-tribromophenyl) ether	35109-60-5	Flame Retardant	-	-	-
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	3268-87-9	Dioxins/Furans	-	-	-
1,2,3,4,6,7,8,9-Octachlorodibenzofuran	39001-02-0	Dioxins/Furans	-	-	-
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	35822-46-9	Dioxins/Furans	-	-	-
1,2,3,4,6,7,8-Heptachlorodibenzofuran	38998-75-3	Dioxins/Furans	-	-	-
1,2,3,4,6,7,9-Heptachlorodibenzo-p-dioxin	58200-70-7	Dioxins/Furans	-	-	-
1,2,3,4,6,7-Hexachlorodibenzo-p-dioxin	58200-66-1	Dioxins/Furans	-	-	-
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	39227-28-6	Dioxins/Furans	-	-	-
1,2,3,4,7,8-Hexachlorodibenzofuran	55684-94-1	Dioxins/Furans	-	-	-
1,2,3,4,7-Pentachlorodibenzo-p-dioxin	39227-61-7	Dioxins/Furans	-	-	-
1,2,3,4-Tetrachlorodibenzo-p-dioxin	30746-58-8	Dioxins/Furans	-	-	-
1,2,3,4-Tetrachlorodibenzofuran	24478-72-6	Dioxins/Furans	-	-	-
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	57653-85-7	Dioxins/Furans	-	Carcinogenicity Assessment: Yes (Last revised: 03-31-1987)	-
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	19408-74-3	Dioxins/Furans	-	-	-
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	40321-76-4	Dioxins/Furans	-	-	-
1,2,3,7,8-Pentachlorodibenzofuran	57117-41-6	Dioxins/Furans,	-	-	-
1,2,3,8,9-Pentachlorodibenzo-p-dioxin	71925-18-3	Dioxins/Furans	-	-	-
1,2,3-Trichlorodibenzo-p-dioxin	54536-17-3	Dioxins/Furans	-	-	-
1,2,4,6,7,9/1,2,4,6,8,9-Hexachlorodibenzo-p-dioxin		Dioxins/Furans	-	-	-
1,2,4,6,8/1,2,4,7,9-Pentachlorodibenzo-p-dioxin	71998-76-0	Dioxins/Furans	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
1,2,4,7,8-Pentachlorodibenzo-p-dioxin	58802-08-7	Dioxins/Furans	-	-	-
1,2,4-Trichlorobenzene	120-82-1	Insecticide, Industrial	-	Oral RfD Assessment: Yes (Last revised: 05-01-1992), Carcinogenicity Assessment: Yes (Last revised: 06-01-1989)	-
1,2,4-Trichlorodibenzo-p-dioxin	39227-58-2	Dioxins/Furans	-	-	-
1,2,5,6,9,10-Hexabromocyclododecane	3194-55-6	Flame Retardant,	-	-	-
1,2,6,7-Tetrachlorodibenzo-p-dioxin	41903-57-5	Dioxins/Furans	-	-	-
1,2,6,8-Tetrachlorodibenzo-p-dioxin	67323-56-2	Dioxins/Furans	-	-	-
1,2,7,8-Tetrachlorodibenzo-p-dioxin	34816-53-0	Dioxins/Furans	-	-	-
1,2,8,9-Tetrachlorodibenzo-p-dioxin	116889-69-1	Dioxins/Furans	-	-	-
1,2-Bis(2,4,6-tribromophenoxy) ethane	37853-59-1	Flame Retardant	-	-	-
1,2-Dibromo-3-chloropropane	96-12-8	Fungicide, Insecticide,	2B(1999)	Inhalation RfC Assessment: Yes (Last revised: 10-01-1991),	cancer (1-Jul-87) male (27-Feb-87)
1,2-Dibromo-4-(1,2-dibromoethyl) cyclohexane	3322-93-8	Flame Retardant,	-	-	-
1,2-Dichlorodibenzo-p-dioxin	54536-18-4	Dioxins/Furans	-	-	-
1,2-dimethylnaphthalene	573-98-8	PAH	-	-	-
1,3,5-Tribromobenzene	626-39-1	Flame Retardant, Industrial	-	-	-
1,3,6,8-Tetrachlorodibenzo-p-dioxin	33423-92-6	Dioxins/Furans	-	-	-
1,3,6,8-Tetrachlorodibenzofuran	30402-14-3	Dioxins/Furans	-	-	-
1,3,7,8-Tetrachlorodibenzo-p-dioxin	50585-46-1	Dioxins/Furans,	-	-	-
1,3,7,9-Tetrachlorodibenzo-p-dioxin	116889-70-4	Dioxins/Furans	-	-	-
1,3-Dichlorobenzene	541-73-1	Insecticide, Industrial,	3(1999)	Carcinogenicity Assessment: Yes (Last revised: 09-01-1990)	-
1,3-Dichlorodibenzo-p-dioxin	50585-39-2	Dioxins/Furans	-	-	-
1,3-Dinitropyrene	75321-20-9	PAH	2B(2013)	-	cancer (2-Nov-12)
1,4-Anthraquinone	635-12-1	OPAH	-	-	-
1,4-Dichlorodibenzo-p-dioxin	54536-19-5	Dioxins/Furans	-	-	-
1,4-dimethylnaphthalene	571-58-4	PAH	-	-	-
1,4-Dioxino(2,3,b,5,6,b')dipyridine	262-16-8	Dioxins/Furans	-	-	-
1,4-Naphthoquinone	130-15-4	OPAH	-	-	-
1,5-dimethylnaphthalene	571-61-9	PAH	-	-	-
1,6-Benzo(a)pyrene-quinone	3067-13-8	OPAH	-	-	-
1,6-Dichlorodibenzo-p-dioxin	38178-38-0	Dioxins/Furans	-	-	-
1,6-dimethylnaphthalene	575-43-9	PAH	-	-	-
1,6-Dinitropyrene	42397-64-8	PAH	2B(2013)	-	cancer (1-Oct-90)
1,7,8-Trichlorodibenzo-p-dioxin	82306-65-8	Dioxins/Furans	-	-	-
1,8-dimethylnaphthalene	569-41-5	PAH	-	-	-
1,8-Dinitropyrene	42397-65-9	PAH	2B(2013)	-	cancer (1-Oct-90)
1-Chlorodibenzo-p-dioxin	39227-53-7	Dioxins/Furans	-	-	-
1-Hydroxynaphthalene	90-15-3	PAH, Industrial,	-	-	-
1-methylnaphthalene	90-12-0	PAH, Industrial	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
1-methylphenanthrene	832-69-9	PAH	3(2010)	-	-
1-methylpyrene	2381-21-7	PAH,	-	-	-
1-Naphthylamine	134-32-7	Industrial	3(1987)	-	cancer (1-Oct-89)
1-Nitronaphthalene	86-57-7	PAH, Industrial	3(1989)	-	-
1-Nitropyrene	5522-43-0	PAH	2A(2014)	-	cancer (1-Oct-90)
17a-Ethynylestradiol	57-63-6	Pharmacological	-	-	cancer (1-Jan-88)
2'-Hydroxy-2,4,4'-tribromodiphenyl ether	N/A	Flame Retardant, PBDE	-	-	-
2'-Hydroxy-4-monobromodiphenyl ether	N/A	Flame Retardant, PBDE	-	-	-
2'-Methoxy-2,4,4'-tribromodiphenyl ether	N/A	Flame Retardant, PBDE	-	-	-
2,3,4,5-Tertrachloronitrobenzene	879-39-0	Fungicide, Industrial	-	-	-
2,3,4,5-Tetrachlorophenol	4901-51-3	General Pesticide,	-	-	-
2,3,4,6-Tetrachlorophenol	58-90-2	Fungicide, Industrial, Pulp/Paper,	-	Oral RfD Assessment: Yes (Last revised: 03-01-1988),	-
2,3,4,7,8-PeCDF	57117-31-4	Dioxins/Furans,	1(2012)	-	-
2,3,4-Tribromophenol	138507-65-0	Flame Retardant,	-	-	-
2,3,4-Trichlorophenyl-4-nitrophenyl ether	142022-61-5	Industrial, Halo Ethers	-	-	-
2,3,5,6-Tetrachloro-p-terphenyl	61576-99-6	Flame Retardant, Industrial	-	-	-
2,3,5,6-Tetrachlorophenol	935-95-5	General Pesticide,	-	-	-
2,3,5-Trichlorophenol	933-78-8	Industrial,	-	-	-
2,3,5-Trichlorophenyl-4-nitrophenyl ether	142022-59-1	Industrial, Halo Ethers	-	-	-
2,3,5-Trimethacarb	2655-15-4	Insecticide	-	-	-
2,3,5-trimethylphenol	697-82-5	Personal Care, Industrial,	-	-	-
2,3,6-Trichloroanisole	50375-10-5	General Pesticide	-	-	-
2,3,6-Trichlorophenyl-4-nitrophenyl ether	142022-58-0	Industrial, Halo Ethers	-	-	-
2,3,6-trimethylphenol	2416-94-6	Industrial,	-	-	-
2,3,7,8-TCDD	1746-01-6	Industrial,	1(2012)	Oral RfD Assessment: Yes (Last revised: 02-17-2012), Carcinogenicity Assessment: Message (Last revised: 02-17-2012)	cancer (1-Jan-88) developmental (1-Apr-91)
2,3,7,8-Tetrabromodibenzo-p-dioxin	50585-41-6	Dioxins/Furans,	-	-	-
2,3,7,8-Tetrachlorodibenzofuran	51207-31-9	Industrial,	-	-	-
2,3,7-Trichlorodibenzo-p-dioxin	33857-28-2	Dioxins/Furans	-	-	-
2,3-Dibromoanisole	95970-22-2	Industrial	-	-	-
2,3-Dibromophenol	57383-80-9	Flame Retardant,	-	-	-
2,3-Dichlorodibenzo-p-dioxin	29446-15-9	Dioxins/Furans	-	-	-
2,3-Dichlorophenyl-4-nitrophenyl ether	82239-20-1	Industrial, Halo Ethers	-	-	-
2,3-dimethylaniline	87-59-2	Industrial,	-	-	-
2,3-dimethylanthracene	613-06-9	PAH	-	-	-
2,3-xylenol	526-75-0	Industrial,	-	-	-
2,4'-DDD	53-19-0	Insecticide, Pharmacological,	-	-	-
2,4'-DDE	3424-82-6	Insecticide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
2,4'-DDT	789-02-6	Insecticide,	-	-	developmental, female, male (15-May-98)
2,4'-Dichlorobenzophenone (2,4'-Dicofol decomposition product)	85-29-0	General Pesticide	-	-	-
2,4,5-T methyl ester	1928-37-6	Herbicide,	-	-	-
2,4,5-Tribromoanisole	N/A	Industrial	-	-	-
2,4,5-Trichloro-p-terphenyl	999008-03-6	Flame Retardant, Industrial	-	-	-
2,4,5-Trichloroaniline	636-30-6	General Pesticide,	-	-	-
2,4,5-Trichlorophenol	95-95-4	Fungicide, Herbicide, Industrial, Pulp/Paper,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987), Inhalation RfC Assessment: Message (Last revised: 07-01-1991),	-
2,4,5-Trichlorophenyl-4-nitrophenyl ether	22532-68-9	Industrial, Halo Ethers	-	-	-
2,4,5-Trimethylaniline	137-17-7	Industrial,	3(1987)	-	-
2,4,6-tri-tert-butylphenol	732-26-3	Industrial,	-	-	-
2,4,6-Tribromoanisole	607-99-8	Industrial	-	-	-
2,4,6-Tribromophenol	118-79-6	Flame Retardant,	-	-	-
2,4,6-Tribromophenyl allyl ether	3278-89-5	Flame Retardant	-	-	-
2,4,6-trichloroaniline	634-93-5	General Pesticide, Industrial,	-	-	-
2,4,6-Trichloroanisole	87-40-1	General Pesticide	-	-	-
2,4,6-Trichlorophenol	88-06-2	Fungicide, Herbicide, Industrial, Pulp/Paper,	2	Inhalation RfC Assessment: Message (Last revised: 07-01-1991), Carcinogenicity Assessment: Yes (Last revised: 06-01-1990)	cancer (1-Jan-88)
2,4,6-triiodophenol	609-23-4	Industrial,	-	-	-
2,4,6-trimethylphenol	527-60-6	Industrial,	-	-	-
2,4,8-Trichlorodibenzofuran	54589-71-8	Dioxins/Furans	-	-	-
2,4-bis(alpha,alpha-dimethylbenzyl) phenol	2772-45-4	Industrial,	-	-	-
2,4-D methyl ester	1928-38-7	General Pesticide	-	-	-
2,4-D sec-butyl ester	94-79-1	Herbicide	-	-	-
2,4-DB methyl ester	18625-12-2	General Pesticide	-	-	-
2,4-di-tert-amylphenol	120-95-6	Industrial,	-	-	-
2,4-di-tert-butylphenol	96-76-4	Industrial,	-	-	-
2,4-Dibromoanisole	21702-84-1	Industrial	-	-	-
2,4-Dibromophenol	615-58-7	Flame Retardant,	-	-	-
2,4-Dibromophenyl-4-nitrophenyl ether	2671-93-4	Industrial, Halo Ethers	-	-	-
2,4-dichloro-3,5-dimethylphenol	133-53-9	Industrial,	-	-	-
2,4-dichloroaniline	554-00-7	Industrial,	-	-	-
2,4-Dichlorophenol	120-83-2	Fungicide, Herbicide, Industrial, Pulp/Paper,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987),	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
2,4-Dichlorophenyl benzenesulfonate	97-16-5	General Pesticide	-	-	-
2,4-Dimethylaniline	95-68-1	Industrial,	3(1987)	-	-
2,4-Dimethylphenol	105-67-9	Pharmacological, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 11-01-1990),	-
2,4-dinitroaniline	97-02-9	Industrial,	-	-	-
2,5-Dibromoanisole	95970-08-4	Industrial	-	-	-
2,5-Dibromophenol	28165-52-8	Flame Retardant,	-	-	-
2,5-dichloroaniline	95-82-9	Industrial,	-	-	-
2,5-dichlorophenol	583-78-8	Industrial,	-	-	-
2,5-Dichlorophenyl-4-nitrophenyl ether	391-48-7	Industrial, Halo Ethers	-	-	-
2,5-dimethylaniline	95-78-3	Industrial,	3(1987)	-	-
2,5-dimethylphenol	95-87-4	Industrial,	-	-	-
2,6-di-tert-butyl-4-dimethylaminomethylphenol	88-27-7	Industrial,	-	-	-
2,6-Di-tert-butyl-4-ethylphenol	4130-42-1	Industrial,	-	-	-
2,6-Di-tert-butylphenol	128-39-2	Industrial,	-	-	-
2,6-dibromo-4-nitroaniline	827-94-1	Industrial,	-	-	-
2,6-Dibromoanisole	38603-09-7	Industrial	-	-	-
2,6-Dibromophenol	608-33-3	Flame Retardant,	-	-	-
2,6-Dichlorobenzamide	2008-58-4	Herbicide	-	-	-
2,6-Dichlorobenzonitrile	1194-65-6	Herbicide	-	-	-
2,6-Dichlorophenol	87-65-0	Industrial, Pulp/Paper,	-	-	-
2,6-Dichlorophenyl-4-nitrophenyl ether	2093-28-9	Industrial, Halo Ethers	-	-	-
2,6-Dichlorosyringaldehyde	76330-06-8	Industrial, Pulp/Paper	-	-	-
2,6-diethylaniline	579-66-8	Industrial,	-	-	-
2,6-diethylnaphthalene	59919-41-4	PAH	-	-	-
2,6-diisopropylaniline	24544-04-5	Industrial,	-	-	-
2,6-dimethoxyphenol	91-10-1	Industrial,	-	-	-
2,6-Dimethylaniline	87-62-7	Industrial,	2B(1993)	-	cancer (1-Jan-91)
2,6-dimethylnaphthalene	581-42-0	PAH	-	-	-
2,6-dimethylphenol	576-26-1	Industrial,	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988),	-
2,6-ditertbutyl-4-methoxyphenol	489-01-0	Industrial,	-	-	-
2,7-Dichlorodibenzo-p-dioxin	33857-26-0	Dioxins/Furans	-	-	-
2,8-Dichlorodibenzo-p-dioxin	38964-22-6	Dioxins/Furans,	-	-	-
2,8-Dichlorodibenzofuran	5409-83-6	Dioxins/Furans,	-	-	-
2-(1-methylbutyl)phenol	87-26-3	Industrial,	-	-	-
2-(1-naphthyl)acetamide	86-86-2	General Pesticide, PAH	-	-	-
2-(2-Butoxyethoxy)ethyl thiocyanate	112-56-1	Insecticide	-	-	-
2-(3-Chlorophenoxy)propionamide	5825-87-6	General Pesticide	-	-	-
2-(4-chlorophenyl)benzothiazole	6265-91-4	Industrial	-	-	-
2-(morpholinothio)benzothiazole	102-77-2	Industrial	-	-	-
2-(Octylthio)ethanol	3547-33-9	Insecticide	-	-	-
2-Amino-4-chlorophenol	95-85-2	Industrial,	-	-	-
2-amino-p-cresol	95-84-1	Industrial,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
2-aminophenol	95-55-6	Industrial,	-	-	-
2-benzothiazolyl sulfide	4074-77-5	Industrial	-	-	-
2-bromo-4,6-dinitroaniline	1817-73-8	Industrial,	-	-	-
2-Bromoanisole	578-57-4	Industrial	-	-	-
2-bromophenol	95-56-7	Industrial,	-	-	-
2-chloro-4,6-dinitroaniline	3531-19-9	Industrial,	-	-	-
2-chloro-4-nitroaniline	121-87-9	Industrial,	-	-	-
2-chloroaniline	95-51-2	Industrial,	-	-	-
2-Chlorodibenzo-p-dioxin	39227-54-8	Dioxins/Furans	-	-	-
2-chlorodibenzofuran	51230-49-0	Dioxins/Furans,	-	-	-
2-Chlorophenol	95-57-8	General Pesticide, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-
2-Chlorophenyl-4-nitrophenyl ether	2303-23-3	Industrial, Halo Ethers	-	-	-
2-Chlorosyringaldehyde	76341-69-0	Industrial, Pulp/Paper	-	-	-
2-ethoxyphenol	94-71-3	Industrial,	-	-	-
2-Ethyl-1,3-hexanediol	94-96-2	Insecticide	-	-	-
2-ethyl-6-methylaniline	24549-06-2	Industrial,	-	-	-
2-ethylaniline	578-54-1	Industrial,	-	-	-
2-ethylnaphthalene	939-27-5	PAH	-	-	-
2-ethylphenol	90-00-6	Industrial,	-	-	-
2-Hydroxyestradiol	362-05-0	Pharmacological	-	-	-
2-isopropylaniline	643-28-7	Industrial,	-	-	-
2-isopropylphenol	88-69-7	Industrial,	-	-	-
2-Mercaptobenzothiazole	149-30-4	Industrial	2	-	-
2-methoxy-4-methylphenol	93-51-6	Natural,	-	-	-
2-methyl-9,10-anthraquinone	84-54-8	OPAH	-	-	-
2-methylanthracene	613-12-7	PAH	-	-	-
2-methylnaphthalene	91-57-6	PAH, Industrial	-	Oral RfD Assessment: Yes (Last revised: 12-22-2003). Inhalation RfC Assessment: Discussion (Last revised: 12-22-2003). Carcinogenicity Assessment: Yes (Last revised: 12-22-2003)	-
2-methylphenanthrene	2531-84-2	PAH	-	-	-
2-Methylphenol	95-48-7	Industrial,	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988). Inhalation RfC Assessment: Message (Last revised: 04-01-1992). Carcinogenicity Assessment: Yes (Last revised: 09-01-1990)	-
2-Naphthylamine	91-59-8	PAH, Industrial	1(2012)	-	cancer (27-Feb-87)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
2-nitro-p-cresol	119-33-5	Industrial,	-	-	-
2-Nitroaniline	88-74-4	Industrial,	-	-	-
2-Nitroanthracene	3586-69-4	PAH	-	-	-
2-Nitrobiphenyl	86-00-0	Fungicide	-	-	-
2-Nitrofluorene	607-57-8	PAH, Industrial	2B(2013)	-	cancer (1-Oct-90)
2-Nitronaphthalene	581-89-5	PAH, Industrial	3(1989)	-	-
2-Nitrophenol	88-75-5	Industrial,	-	-	-
2-Nitropyrene	789-07-1	PAH	3(1989)	-	-
2-Phenoxypropionic acid	940-31-8	Pharmacological	-	-	-
2-propylphenol	644-35-9	Industrial,	-	-	-
2-sec-butylphenol	89-72-5	Industrial,	-	-	-
3,3'-Dimethoxybenzidine	119-90-4	Industrial	2B(1987)	-	cancer (1-Jan-88)
3,4,5-trichlorophenol	609-19-8	Industrial,	-	-	-
3,4,5-Trichlorophenyl-4-nitrophenyl ether		Industrial, Halo Ethers	-	-	-
3,4,5-Trimethacarb	2686-99-9	Insecticide	-	-	-
3,4,6-Trichloroguaiacol	60712-44-9	Industrial, Pulp/Paper	-	-	-
3,4-Dichloroaniline	95-76-1	General Pesticide, Pharmacological, Industrial,	-	-	-
3,4-Dichlorocatechol	3978-67-4	Industrial, Pulp/Paper	-	-	-
3,4-Dichloroguaiacol	77102-94-4	Industrial, Pulp/Paper	-	-	-
3,4-Dichlorophenyl-4-nitrophenyl ether	22532-80-5	Industrial, Halo Ethers	-	-	-
3,4-dimethylaniline	95-64-7	Industrial,	-	-	-
3,4-dimethylphenol	95-65-8	Industrial,	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988).	-
3,5-Dibromoanisole	74137-36-3	Industrial	-	-	-
3,5-Dibromophenol	626-41-5	Flame Retardant,	-	-	-
3,5-Dichloroaniline	626-43-7	General Pesticide, Pharmacological, Industrial,	-	-	-
3,5-Dichlorophenyl-4-nitrophenyl ether	21105-77-1	Industrial, Halo Ethers	-	-	-
3,5-dimethylaniline	108-69-0	Industrial,	-	-	-
3,5-dimethylphenol	108-68-9	Industrial,	-	-	-
3,6-dimethylphenanthrene	1576-67-6	PAH	-	-	-
3-(dimethylamino)phenol	99-07-0	Industrial,	-	-	-
3-Aminophenol	591-27-5	Personal Care, Industrial,	-	-	-
3-Bromoanisole	2398-37-0	Industrial	-	-	-
3-Bromophenol	591-20-8	Flame Retardant,	-	-	-
3-Bromostyrene	2039-86-3	Flame Retardant	-	-	-
3-Chloro-4-fluoroaniline	367-21-5	Industrial,	-	-	-
3-Chloro-4-methoxyaniline	5345-54-0	General Pesticide,	-	-	-
3-Chloroaniline	108-42-9	Industrial,	-	-	-
3-chlorophenol	108-43-0	Pharmacological, Industrial, Pulp/Paper,	-	-	-
3-Chlorophenyl-4-nitrophenyl ether	2303-23-3	Industrial, Halo Ethers	-	-	-
3-ethylphenol	620-17-7	Industrial,	-	-	-
3-hydroxybiphenyl	580-51-8	Industrial,	-	-	-
3-Hydroxycarbofuran	16655-82-6	Industrial	-	-	-
3-Indolylacetonitrile	771-51-7	General Pesticide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
3-Methoxy-2,2',4,4',6-pentabromodiphenyl ether	N/A	Flame Retardant, PBDE	-	-	-
3-methoxyphenol	150-19-6	Pharmacological, Industrial, Natural,	-	-	-
3-Nitroaniline	99-09-2	Industrial,	-	-	-
3-Nitrobenzanthrone	17117-34-9	PAH	2B(2014)	-	-
3-Nitrobiphenyl	2113-58-8	Industrial	-	-	-
3-nitrodibenzofuran	5410-97-9	Dioxins/Furans	-	-	-
3-Nitrofluoranthene	892-21-7	PAH	3(1987)	-	-
3-Nitrophenanthrene	17024-19-0	PAH	-	-	-
3-nitrophenol	554-84-7	Industrial,	-	-	-
3-tert-butylphenol	585-34-2	Industrial,	-	-	-
3-Trifluormethylaniline	98-16-8	Herbicide, Industrial,	-	-	-
4,4'-DDD	72-54-8	Insecticide,	-	Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	cancer (1-Jan-89)
4,4'-DDE	72-55-9	Insecticide,	-	Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	developmental, male (30-Mar-10) cancer (1-Jan-89)
4,4'-DDT	50-29-3	Insecticide,	2	Oral RfD Assessment: Yes (Last revised: 03-31-1987), Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	cancer (1-Oct-87) developmental, female, male (15-May-98)
4,4'-Dichlorobenzophenone	90-98-2	General Pesticide	-	-	-
4,4'-Methylenedianiline	101-77-9	Industrial,	2B(1987)	-	cancer (1-Jan-88)
4,4'-Oxydianiline	101-80-4	Industrial,	2B(1987)	-	cancer (1-Jan-88)
4,5-Dichloroguaiacol	2460-49-3	Industrial, Pulp/Paper	-	-	-
4,6-Dichloroguaiacol	16766-31-7	Industrial, Pulp/Paper	-	-	-
4,6-Dinitro-o-cresol (DNOC)	534-52-1	Fungicide, Herbicide, Insecticide, Industrial,	-	-	-
4-(2-benzothiazolyldithio)morpholine	95-32-9	Industrial	-	-	-
4-alpha-cumylphenol	599-64-4	Industrial,	-	-	-
4-amino-2,6-dichlorophenol	5930-28-9	Industrial,	-	-	-
4-Aminobiphenyl	92-67-1	Industrial	1(2012)	-	cancer (27-Feb-87)
4-aminophenol	123-30-8	Industrial,	-	-	-
4-benzylphenol	101-53-1	Industrial,	-	-	-
4-Bromoaniline	106-40-1	Herbicide, Industrial,	-	-	-
4-Bromoanisole	104-92-7	Industrial	-	-	-
4-Bromophenol	106-41-2	Flame Retardant,	-	-	-
4-Bromostyrene	2039-82-9	Flame Retardant	-	-	-
4-butylphenol	1638-22-8	Industrial,	-	-	-
4-Chloro-2-methylaniline	95-69-2	General Pesticide, Industrial,	2A(2010)	-	cancer (1-Jan-90)
4-chloro-2-methylphenol	1570-64-5	Industrial,	-	-	-
4-chloro-2-nitroaniline	89-63-4	Industrial,	-	-	-
4-chloro-3,5-dimethylphenol	88-04-0	Industrial,	-	-	-
4-Chloro-3-methylphenol	59-50-7	Fungicide, Industrial,	-	-	-
4-Chloroaniline	106-47-8	Herbicide, Industrial,	2B(1993)	Oral RfD Assessment: Yes (Last revised: 08-22-1988),	cancer (1-Oct-94)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
4-Chlorocatechol	2138-22-9	Industrial, Pulp/Paper	-	-	-
4-Chlorodibenzofuran	74992-96-4	Dioxins/Furans,	-	-	-
4-Chloroguaiacol	16766-30-6	Industrial, Pulp/Paper	-	-	-
4-Chlorophenol	106-48-9	Industrial, Pulp/Paper,	-	-	-
4-Chlorophenyl isocyanate	104-12-1	Herbicide, Industrial	-	-	-
4-Chlorophenyl phenyl ether	7005-72-3	Industrial, Halo Ethers	-	-	-
4-Chlorophenyl-4-nitrophenyl ether	1836-74-4	Industrial, Halo Ethers	-	-	-
4-ethoxyphenol	622-62-8	Industrial,	-	-	-
4-ethylphenol	123-07-9	Personal Care, Industrial,	-	-	-
4-hydroxy-3-chlorobiphenyl	92-04-6	Industrial,	-	-	-
4-hydroxybiphenyl	92-69-3	Industrial,	-	-	-
4-Isopropylaniline	99-88-7	Industrial,	-	-	-
4-isopropylphenol	99-89-8	Personal Care, Industrial,	-	-	-
4-methoxyphenol	150-76-5	Pharmacological, Industrial,	-	-	-
4-Methylphenol	106-44-5	Industrial,	-	Oral RfD Assessment: Withdrawn (Last revised: 08-01-1991). Inhalation RfC Assessment: Message (Last revised: 04-01-1992). Carcinogenicity Assessment: Yes (Last revised: 09-01-1990)	-
4-n-octylphenol	1806-26-4	Industrial,	-	-	-
4-Nitroaniline	100-01-6	Industrial,	-	-	-
4-Nitrobiphenyl	92-93-3	Industrial	3(1987)	-	cancer (1-Apr-88)
4-Nitrophenol	100-02-7	Fungicide, Industrial,	-	Inhalation RfC Assessment: Message (Last revised: 10-01-1991).	-
4-Nitrophenyl phenyl ether	620-88-2	Industrial, Halo Ethers	-	-	-
4-Nonylphenol	104-40-5	Fungicide, Industrial,	-	-	-
4-tert-butylphenol	98-54-4	Industrial,	-	-	-
4H-cyclopenta[def]phenanthren-4-one	5737-13-3	OPAH, Industrial	-	-	-
5,12-Naphthacene-quinone	1090-13-7	OPAH	-	-	-
5,6-Dichlorovanillin	18268-69-4	Industrial, Pulp/Paper	-	-	-
5,7-Dihydroxy-4'-methoxyisoflavone	491-80-5	Pharmacological,	-	-	-
5-Chlorovanillin	19463-48-0	Industrial, Pulp/Paper	-	-	-
5-methylchrysene	3697-24-3	PAH,	2B(2010)	-	cancer (1-Apr-88)
5-Nitroacenaphthene	602-87-9	PAH	2B(1987)	-	cancer (1-Apr-88)
6-chloro-m-cresol	615-74-7	Industrial,	-	-	-
6-Chlorovanillin	18268-76-3	Industrial, Pulp/Paper	-	-	-
6-methylchrysene	1705-85-7	PAH	3(2010)	-	-
6-Nitrobenzo(a)pyrene	63041-90-7	PAH	3(1989)	-	-
6-Nitrochrysene	7496-02-8	PAH	2A(2014)	-	cancer (1-Oct-90)
7,12-dimethylbenz[a]anthracene	57-97-6	PAH,	-	-	cancer (1-Jan-90)
7-Nitrobenz(a)anthracene	20268-51-3	PAH	3(1989)	-	-
9,10-Anthraquinone	84-65-1	OPAH, Industrial	2B(2013)	-	cancer (28-Sep-07)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
9,10-dimethylanthracene	781-43-1	PAH	-	-	-
9,10-Phenanthrenequinone	84-11-7	Fungicide, OPAH, Industrial	-	-	-
9-Fluorenone	486-25-9	OPAH, Industrial	-	-	-
9-methylanthracene	779-02-2	PAH	-	-	-
9-Nitroanthracene	602-60-8	PAH	3(1987)	-	-
9-Nitrophenanthrene	954-46-1	PAH	-	-	-
a-Ionone	127-41-3	Personal Care, Natural	-	-	-
acenaphthene	83-32-9	PAH	3(2010)	Oral RfD Assessment: Yes (Last revised: 11-01-1990).	-
Acenaphthenequinone	82-86-0	OPAH	-	-	-
acenaphthylene	208-96-8	PAH	-	Carcinogenicity Assessment: Yes (Last revised: 01-01-1991)	-
Acephate	30560-19-1	Insecticide,	-	-	-
Acequinocyl	57960-19-7	General Pesticide, OPAH	-	-	-
acetamiprid	135410-20-7	Insecticide	-	-	-
Acetochlor	34256-82-1	Herbicide, Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-01-1993).	cancer (1-Jan-89)
Acifluorfen methyl ester	50594-67-7	Herbicide,	-	-	-
Aclonifen	74070-46-5	Herbicide	-	-	-
Acrinathrin	101007-06-1	Insecticide	-	-	-
Alachlor	15972-60-8	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-01-1993).	cancer (1-Jan-89)
Aldrin	309-00-2	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987). Carcinogenicity Assessment: Yes (Last revised: 09-30-1987)	cancer (1-Jul-88)
Allidochlor	93-71-0	Herbicide	-	-	-
alpha, alpha-Dibromo-m-xylene	626-15-3	Flame Retardant, Industrial	-	-	-
alpha-BHC	319-84-6	Insecticide, Pharmacological,	-	Carcinogenicity Assessment: Yes (Last revised: 03-31-1987)	-
alpha-Chlordane	5103-71-9	Insecticide,	-	-	-
Ametryne	834-12-8	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Amidithion	919-76-6	Insecticide	-	-	-
Aminocarb	2032-59-9	Insecticide	-	-	-
Amitraz	33089-61-1	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	developmental (30-Mar-99)
Amitraz metabolite [Methanimidamide, N-(2,4-dimethylphenyl)-N'-methyl-]	33089-74-6	Insecticide	-	-	-
amyl cinnamal	122-40-7	Personal Care	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
amylcinnamyl alcohol	101-85-9	Personal Care	-	-	-
Ancymidol	12771-68-5	General Pesticide	-	-	-
Anilazine	101-05-3	Fungicide	-	-	-
Aniline	62-53-3	Industrial,	3(1987)	Inhalation RfC Assessment: Yes (Last revised: 11-01-1990), Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	cancer (1-Jan-90)
Anilofos	64249-01-0	Herbicide,	-	-	-
Anisyl alcohol	105-13-5	Personal Care, Natural	-	-	-
anthanthrene	191-26-4	PAH	3(2010)	-	-
anthracene	120-12-7	PAH,	3(2010)	Oral RfD Assessment: Yes (Last revised: 09-01-1990), Carcinogenicity Assessment: Yes (Last revised: 01-01-1991)	-
Aramite	140-57-8	General Pesticide	2B(1987)	Carcinogenicity Assessment: Yes (Last revised: 06-01-1991)	cancer (1-Jul-87)
Aramite II	999009-03-9	General Pesticide	-	-	-
Atraton	1610-17-9	Herbicide	-	-	-
Atrazine	1912-24-9	Herbicide, Pharmacological,	3(1999)	Oral RfD Assessment: Yes (Last revised: 10-01-1993),	developmental, female (15-Jul-16)
Atrazine-desethyl	6190-65-4	Herbicide,	-	-	developmental, female (15-Jul-16)
Azaconazole	60207-31-0	Fungicide	-	-	-
Azamethiphos	35575-96-3	Insecticide	-	-	-
Azibenzolar-S-methyl	135158-54-2	Fungicide	-	-	-
Azinphos-ethyl	2642-71-9	Insecticide	-	-	-
Azinphos-methyl	86-50-0	Insecticide, Pharmacological,	-	-	-
Aziprotryn metabolite [2-Amino-4-isopropylamino-6-methylthio-1,3,5-triazine]	4147-57-3	Pesticide Product	-	-	-
Aziprotryne	4658-28-0	Herbicide	-	-	-
Azobenzene	103-33-3	Fungicide, Insecticide, Industrial	3(1987)	Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	cancer (1-Jan-90)
Azoxybenzene	495-48-7	Insecticide, Industrial	-	-	-
Azoxystrobin	131860-33-8	Fungicide	-	-	-
b-citronellol	106-22-9	Personal Care, Natural	-	-	-
b-Estradiol	50-28-2	Pharmacological	-	-	cancer (1-Jan-88)
b-Ionone	79-77-6	Personal Care, Natural	-	-	-
Barban	101-27-9	Herbicide	-	-	-
Beflubutamid	113614-08-7	Herbicide	-	-	-
Benalaxyl	71626-11-4	Fungicide	-	-	-
Benazolin-ethyl	25059-80-7	Herbicide	-	-	-
Bendiocarb	22781-23-3	Insecticide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Benfluralin	1861-40-1	Herbicide	-	-	-
Benfuracarb	82560-54-1	Insecticide	-	-	-
Benfuresate	68505-69-1	Herbicide	-	-	-
Benodanil	15310-01-7	Fungicide	-	-	-
Benoxacor	98730-04-2	Herbicide	-	-	-
Bentazone	25057-89-0	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 03-02-1998), Inhalation RfC Assessment: Message (Last revised: 03-02-1998), Carcinogenicity Assessment: Yes (Last revised: 03-02-1998)	-
Bentazone methyl derivative	61592-45-8	Herbicide	-	-	-
Benthiocarb	28249-77-6	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987),	-
Benz(a)anthracene-7,12-dione	2498-66-0	OPAH	-	-	-
benz[a]anthracene	56-55-3	PAH,	2B(2010)	Inhalation RfC Assessment: No (Last revised: 09-01-1994), Carcinogenicity Assessment: Yes (Last revised: 03-01-1994)	cancer (1-Jul-87)
benz[j]and[e]aceanthrylene	202-33-5 and 199-54-2	PAH	2B(2010)	-	-
Benzanthrone	82-05-3	OPAH, Industrial,	-	-	-
Benzenesulfonamide	98-10-2	Pharmacological	-	-	-
Benzidine	92-87-5	Industrial	1(2012)	Oral RfD Assessment: Yes (Last revised: 01-01-1989), Inhalation RfC Assessment: Message (Last revised: 07-01-1991), Carcinogenicity Assessment: Yes (Last revised: 03-31-1987)	cancer (27-Feb-87)
Benzo(a)fluoren-11-one	479-79-8	OPAH	-	-	-
Benzo(a)pyrene-7,8-dione	65199-11-3	OPAH	-	-	-
Benzo(c)phenanthrene(1,4)quinone	109699-80-1	OPAH	-	-	-
benzo[a]chrysene	213-46-7	PAH,	3(2010)	-	-
benzo[a]fluorene	238-84-6	PAH	3(2010)	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
benzo[a]pyrene	50-32-8	PAH,	1(2012)	Oral RfD Assessment: Yes (Last revised: 01-19-2017). Inhalation RfC Assessment: Yes (Last revised: 01-19-2017). Carcinogenicity Assessment: Yes (Last revised: 01-19-2017)	cancer (1-Jul-87)
benzo[b]fluoranthene	205-99-2	PAH,	2B(2010)	Carcinogenicity Assessment: Yes (Last revised: 03-01-1994)	cancer (1-Jul-87)
benzo[b]fluorene	243-17-4	PAH,	3(2010)	-	-
benzo[b]perylene	197-70-6	PAH	-	-	-
benzo[c]fluorene	205-12-9	PAH	3(2010)	-	-
benzo[e]pyrene	192-97-2	PAH,	3(2010)	-	-
benzo[ghi]perylene	191-24-2	PAH	3(2010)	Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	-
benzo[j]fluoranthene	205-82-3	PAH,	2B(2010)	-	cancer (1-Jul-87)
benzo[k]fluoranthene	207-08-9	PAH,	2B(2010)	Carcinogenicity Assessment: Yes (Last revised: 03-01-1994)	cancer (1-Jul-87)
Benzophenone	119-61-9	Personal Care, Industrial,	2B(2013)	-	cancer (22-Jun-12)
Benzothiazole	95-16-9	Industrial	-	-	-
benzotriazole	95-14-7	Industrial	-	-	-
Benzoximate metabolite	55440-55-6	General Pesticide	-	-	-
Benzoylprop ethyl	22212-55-1	Herbicide	-	-	-
Benzyl alcohol	100-51-6	Pharmacological, Personal Care, Industrial, Natural	-	-	-
Benzyl benzoate	120-51-4	Insecticide	-	-	-
Benzyl cinnamate	103-41-3	Personal Care, Natural	-	-	-
benzyl salicylate	118-58-1	Personal Care, Natural	-	-	-
beta-BHC	319-85-7	Insecticide,	-	Carcinogenicity Assessment: Yes (Last revised: 09-30-1987)	-
BHC epsilon isomer	6108-10-7	Insecticide	-	Carcinogenicity Assessment: Yes (Last revised: 03-31-1987)	-
Bifenazate metabolite (5-Phenyl-o-anisidine)	39811-17-1	General Pesticide	-	-	-
Bifenox	42576-02-3	Herbicide,	-	-	-
Bifenthrin	82657-04-3	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-
Bifenthrin	82657-04-3	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Bifenthrin	82657-04-3	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-
Binapacryl	485-31-4	Fungicide	-	-	-
Bioallethrin	584-79-2	Insecticide	-	-	-
Bioallethrin S-cyclopentenyl isomer	28434-00-6	Insecticide	-	-	-
Bioresmethrin	28434-01-7	Insecticide	-	-	-
Biphenyl	92-52-4	Fungicide, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 08-27-2013). Inhalation RfC Assessment: Discussion (Last revised: 08-27-2013). Carcinogenicity Assessment: Yes (Last revised: 08-27-2013)	-
Bis(2,3,3,3-tetrachloropropyl) ether	127-90-2	Insecticide	-	-	-
Bis(2-butoxyethyl) phthalate	117-83-9	Industrial	-	-	-
Bis(2-ethylhexyl)phthalate	117-81-7	Insecticide, Industrial,	2B(2013)	Oral RfD Assessment: Yes (Last revised: 01-31-1987). Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	cancer (1-Jan-88) developmental, male (24-Oct-03)
Bisphenol A	80-05-7	Industrial,	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988).	female (11-May-15)
bisphenol B	77-40-7	Industrial,	-	-	-
bisphenol E	2081-08-5	Industrial,	-	-	-
bisphenol Z	843-55-0	Industrial,	-	-	-
bisphenol AF	1478-61-1	Industrial,	-	-	-
Bitertanol I	55179-31-2	Fungicide,	-	-	-
Bitertanol II	999027-03-1	Fungicide	-	-	-
Boscalid (Nicobifen)	188425-85-6	Fungicide	-	-	-
Bromacil	314-40-9	Herbicide,	-	-	-
Bromfenvinphos-(E)	58580-14-6	Insecticide	-	-	-
Bromfenvinphos-(Z)	58580-13-5	Insecticide	-	-	-
Bromobutide	74712-19-9	Herbicide	-	-	-
Bromocyclen	1715-40-8	Insecticide	-	-	-
Bromophos	2104-96-3	Insecticide,	-	-	-
Bromophos-ethyl	4824-78-6	Insecticide,	-	-	-
Bromopropylate	18181-80-1	General Pesticide,	-	-	-
Bromoxynil	1689-84-5	Herbicide,	-	-	developmental (1-Oct-90)
Bromoxynil octanoic acid ester	1689-99-2	Herbicide	-	-	developmental (18-May-99)
Bromuconazole I	116255-48-2	Fungicide	-	-	-
Bromuconazole II	999010-03-6	Fungicide	-	-	-
Bufencarb	8065-36-9	Insecticide	-	-	-
Bupirimate	41483-43-6	Fungicide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Buprofezin	69327-76-0	Insecticide	-	-	-
Butachlor	23184-66-9	Herbicide,	-	-	-
Butafenacil	134605-64-4	Herbicide	-	-	-
Butamifos	36335-67-8	Herbicide,	-	-	-
Butoxycarboxim	34681-23-7	Insecticide	-	-	-
Butralin	33629-47-9	Herbicide	-	-	-
Butyl benzyl phthalate	85-68-7	Industrial,	3(1999)	Oral RfD Assessment: Yes (Last revised: 09-01-1989). Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	developmental (2-Dec-05)
Butylate	2008-41-5	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 10-01-1994).	-
Butylated hydroxyanisole	25013-16-5	Pharmacological, Personal Care,	2B(1987)	-	cancer (1-Jan-90)
butylated hydroxytoluene	128-37-0	Industrial,	3(1987)	-	-
Cadusafos	95465-99-9	Insecticide	-	-	-
Cafenstrole	125306-83-4	Herbicide	-	-	-
Caffeine	58-08-2	Pharmacological,	3(1991)	-	-
Captafol	2425-06-1	Fungicide	2A(1991)	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	cancer (1-Oct-88)
Captan	133-06-2	Fungicide,	3(1987)	Oral RfD Assessment: Yes (Last revised: 03-01-1989).	cancer (1-Jan-90)
Carbaryl	63-25-2	Insecticide, PAH,	3(1987)	Oral RfD Assessment: Yes (Last revised: 01-31-1987). Inhalation RfC Assessment: Message (Last revised: 11-01-1991).	cancer (5-Feb-10) developmental, female, male (7-Aug-09)
Carbazole	86-74-8	Industrial	2B(2013)	-	cancer (1-May-96)
Carbetamide	16118-49-3	Herbicide	-	-	-
Carbofuran	1563-66-2	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Carbofuran-3-keto	16709-30-1	General Pesticide	-	-	-
Carbofuran-7-phenol	1563-38-8	Pesticide Product,	-	-	-
Carbophenothion	786-19-6	Insecticide	-	-	-
Carbosulfan	55285-14-8	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Carboxin	5234-68-4	Fungicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Carfentrazone-ethyl	128639-02-1	Herbicide	-	-	-
Carpropamid	104030-54-8	Fungicide	-	-	-
Carvone	99-49-0	Fungicide, Personal Care	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Cashmeran	33704-61-9	Personal Care,	-	-	-
Cekafix	121227-99-4	Fungicide, Insecticide	-	-	-
Celestolide	13171-00-1	PAH, Personal Care,	-	-	-
Chinomethionat	2439-01-2	Fungicide	-	-	cancer (20-Aug-99) developmental (6-Nov-98)
Chloramben methyl ester	7286-84-2	Herbicide	-	-	-
Chloranocryl	2164-09-2	Herbicide	-	-	-
Chlorbenside	103-17-3	General Pesticide	-	-	-
Chlorbenside sulfone	7082-99-7	General Pesticide	-	-	-
Chlorbicyclen	2550-75-6	Insecticide	-	-	-
Chlorbromuron	13360-45-7	Herbicide	-	-	-
Chlorbufam	1967-16-4	Herbicide	-	-	-
Chlordene, trans-	3734-48-3	Insecticide, Industrial	-	-	-
Chlordimeform	6164-98-3	Insecticide,	3(1987)	-	cancer (1-Jan-89)
Chlorethoxyfos	54593-83-8	Insecticide	-	-	-
Chlorfenapyr	122453-73-0	Insecticide	-	-	-
Chlorfenethol	80-06-8	General Pesticide	-	-	-
Chlorfenprop-methyl	14437-17-3	Herbicide	-	-	-
Chlorfenson	80-33-1	Fungicide, Insecticide	-	-	-
Chlorfenvinphos	470-90-6	Insecticide,	-	-	-
Chlorfenvinphos, cis-	18708-87-7	Insecticide	-	-	-
Chlorfenvinphos, trans-	18708-86-6	Insecticide	-	-	-
Chlorflurecol-methyl ester	2536-31-4	Herbicide, PAH	-	-	-
Chlormefos	24934-91-6	Insecticide	-	-	-
Chlornitrofen	1836-77-7	Herbicide,	-	-	-
Chlorobenzilate	510-15-6	General Pesticide,	3(1987)	Oral RfD Assessment: Yes (Last revised: 12-01-1989).	cancer (1-Jan-90)
Chloroneb	2675-77-6	Fungicide	-	-	-
Chloropropylate	5836-10-2	General Pesticide,	-	-	-
Chlorothalonil	1897-45-6	General Pesticide, Fungicide, Insecticide,	2B(1999)	Oral RfD Assessment: Yes (Last revised: 03-01-1988).	cancer (1-Jan-89)
Chlorotoluron	15545-48-9	Herbicide	-	-	-
Chlorpropham	101-21-3	Herbicide,	3(1987)	-	-
Chlorpyrifos	2921-88-2	Insecticide, Pharmacological,	-	Oral RfD Assessment: No (Last revised: 03-01-1988).	-
Chlorpyrifos Methyl	5598-13-0	Insecticide,	-	-	-
Chlorthiamid	1918-13-4	Herbicide	-	-	-
Chlorthion	500-28-7	Insecticide	-	-	-
Chlorthiophos	60238-56-4	Insecticide	-	-	-
Chlorthiophos sulfone	999053-03-1	General Pesticide	-	-	-
Chlorthiophos sulfoxide	29185-21-5	Pesticide Product	-	-	-
Chlozolinate	84332-86-5	Fungicide	-	-	-
chrysene	218-01-9	PAH,	2B(2010)	Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	cancer (1-Jan-90)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Cinerin I	25402-06-6	Insecticide	-	-	-
Cinerin II	121-20-0	Insecticide	-	-	-
Cinidon-ethyl	142891-20-1	Herbicide	-	-	-
cinnamal	104-55-2	Fungicide, Personal Care, Industrial, Natural	-	-	-
cinnamyl alcohol	104-54-1	Personal Care, Natural	-	-	-
cis-Nonachlor	5103-73-1	Insecticide,	-	-	-
Citral A	5392-40-5	Personal Care, Natural	-	-	-
Citral B	5392-40-5	Personal Care, Natural	-	-	-
Clodinafop-propargyl	105512-06-9	Herbicide	-	-	-
Clomazone	81777-89-1	Herbicide	-	-	-
Cloquintocet-mexyl	99607-70-2	Herbicide	-	-	-
coronene	191-07-1	PAH	3(1987)	-	-
Coumaphos	56-72-4	Insecticide	-	-	-
Coumarin	91-64-5	Personal Care, Natural	3(2000)	-	-
Crimidine	535-89-7	Rodenticide	-	-	-
Crotoxyphos	7700-17-6	Insecticide	-	-	-
Crufomate	299-86-5	Insecticide	-	-	-
Cyanazine	21725-46-2	Herbicide,	-	Oral RfD Assessment: Withdrawn (Last revised: 07-01-1992).	developmental (1-Apr-90)
Cyanofenphos	13067-93-1	Insecticide,	-	-	-
Cyanophos	2636-26-2	Insecticide,	-	-	-
Cyclafuramid	34849-42-8	Fungicide	-	-	-
Cycloate	1134-23-2	Herbicide	-	-	developmental (19-Mar-99)
cyclopenta[cd]pyrene	27208-37-3	PAH,	2A(2010)	-	cancer (29-Apr-11)
Cyclopentadecanone	502-72-7	Personal Care	-	-	-
Cycluron	2163-69-1	Herbicide	-	-	-
Cyflufenamid	180409-60-3	Fungicide	-	-	-
Cyfluthrin I	68359-37-5	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 03-01-1988).	-
Cyfluthrin II	999028-03-4	Insecticide	-	-	-
Cyfluthrin III	999029-03-7	Insecticide	-	-	-
Cyfluthrin IV	999030-03-4	Insecticide	-	-	-
Cyhalofop-butyl	122008-85-9	Herbicide	-	-	-
Cyhalothrin (Gamma)	76703-62-3	Insecticide	-	-	-
Cyhalothrin I (lambda)	68085-85-8	Fungicide, Insecticide,	-	-	-
Cymiazole	61676-87-7	General Pesticide	-	-	-
Cymoxanil	57966-95-7	Fungicide	-	-	-
Cypermethrin-1	52315-07-8	Insecticide,	-	-	-
Cypermethrin-2	52315-07-8	Insecticide,	-	-	-
Cypermethrin-3	52315-07-8	Insecticide,	-	-	-
Cypermethrin-4	52315-07-8	Insecticide,	-	-	-
Cyphenothrin cis-	39515-40-7	Insecticide	-	-	-
Cyphenothrin trans-	999011-03-9	Insecticide	-	-	-
Cyprazine	22936-86-3	Herbicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Cyproconazole	113096-99-4	Insecticide	-	-	-
Cyprodinil	121552-61-2	Fungicide,	-	-	-
Cyprofuram	69581-33-5	Fungicide	-	-	-
Cyromazine	66215-27-8	Insecticide	-	-	-
d-(cis-trans)-Phenothrin-I	26002-80-2	Insecticide,	-	-	-
d-(cis-trans)-Phenothrin-II	999034-03-6	Insecticide	-	-	-
d-Limonene	5989-27-5	Personal Care, Natural	3(1999)	Inhalation RfC Assessment: Discussion (Last revised: 12-01-1993).	-
Dacthal	1861-32-1	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 08-01-1994).	-
Dazomet	533-74-4	Fungicide, Herbicide, Insecticide, Industrial	-	-	-
DDMU [1-Chloro-2,2-bis(4'-chlorophenyl)ethylene]	1022-22-6	General Pesticide	-	-	-
delta-BHC	319-86-8	Insecticide,	-	Carcinogenicity Assessment: Yes (Last revised: 03-31-1987)	-
Deltamethrin	52918-63-5	Insecticide,	3(1991)	-	-
Demephion	8065-62-1	General Pesticide	-	-	-
Demeton-s	126-75-0	Insecticide	-	-	-
Demeton-S-methyl	919-86-8	Insecticide	-	-	-
Demeton-S-methylsulfon	17040-19-6	Insecticide	-	-	-
Desbromo-bromobutide	999055-03-7	Herbicide	-	-	-
Desmedipham	13684-56-5	Herbicide	-	-	-
Desmetryn	1014-69-3	Herbicide	-	-	-
Di-n-butyl phthalate	84-74-2	Insecticide, Personal Care, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987). Inhalation RfC Assessment: Message (Last revised: 10-01-1990). Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	developmental, female, male (2-Dec-05)
Di-n-hexyl phthalate	84-75-3	Industrial,	-	-	female, male (2-Dec-05)
Di-n-nonyl phthalate	84-76-4	Industrial	-	-	-
Di-n-octyl phthalate	117-84-0	Industrial,	-	-	-
Di-n-propyl phthalate	131-16-8	Pharmacological, Industrial,	-	-	-
Dialifos	10311-84-9	Insecticide	-	-	-
Diallate I	2303-16-4	Herbicide	3(1987)	-	-
Diallate II	999035-03-9	Herbicide	-	-	-
Diamyl phthalate	131-18-0	Industrial,	-	-	-
Diazinon	333-41-5	Insecticide, Pharmacological,	2	-	-
Diazinon-oxon	962-58-3	General Pesticide	-	-	-
Dibenzo-p-dioxin	262-12-4	Dioxins/Furans	3(1997)	-	-
dibenzo[a,e]fluoranthene	5385-75-1	PAH,	3(2010)	-	-
dibenzo[a,e]pyrene	192-65-4	PAH,	3(2010)	-	cancer (1-Jan-88)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
dibenzo[a,h]anthracene	53-70-3	PAH,	2A(2010)	Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	cancer (1-Jan-88)
dibenzo[a,h]pyrene	189-64-0	PAH,	2B(2010)	-	cancer (1-Jan-88)
dibenzo[a,i]pyrene	189-55-9	PAH,	2B(2010)	-	cancer (1-Jan-88)
dibenzo[a,l]pyrene	191-30-0	PAH,	2A(2010)	-	cancer (1-Jan-88)
dibenzo[e,l]pyrene	192-51-8	PAH	3(2010)	-	-
Dibenzofuran	132-64-9	Dioxins/Furans	-	Carcinogenicity Assessment: Yes (Last revised: 10-01-1990)	-
dibenzothiophene	132-65-0	PAH, Pharmacological, Personal Care, Industrial,	3(2013)	-	-
Dicamba	1918-00-9	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988),	-
Dicamba methyl ester	6597-78-0	Pharmacological	-	-	-
Dicapthon	2463-84-5	Insecticide	-	-	-
Dichlofenthion	97-17-6	Insecticide,	-	-	-
Dichlofluanid	1085-98-9	Fungicide	-	-	-
Dichlofluanid metabolite (DMSA)	4710-17-2	Pharmacological	-	-	-
Dichlone	117-80-6	Fungicide, Herbicide, OPAH,	-	-	-
Dichloran	99-30-9	Fungicide, Industrial	-	-	-
Dichlormid	37764-25-3	Herbicide, Insecticide	-	-	-
Dichlorophen	97-23-4	Fungicide, Pharmacological,	-	-	developmental (27-Apr-99)
Dichlorprop	120-36-5	Herbicide,	-	-	-
Dichlorprop methyl ester	57153-17-0	General Pesticide	-	-	-
Dichlorvos	62-73-7	Insecticide,	2B(1991)	Oral RfD Assessment: Yes (Last revised: 11-01-1993), Inhalation RfC Assessment: Yes (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1989)	cancer (1-Jan-89)
Diclobutrazol	75736-33-3	Fungicide,	-	-	-
Diclocymet I	139920-32-4	Fungicide	-	-	-
Diclocymet II	999059-03-9	Fungicide	-	-	-
Diclofop methyl	51338-27-3	Herbicide,	-	-	developmental (5-Mar-99) cancer (6-Apr-10)
Dicrotophos	141-66-2	Insecticide	-	-	-
Dicyclohexyl phthalate	84-61-7	Industrial,	-	-	-
Dicyclopentadiene	77-73-6	PAH, Industrial	-	-	-
Dieldrin	60-57-1	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988), Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	cancer (1-Jul-88)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Diethatyl ethyl	38727-55-8	Herbicide	-	-	-
Diethofencarb	87130-20-9	Fungicide,	-	-	-
Diethyl dithiobis(thionoformate) (EXD)	502-55-6	Herbicide	-	-	-
Diethyl phthalate	84-66-2	Insecticide, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987), Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	-
Diethylene glycol	111-46-6	Industrial	-	-	-
Diethylstilbestrol	56-53-1	Pharmacological	1(2012)	-	cancer (27-Feb-87) developmental (1-Jul-87)
Difenoconazol I	119446-68-3	Fungicide,	-	-	-
Difenoconazol II	999036-03-2	Fungicide	-	-	-
Difenoxuron	14214-32-5	Herbicide	-	-	-
Diflufenican	83164-33-4	Herbicide	-	-	-
Diisobutyl phthalate	84-69-5	Industrial,	-	-	-
Dimefox	115-26-4	Insecticide	-	-	-
Dimepiperate	61432-55-1	Herbicide	-	-	-
Dimethachlor	50563-36-5	Herbicide	-	-	-
Dimethametryn	22936-75-0	Herbicide	-	-	-
Dimethenamid	87674-68-8	Herbicide	-	-	-
Dimethipin	55290-64-7	General Pesticide	-	-	-
Dimethoate	60-51-5	Insecticide, Pharmacological,	-	-	-
Dimethomorph-(E)	110488-70-5	Fungicide,	-	-	-
Dimethomorph-(Z)	999012-03-2	Fungicide	-	-	-
Dimethyl phthalate	131-11-3	Insecticide, Industrial	-	Inhalation RfC Assessment: Message (Last revised: 09-01-1990), Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	-
Dimethylvinphos(E)	71363-52-5	Insecticide	-	-	-
Dimethylvinphos(Z)	67628-93-7	Insecticide	-	-	-
Dimetilan	644-64-4	Insecticide	-	-	-
Dimoxystrobin	149961-52-4	Fungicide	-	-	-
Diniconazole	83657-24-3	Fungicide	-	-	-
Dinitramine	29091-05-2	Herbicide	-	-	-
Dinobuton	973-21-7	Fungicide	-	-	-
Dinocap	39300-45-3	Fungicide,	-	-	developmental (1-Apr-90)
Dinocap II	999037-03-5	Fungicide	-	-	-
Dinocap III	999038-03-8	Fungicide	-	-	-
Dinocap IV	999039-03-1	Fungicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Dinoseb	88-85-7	Fungicide, Herbicide, Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987). Carcinogenicity Assessment: Yes (Last revised: 08-01-1989)	developmental, male (1-Jan-89)
Dinoseb acetate	2813-95-8	Herbicide	-	-	-
Dinoseb methyl ether	6099-79-2	General Pesticide	-	-	-
Dinoterb	1420-07-1	Herbicide	-	-	-
Dinoterb acetate	3204-27-1	Herbicide, Insecticide	-	-	-
Diofenolan I	63837-33-2	Insecticide	-	-	-
Diofenolan II	999013-03-5	Insecticide	-	-	-
Dioxabenzofos	3811-49-2	Insecticide	-	-	-
Dioxacarb	6988-21-2	Insecticide	-	-	-
Dioxathion	78-34-2	Insecticide	-	-	-
Diphacinone	82-66-6	Rodenticide, PAH	-	-	-
Diphenamid	957-51-7	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Diphenyl phthalate	84-62-8	General Pesticide, Industrial,	-	-	-
Diphenylamine	122-39-4	Fungicide	-	-	-
Dipropetryn	4147-51-7	Herbicide	-	-	-
Dipropyl isocinchomeronate	136-45-8	Insecticide	-	-	cancer (1-May-96)
Disulfoton	298-04-4	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Disulfoton sulfone	2497-06-5	Insecticide	-	-	-
Ditalimfos	5131-24-8	Fungicide	-	-	-
Dithiopyr	97886-45-8	Herbicide	-	-	-
Diuron	330-54-1	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	cancer (31-May-02)
Diuron Metabolite [3,4-Dichlorophenyl isocyanate]	102-36-3	Industrial	-	-	-
Dodemorph I	1593-77-7	Fungicide,	-	-	-
Dodemorph II	999040-03-8	Fungicide	-	-	-
Drazoxolon	5707-69-7	Fungicide	-	-	-
drometrizole	2440-22-4	Industrial	-	-	-
Edifenphos	17109-49-8	Fungicide	-	-	-
Empenthrin I	54406-48-3	Insecticide	-	-	-
Empenthrin II	999014-03-8	Insecticide	-	-	-
Empenthrin III	999015-03-1	Insecticide	-	-	-
Empenthrin IV	999016-03-4	Insecticide	-	-	-
Empenthrin V	999017-03-7	Insecticide	-	-	-
Endosulfan ether	3369-52-6	General Pesticide	-	-	-
Endosulfan I	959-98-8	Insecticide,	-	-	-
Endosulfan II	33213-65-9	Insecticide,	-	-	-
Endosulfan lactone	3868-61-9	General Pesticide	-	-	-
Endosulfan sulfate	1031-07-8	Insecticide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Endrin	72-20-8	Fungicide, Insecticide, Rodenticide,	3(1987)	Oral RfD Assessment: Yes (Last revised: 09-07-1988), Carcinogenicity Assessment: Yes (Last revised: 10-01-1989)	developmental (15-May-98)
Endrin aldehyde	7421-93-4	Fungicide, Insecticide, Rodenticide	-	-	-
Endrin ketone	53494-70-5	General Pesticide	-	-	-
EPN	2104-64-5	Insecticide, Pharmacological,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987),	-
Epoxiconazole	106325-08-0	General Pesticide,	-	-	cancer (15-Apr-11)
EPTC	759-94-4	Herbicide,	-	-	developmental (27-Apr-99)
Erbon	136-25-4	Herbicide	-	-	-
Esfenvalerate	66230-04-4	Insecticide,	-	-	-
Esprocarb	85785-20-2	Herbicide	-	-	-
Etaconazole	60207-93-4	Fungicide	-	-	-
Ethalfluralin	55283-68-6	Herbicide	-	-	-
Ethidimuron	30043-49-3	Herbicide	-	-	-
Ethiofencarb	29973-13-5	Insecticide	-	-	-
Ethiolate	2941-55-1	Herbicide	-	-	-
Ethion	563-12-2	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-01-1989),	-
Ethofenprox	80844-07-1	Insecticide,	-	-	-
Ethofumesate	26225-79-6	Herbicide	-	-	-
Ethofumesate, 2-Keto	26244-33-7	General Pesticide	-	-	-
Ethoprophos	13194-48-4	Insecticide	-	-	cancer (27-Feb-01)
Ethoxyfen-ethyl	131086-42-5	Herbicide	-	-	-
Ethoxyquin	91-53-2	Fungicide,	-	-	-
Ethylene brassylate	105-95-3	Personal Care	-	-	-
Ethylenethiourea	96-45-7	Fungicide, Industrial,	3(2001)	Oral RfD Assessment: Yes (Last revised: 05-01-1991),	cancer (1-Jan-88) developmental (1-Jan-93)
Etoxazole	153233-91-1	General Pesticide	-	-	-
Etridiazole, deschloro- (5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole)	999001-03-5	Pesticide Product	-	-	-
Etrimfos	38260-54-7	Insecticide	-	-	-
Eugenol	97-53-0	Insecticide, Pharmacological, Personal Care, Industrial	3(1987)	-	-
Exaltolide [15-Pentadecanolide]	106-02-5	Personal Care	-	-	-
Famoxadon	131807-57-3	Fungicide	-	-	-
Famphur	52-85-7	Fungicide, Insecticide	-	-	-
Farnesol I	4602-84-0	Personal Care, Natural	-	-	-
Farnesol II	4602-84-0	Personal Care, Natural	-	-	-
Farnesol III	4602-84-0	Personal Care, Natural	-	-	-
Farnesol IV	4602-84-0	Personal Care, Natural	-	-	-
Fenamidone	161326-34-7	Fungicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Fenamiphos	22224-92-6	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Fenamiphos sulfoxide	31972-43-7	General Pesticide	-	-	-
Fenamiphos-sulfone	31972-44-8	General Pesticide	-	-	-
Fenarimol	60168-88-9	Fungicide, Industrial,	-	-	-
Fenazaflor	14255-88-0	Insecticide	-	-	-
Fenazaflor metabolite	4228-88-0	Insecticide	-	-	-
Fenazaquin	120928-09-8	General Pesticide	-	-	-
Fenbuconazole	119611-00-6	Fungicide	-	-	-
Fenchlorazole-ethyl	103112-35-2	Herbicide	-	-	-
Fenchlorphos	299-84-3	Insecticide,	-	-	-
Fenchlorphos-oxon	3983-45-7	Pesticide Product	-	-	-
Fenclorim	3740-92-9	Herbicide	-	-	-
Fenfuram	24691-80-3	Fungicide	-	-	-
Fenhexamid	126833-17-8	Fungicide,	-	-	-
Fenitrothion	122-14-5	Insecticide,	-	-	-
Fenitrothion-oxon	2255-17-6	Insecticide	-	-	-
Fenobucarb	3766-81-2	Insecticide	-	-	-
Fenoprop	93-72-1	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988). Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	-
Fenoprop methyl ester	4841-20-7	Herbicide	-	-	-
Fenothiocarb	62850-32-2	General Pesticide	-	-	-
Fenoxanil	115852-48-7	Fungicide	-	-	-
Fenoxaprop-ethyl	66441-23-4	Herbicide	-	-	developmental (26-Mar-99)
Fenoxycarb	79127-80-3	Insecticide,	-	-	cancer (2-Jun-00)
Fenpiclonil	74738-17-3	Fungicide	-	-	-
Fenpropathrin	64257-84-7	Insecticide	-	-	-
Fenpropidin	67306-00-7	Fungicide	-	-	-
Fenson	80-38-6	General Pesticide	-	-	-
Fensulfothion	115-90-2	Insecticide	-	-	-
Fensulfothion-oxon	6552-21-2	Insecticide	-	-	-
Fensulfothion-oxon -sulfone	6132-17-8	Insecticide	-	-	-
fensulfothion-sulfone	14255-72-2	Insecticide	-	-	-
Fenthion	55-38-9	Insecticide,	-	-	-
Fenthion sulfoxide	3761-41-9	Insecticide	-	-	-
Fenthion-sulfone	3761-42-0	Insecticide	-	-	-
Fenuron	101-42-8	Herbicide	-	-	-
Fenvalerate	51630-58-1	Insecticide,	3(1991)	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Fenvalerate II	999041-03-1	Insecticide	-	-	-
Fepropimorph	67564-91-4	Fungicide	-	-	-
Fipronil	120068-37-3	Insecticide, Pharmacological,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Fipronil, Desulfinyl-	205650-65-3	General Pesticide	-	-	-
Fipronil-sulfide	120067-83-6	Insecticide	-	-	-
Fipronil-sulfone	120068-36-2	Insecticide	-	-	-
Flamprop-isopropyl	52756-22-6	Herbicide	-	-	-
Flamprop-methyl	52756-25-9	Herbicide	-	-	-
Fluacrypyrim	229977-93-9	General Pesticide	-	-	-
Fluazifop-p-butyl	79241-46-6	Herbicide	-	-	-
Fluazinam	79622-59-6	Fungicide	-	-	-
Fluazolate	174514-07-9	Herbicide	-	-	-
Flubenzimine	37893-02-0	Fungicide	-	-	-
Fluchloralin	33245-39-5	Herbicide	-	-	-
Flucythrinate I	70124-77-5	Insecticide,	-	-	-
Flucythrinate II	999042-03-4	Insecticide	-	-	-
Fludioxonil	131341-86-1	Fungicide,	-	-	-
Flufenacet	142459-58-3	Herbicide,	-	-	-
Flumetralin	62924-70-3	Herbicide	-	-	-
Flumiclorac-pentyl	87546-18-7	Herbicide	-	-	-
Flumioxazin	103361-09-7	Herbicide	-	-	-
Fluometuron	2164-17-2	Herbicide	3(1987)	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
fluoranthene	206-44-0	PAH, Pharmacological,	3(2010)	Oral RfD Assessment: Yes (Last revised: 09-01-1990). Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	-
fluorene	86-73-7	PAH,	3(2010)	Oral RfD Assessment: Yes (Last revised: 11-01-1990). Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	-
Fluorodifen	15457-05-3	Herbicide	-	-	-
Fluoroglycofen-ethyl	77501-90-7	Herbicide	-	-	-
Fluoroimide	41205-21-4	Fungicide	-	-	-
Fluotrimazole	31251-03-3	Fungicide	-	-	-
Fluoxastrobin cis-	361377-29-9	Fungicide, Herbicide	-	-	-
Fluquinconazole	136426-54-5	Fungicide	-	-	-
Flurenol-butyl ester	2314-09-2	Herbicide	-	-	-
Flurenol-methylester	1216-44-0	General Pesticide	-	-	-
Fluridone	59756-60-4	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-
Flurochloridone I	61213-25-0	Herbicide	-	-	-
Flurochloridone II	999043-03-7	Herbicide	-	-	-
Flurochloridone, deschloro-	999003-03-1	Pesticide Product	-	-	-
Fluroxypyr-1-methylheptyl ester	81406-37-3	Herbicide	-	-	-
Flurprimidol	56425-91-3	General Pesticide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Flurtamone	96525-23-4	Herbicide	-	-	-
Flusilazole	85509-19-9	Fungicide,	-	-	-
Fluthiacet-methyl	117337-19-6	Herbicide	-	-	-
Flutolanil	66332-96-5	Fungicide,	-	-	-
Flutriafol	76674-21-0	Fungicide,	-	-	-
Fluvalinate-tau-I	102851-06-9	Insecticide	-	-	-
Fluvalinate-tau-II	999044-03-0	Insecticide	-	-	-
Folpet	133-07-3	Fungicide	-	-	cancer (1-Jan-89)
Fonofos	944-22-9	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Formothion	2540-82-1	Insecticide	-	-	-
Fosthiazate I	98886-44-3	Insecticide	-	-	-
Fosthiazate II	999018-03-0	Insecticide	-	-	-
Fuberidazole	3878-19-1	Fungicide	-	-	-
Furalaxyl	57646-30-7	Fungicide	-	-	-
Furathiocarb	65907-30-4	Insecticide	-	-	-
Furilazole	121776-33-8	Herbicide	-	-	cancer (22-Mar-11)
Furmecyclox	60568-05-0	Fungicide	-	Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	cancer (1-Jan-90)
Galaxolide	1222-05-5	Personal Care, Industrial,	-	-	-
gamma-Chlordane	5103-74-2	Insecticide,	-	-	-
Geraniol	106-24-1	Personal Care, Natural	-	-	-
guaiacol	90-05-1	Industrial, Natural,	-	-	-
Halfenprox	111872-58-3	Insecticide	-	-	-
Haloxyfop-methyl	69806-40-2	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 05-01-1990).	-
Heptachlor	76-44-8	Fungicide, Insecticide,	2B(2001)	Oral RfD Assessment: Yes (Last revised: 09-30-1987). Carcinogenicity Assessment: Yes (Last revised: 09-30-1987)	cancer (1-Jul-88) developmental (20-Aug-99)
Heptachlor epoxide	1024-57-3	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987). Carcinogenicity Assessment: Yes (Last revised: 09-30-1987)	cancer (1-Jul-88)
Heptachlor epoxide isomer A	28044-83-9	General Pesticide	-	-	-
Heptenophos	23560-59-0	Insecticide	-	-	-
Hexabromobenzene	87-82-1	Flame Retardant	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Hexachlorobenzene	118-74-1	Fungicide, Industrial,	2B(2001)	Oral RfD Assessment: Yes (Last revised: 09-26-1988). Inhalation RfC Assessment: Message (Last revised: 03-01-1991). Carcinogenicity Assessment: Yes (Last revised: 03-01-1991)	cancer (1-Oct-87) developmental (1-Jan-89)
Hexachlorophene	70-30-4	Fungicide, Pharmacological,	3(1987)	Oral RfD Assessment: Yes (Last revised: 08-22-1988).	-
Hexaconazole	79983-71-4	Fungicide,	-	-	-
Hexazinone	51235-04-2	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Hexestrol	84-16-2	Pharmacological	-	-	-
Hydroprene	41096-46-2	Insecticide,	-	-	-
hydroxy-citronellal	107-75-5	Personal Care	-	-	-
Imazalil	35554-44-0	Fungicide, Industrial,	-	-	cancer (20-May-11)
Imazamethabenz-methyl I	81405-85-8	Herbicide	-	-	-
Imazamethabenz-methyl II	999019-03-3	Herbicide	-	-	-
Imibenconazole	86598-92-7	Fungicide	-	-	-
Imibenconazole-desbenzyl	199338-48-2	Pesticide Product	-	-	-
Imidan	732-11-6	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
indeno[1,2,3-cd]pyrene	193-39-5	PAH,	2B(2010)	Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	cancer (1-Jan-88)
indole	120-72-9	Personal Care, Industrial,	-	-	-
Indoxacarb and Dioxacarb decomposition product [Phenol, 2-(1,3-dioxolan-2-yl)-]	999058-03-6	Insecticide,	-	-	-
Ioxynil	1689-83-4	Herbicide,	-	-	-
Ioxynil octanoate	3861-47-0	Herbicide	-	-	-
Ipconazole	125225-28-7	Fungicide	-	-	-
Iprobenfos	26087-47-8	Fungicide	-	-	-
Iprodione	36734-19-7	Fungicide,	-	Oral RfD Assessment: Yes (Last revised: 06-30-1988).	cancer (1-May-96)
Iprovalicarb I	140923-25-7	Fungicide	-	-	cancer (1-Jun-07)
Iprovalicarb II	999020-03-0	Fungicide	-	-	-
Irgarol	28159-98-0	General Pesticide	-	-	-
Isazophos	42509-80-8	Fungicide, Insecticide	-	-	-
Isobenzan	297-78-9	Insecticide	-	-	-
Isobornyl thiocyanoacetate	115-31-1	Insecticide	-	-	-
Isocarbamide	30979-48-7	Herbicide	-	-	-
Isocarbophos	24353-61-5	Insecticide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Isodrin	465-73-6	Insecticide	-	-	-
isoeugenol	97-54-1	Personal Care, Natural	-	-	-
Isofenphos	25311-71-1	Insecticide,	-	-	-
Isofenphos-oxon	31120-85-1	General Pesticide	-	-	-
Isomethiozin	57052-04-7	Herbicide	-	-	-
Isoprocarb	2631-40-5	Insecticide	-	-	-
Isopropalin	33820-53-0	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Isoprothiolane	50512-35-1	Fungicide, Insecticide,	-	-	-
Isoproturon	34123-59-6	Herbicide,	-	-	-
Isoxaben	82558-50-7	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988). Carcinogenicity Assessment: Yes (Last revised: 09-01-1991)	-
Isoxadifen-ethyl	163520-33-0	Herbicide	-	-	-
Isoxaflutole	141112-29-0	Herbicide	-	-	cancer (22-Dec-00)
Isoxathion	18854-01-8	Insecticide,	-	-	-
Jasmolin I	4466-14-2	Insecticide	-	-	-
Jasmolin II	1172-63-0	Insecticide	-	-	-
Jodfenphos	18181-70-9	Insecticide	-	-	-
Kepone	143-50-0	Fungicide, Insecticide,	2B(1987)	Oral RfD Assessment: Yes (Last revised: 09-22-2009). Inhalation RfC Assessment: Discussion (Last revised: 09-22-2009). Carcinogenicity Assessment: Yes (Last revised: 09-22-2009)	cancer (1-Jan-88) developmental (1-Jan-89)
Kinoprene	42588-37-4	Insecticide,	-	-	-
Kresoxim-methyl	143390-89-0	Fungicide	-	-	cancer (3-Feb-12)
Lactofen	77501-63-4	Herbicide	-	-	cancer (1-Jan-89)
Lenacil	2164-08-1	Herbicide	-	-	-
Leptophos	21609-90-5	Insecticide,	-	-	-
Leptophos oxon	25006-32-0	Insecticide	-	-	-
lilial	80-54-6	Personal Care,	-	-	-
Linalool	78-70-6	Insecticide, Personal Care	-	-	-
Lindane	58-89-9	Fungicide, Insecticide,		Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Linuron	330-55-2	Herbicide,	-	-	developmental (19-Mar-99)
lyral	31906-04-4	Personal Care	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
m-Cresol	108-39-4	Industrial	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988). Inhalation RfC Assessment: Message (Last revised: 04-01-1992). Carcinogenicity Assessment: Yes (Last revised: 09-01-1990)	-
m-toluidine	108-44-1	Industrial,	-	-	-
Malathion	121-75-5	Insecticide, Pharmacological,	2	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	cancer (20-May-16)
Malathion-o-analog	1634-78-2	Insecticide,	-	-	-
MCPA methyl ester	2436-73-9	General Pesticide	-	-	-
MCPA-butoxyethyl ester	19480-43-4	Herbicide	-	-	-
MCPB methyl ester	57153-18-1	Herbicide	-	-	-
Mecarbam	2595-54-2	Insecticide	-	-	-
Mecoprop methyl ester	23844-56-6	General Pesticide	-	-	-
Mefenacet	73250-68-7	Herbicide,	-	-	-
Mefenpyr-diethyl	135590-91-9	Herbicide	-	-	-
Mefluidide	53780-34-0	Herbicide	-	-	-
Menazon	78-57-9	Insecticide	-	-	-
Mepanipyrim	110235-47-7	Fungicide	-	-	cancer (1-Jul-08)
Mephosfolan	950-10-7	Insecticide	-	-	-
Mepronil	55814-41-0	Fungicide	-	-	-
Merphos	150-50-5	General Pesticide	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988). Inhalation RfC Assessment: Message (Last revised: 11-01-1992).	-
Metalaxyl	57837-19-1	Fungicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Metamitron	41394-05-2	Herbicide	-	-	-
Metazachlor	67129-08-2	Herbicide	-	-	-
Metconazole I	125116-23-6	Fungicide	-	-	-
Metconazole II	999021-03-3	Fungicide	-	-	-
Methabenzthiazuron [decomposition product]	16954-69-1	Herbicide	-	-	-
Methacrifos	62610-77-9	Insecticide	-	-	-
Methamidophos	10265-92-6	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Methfuroxam	28730-17-8	Fungicide	-	-	-
Methidathion	950-37-8	Insecticide	-	-	-
Methiocarb	2032-65-7	Insecticide,	-	-	-
Methiocarb Sulfone	2179-25-1	General Pesticide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Methiocarb sulfoxide	2635-10-1	General Pesticide	-	-	-
Methomyl	16752-77-5	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Methoprene I	40596-69-8	Insecticide,	-	-	-
Methoprene II	999045-03-3	Insecticide	-	-	-
Methoprotryne	841-06-5	Herbicide	-	-	-
Methoxychlor	72-43-5	Insecticide, Pharmacological,	3(1987)	Oral RfD Assessment: Yes (Last revised: 09-01-1990). Inhalation RfC Assessment: Discussion (Last revised: 12-01-1993). Carcinogenicity Assessment: Yes (Last revised: 09-07-1988)	-
Methoxychlor olefin	2132-70-9	General Pesticide	-	-	-
Methyl (2-naphthoxy)acetate	1929-87-9	Herbicide	-	-	-
METHYL 2-OCTYNOATE	111-12-6	Personal Care	-	-	-
Methyl paraoxon	950-35-6	Insecticide,	-	-	-
Methyl-1-naphthalene acetate	2876-78-0	General Pesticide	-	-	-
Methyldymron	42609-73-4	Herbicide	-	-	-
Methyleugenol	93-15-2	Personal Care, Natural	2B(2013)	-	cancer (16-Nov-01)
Metobromuron	3060-89-7	Herbicide	-	-	-
Metolachlor	51218-45-2	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 10-01-1990). Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	-
Metolcarb	1129-41-5	Insecticide	-	-	-
Metominostrobin (E)	133408-50-1	Fungicide	-	-	-
Metominostrobin (Z)	999022-03-6	Fungicide	-	-	-
Metrafenone	220899-03-6	Fungicide	-	-	-
Metribuzin	21087-64-9	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987). Carcinogenicity Assessment: Yes (Last revised: 12-01-1993)	-
Mevinphos	7786-34-7	Insecticide,	-	-	-
Mirex	2385-85-5	Insecticide,	2B(1987)	Oral RfD Assessment: Yes (Last revised: 10-01-1992).	cancer (1-Jan-88)
Molinate	2212-67-1	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988).	developmental, female, male (11-Dec-09)
Monalide	7287-36-7	Herbicide	-	-	-
Monocrotophos	6923-22-4	Insecticide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Monolinuron	1746-81-2	Herbicide	-	-	-
Musk amberette	83-66-9	Personal Care	3(1996)	-	-
Musk Ketone	81-14-1	Personal Care,	-	-	-
Musk Moskene	116-66-5	Personal Care	-	-	-
Musk Tibetene (Moschustibeten)	145-39-1	Personal Care	-	-	-
Musk xylene	81-15-2	Personal Care,	3(1996)	-	-
Myclobutanil	88671-89-0	Fungicide,	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988).	developmental, male (16-Apr-99)
n,n-diethyl-3-aminophenol	91-68-9	Industrial,	-	-	-
N,N-Diethyl-m-toluamide	134-62-3	Insecticide	-	-	-
N-1-Naphthylacetamide	575-36-0	PAH	-	-	-
N-Cyclohexyl-2-benzothiazolylsulfenamide	95-33-0	Industrial	-	-	-
N-Methyl-N-1-naphthyl acetamide	5903-13-9	Insecticide, PAH	-	-	-
n-phenyl-1-naphthylamine	90-30-2	Industrial	-	-	-
n-phenyl-2-naphthylamine	135-88-6	Industrial	3(1987)	-	-
Naled	300-76-5	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
naphthalene	91-20-3	PAH,	2B(2002)	Oral RfD Assessment: Yes (Last revised: 09-17-1998). Inhalation RfC Assessment: Yes (Last revised: 09-17-1998). Carcinogenicity Assessment: Yes (Last revised: 09-17-1998)	cancer (19-Apr-02)
Naphthalic anhydride	81-84-5	Herbicide, PAH, Industrial	-	-	-
Naphthanthrone	3074-00-8	OPAH	-	-	-
naphtho[1,2-b]fluoranthene	111189-32-3	PAH	3(2010)	-	-
naphtho[2,3-a]pyrene	196-42-9	PAH,	-	-	-
naphtho[2,3-e]pyrene	193-09-9	PAH	3(2010)	-	-
naphtho[2,3-j]fluoranthene	205-83-4	PAH	-	-	-
naphtho[2,3-k]fluoranthene	207-18-1	PAH	-	-	-
Naproanilide	52570-16-8	Herbicide, PAH	-	-	-
Napropamide	15299-99-7	Herbicide, PAH	-	-	-
Nickel dibutyldithiocarbamate	13927-77-0	Personal Care, Industrial	-	-	-
Nicotine	54-11-5	Insecticide, Pharmacological,	-	-	developmental (1-Apr-90)
Nitralin	4726-14-1	Herbicide	-	-	-
Nitrapyrin	1929-82-4	Fungicide, Insecticide	-	Oral RfD Assessment: Withdrawn (Last revised: 07-01-1992).	developmental (30-Mar-99) cancer (5-Oct-05)
Nitrofen	1836-75-5	Herbicide,	2B(1987)	-	cancer (1-Jan-88)
Nitrothal-isopropyl	10552-74-6	Fungicide	-	-	-
Norflurazon	27314-13-2	Herbicide	-	-	-
Norflurazon, Desmethyl-	23576-24-1	Herbicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Nuarimol	63284-71-9	Fungicide,	-	-	-
o-Dichlorobenzene	95-50-1	Herbicide, Insecticide, Industrial,	3(1999)	Oral RfD Assessment: Yes (Last revised: 08-01-1989). Carcinogenicity Assessment: Yes (Last revised: 11-01-1990)	-
o-Phenylphenol	90-43-7	Fungicide, Insecticide, Industrial,	3(1999)	-	cancer (4-Aug-00)
o-Toluidine	95-53-4	Industrial,	1(2012)	-	cancer (1-Jan-88)
Octachlorostyrene	29082-74-4	Industrial,	-	-	-
Octamethyl pyrophosphoramide	152-16-9	Insecticide	-	-	-
Ofurace	58810-48-3	Fungicide	-	-	-
Omethoate	1113-02-6	Insecticide	-	-	-
Orbencarb	34622-58-7	Herbicide	-	-	-
ortho-Aminoazotoluene	97-56-3	Industrial	2B(1987)	-	cancer (1-Jul-87)
Oryzalin	19044-88-3	Herbicide,	-	-	cancer (12-Sep-08)
Oxabetrinil	74782-23-3	Herbicide	-	-	-
Oxadiazon	19666-30-9	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	cancer (1-Jul-91) developmental (15-May-98)
Oxadixyl	77732-09-3	Fungicide	-	-	-
Oxamyl	23135-22-0	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Oxycarboxin	5259-88-1	Fungicide	-	-	-
Oxychlordane	27304-13-8	Insecticide	-	-	-
Oxydemeton-methyl	301-12-2	Insecticide	-	-	female, male (6-Nov-98)
Oxyfluorfen	42874-03-3	Herbicide,	-	-	-
p,p'-DDM [bis(4-chlorophenyl) methane]	101-76-8	Industrial	-	-	-
p,p'-Dibromobenzophenone	3988-03-2	General Pesticide	-	-	-
p,p'-Dicofol	115-32-2	Insecticide,	3(1987)	Carcinogenicity Assessment: Withdrawn (Last revised: 04-01-1992)	-
p-Dichlorobenzene	106-46-7	Fungicide, Insecticide, Industrial	2B(1999)	Inhalation RfC Assessment: Yes (Last revised: 01-01-1994).	cancer (1-Jan-89)
p-Nitrotoluene	99-99-0	Industrial	3(1996)	-	-
p-toluidine	106-49-0	Industrial,	-	-	-
Paclobutrazol	76738-62-0	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Paclobutrazol	76738-62-0	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Paraoxon	311-45-5	Insecticide, Pharmacological,	-	Carcinogenicity Assessment: Yes (Last revised: 10-01-1992)	-
Parathion-ethyl	56-38-2	Insecticide,	2	Oral RfD Assessment: No (Last revised: 08-22-1988). Carcinogenicity Assessment: Yes (Last revised: 10-01-1993)	cancer (20-May-16)
Parathion-methyl	298-00-0	Fungicide, Insecticide,	3(1987)	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
PBB 1	2052-07-5	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 10	59080-32-9	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 101	67888-96-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 15	92-86-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 169 Hexabrombiphenyl	60044-26-0	Flame Retardant, PBB,	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 18	59080-34-1	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 2	2113-57-7	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 26	59080-35-2	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 29	115245-07-3	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 3	92-66-0	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 30	59080-33-0	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 31	59080-36-3	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 4	13029-09-9	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 49	60044-24-8	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 52	59080-37-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 53	60044-25-9	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PBB 7	53592-10-2	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 77	77102-82-0	Flame Retardant, PBB,	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 80	16400-50-3	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB 9	57422-77-2	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-101	67888-96-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-103	59080-39-6	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-114	96551-70-1	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-137	81381-52-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-141	120991-47-1	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-153	59080-40-9	Flame Retardant, PBB,	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-155	59261-08-4	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-156	77607-09-1	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-159	120991-48-2	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-169	60044-26-0	Flame Retardant, PBB,	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-180	67733-52-2	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-189	88700-06-5	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBB-200	119264-60-7	Flame Retardant, PBB	2A(2016)	-	cancer (1-Jan-88) developmental (1-Oct-94)
PBDE 103	446254-67-7	Flame Retardant, PBDE	-	-	-
PBDE 108	446254-71-3	Flame Retardant, PBDE	-	-	-
PBDE 115	446254-78-0	Flame Retardant, PBDE	-	-	-
PBDE 127	N/A	Flame Retardant, PBDE	-	-	-
PBDE 128	N/A	Flame Retardant, PBDE	-	-	-
PBDE 142	N/A	Flame Retardant, PBDE	-	-	-
PBDE 144	N/A	Flame Retardant, PBDE	-	-	-
PBDE 160	N/A	Flame Retardant, PBDE	-	-	-
PBDE 185	N/A	Flame Retardant, PBDE	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PBDE 19	147217-73-0	Flame Retardant, PBDE,	-	-	-
PBDE 201	N/A	Flame Retardant, PBDE	-	-	-
PBDE 21	337513-67-4	Flame Retardant, PBDE	-	-	-
PBDE 26	337513-75-4	Flame Retardant, PBDE	-	-	-
PBDE 27	337513-53-8	Flame Retardant, PBDE	-	-	-
PBDE 31	65075-08-3	Flame Retardant, PBDE	-	-	-
PBDE 4	51452-87-0	Flame Retardant, PBDE	-	-	-
PBDE 50	446254-23-5	Flame Retardant, PBDE	-	-	-
PBDE 51	189084-57-9	Flame Retardant, PBDE,	-	-	-
PBDE 6	147217-72-9	Flame Retardant, PBDE	-	-	-
PBDE 62	446254-33-7	Flame Retardant, PBDE	-	-	-
PBDE 69	327185-09-1	Flame Retardant, PBDE	-	-	-
PBDE 88	446254-55-3	Flame Retardant, PBDE	-	-	-
PBDE 89	N/A	Flame Retardant, PBDE	-	-	-
PBDE 9	337513-66-3	Flame Retardant, PBDE	-	-	-
PBDE 1	7025-06-1	Flame Retardant, PBDE	-	-	-
PBDE 10	51930-04-2	Flame Retardant, PBDE	-	-	-
PBDE 100	189084-64-8	Flame Retardant, PBDE,	-	-	-
PBDE 11	6903-63-5	Flame Retardant, PBDE	-	-	-
PBDE 116	189084-65-9	Flame Retardant, PBDE	-	-	-
PBDE 118	446254-80-4	Flame Retardant, PBDE	-	-	-
PBDE 119	189084-66-0	Flame Retardant, PBDE,	-	-	-
PBDE 12	189084-59-1	Flame Retardant, PBDE	-	-	-
PBDE 13	83694-71-7	Flame Retardant, PBDE	-	-	-
PBDE 138	182677-30-1	Flame Retardant, PBDE	-	-	-
PBDE 15	2050-47-7	Flame Retardant, PBDE	-	Carcinogenicity Assessment: Yes (Last revised: 08-01-1990)	-
PBDE 153	68631-49-2	Flame Retardant, PBDE,	-	Oral RfD Assessment: Yes (Last revised: 06-30-2008). Inhalation RfC Assessment: Discussion (Last revised: 06-30-2008). Carcinogenicity Assessment: Yes (Last revised: 06-30-2008)	-
PBDE 154	207122-15-4	Flame Retardant, PBDE	-	-	-
PBDE 155	35854-94-5	Flame Retardant, PBDE,	-	-	-
PBDE 166	189084-58-0	Flame Retardant, PBDE,	-	-	-
PBDE 17	147217-75-2	Flame Retardant, PBDE	-	-	-
PBDE 2	6876-00-2	Flame Retardant, PBDE	-	-	-
PBDE 25	147217-77-4	Flame Retardant, PBDE	-	-	-
PBDE 28	41318-75-6	Flame Retardant, PBDE,	-	-	-
PBDE 3	101-55-3	Flame Retardant, PBDE	-	Carcinogenicity Assessment: Yes (Last revised: 08-01-1990)	-
PBDE 30	155999-95-4	Flame Retardant, PBDE,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PBDE 32	189084-60-4	Flame Retardant, PBDE,	-	-	-
PBDE 33	147217-78-5	Flame Retardant, PBDE	-	-	-
PBDE 35	147217-80-9	Flame Retardant, PBDE	-	-	-
PBDE 37	147217-81-0	Flame Retardant, PBDE	-	-	-
PBDE 47	5436-43-1	Flame Retardant, PBDE,	-	Oral RfD Assessment: Yes (Last revised: 06-30-2008), Inhalation RfC Assessment: Discussion (Last revised: 06-30-2008), Carcinogenicity Assessment: Yes (Last revised: 06-30-2008)	-
PBDE 49	243982-82-3	Flame Retardant, PBDE,	-	-	-
PBDE 66	189084-61-5	Flame Retardant, PBDE	-	-	-
PBDE 7	171977-44-9	Flame Retardant, PBDE	-	-	-
PBDE 71	189084-62-6	Flame Retardant, PBDE,	-	-	-
PBDE 75	189084-63-7	Flame Retardant, PBDE,	-	-	-
PBDE 77	93703-48-1	Flame Retardant, PBDE	-	-	-
PBDE 8	147217-71-8	Flame Retardant, PBDE	-	-	-
PBDE 85	182346-21-0	Flame Retardant, PBDE,	-	-	-
PBDE 99	60348-60-9	Flame Retardant, PBDE,	-	Oral RfD Assessment: Yes (Last revised: 06-30-2008), Inhalation RfC Assessment: Discussion (Last revised: 06-30-2008), Carcinogenicity Assessment: Yes (Last revised: 06-30-2008)	-
PCB 1	2051-60-7	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 10	33146-45-1	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 101	37680-73-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 102	68194-06-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 103	60145-21-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 104	56558-16-8	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 105	32598-14-4	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 106	70424-69-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 107	70424-68-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 108	70362-41-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 109	74472-35-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 11	2050-67-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 110	38380-03-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 111	39635-32-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 112	74472-36-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 113	68194-10-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 114	74472-37-0	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 115	74472-38-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 116	18259-05-7	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 117	68194-11-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 118	31508-00-6	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 119	56558-17-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 12	2974-92-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 120	68194-12-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 121	56558-18-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 122	76842-07-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 123	65510-44-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 124	70424-70-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 125	74472-39-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 126	57465-28-8	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 127	39635-33-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 128	38380-07-3	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 129	55215-18-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 13	2974-90-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 130	52663-66-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 131	61798-70-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 132	38380-05-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 133	35694-04-3	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 134	52704-70-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 135	52744-13-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 136	38411-22-2	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 137	35694-06-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 138	35065-28-2	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 139	56030-56-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 14	34883-41-5	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 140	59291-64-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 141	52712-04-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 142	41411-61-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 143	68194-15-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 144	68194-14-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 145	74472-40-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 146	51908-16-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 147	68194-13-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 148	74472-41-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 149	38380-04-0	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 15	2050-68-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 150	68194-08-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 151	52663-63-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 152	68194-09-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 153	35065-27-1	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 154	60145-22-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 155	33979-03-2	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 156	38380-08-4	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 157	69782-90-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 158	74472-42-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 159	39635-35-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 16	38444-78-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 160	41411-62-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 161	74472-43-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 162	39635-34-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 163	74472-44-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 164	74472-45-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 165	74472-46-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 166	41411-63-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 167	52663-72-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 168	59291-65-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 169	32774-16-6	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 17	37680-66-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 170	35065-30-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 171	52663-71-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 172	52663-74-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 173	68194-16-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 174	38411-25-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 175	40186-70-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 176	52663-65-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 177	52663-70-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 178	52663-67-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 179	52663-64-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 18	37680-65-2	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 180	35065-29-3	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 181	74472-47-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 182	60145-23-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 183	52663-69-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 184	74472-48-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 185	52712-05-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 186	74472-49-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 187	52663-68-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 188	74487-85-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 189	39635-31-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 19	38444-73-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 190	41411-64-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 191	74472-50-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 192	74472-51-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 193	69782-91-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 194	35694-08-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 195	52663-78-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 196	42740-50-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 197	33091-17-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 198	68194-17-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 199	52663-75-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 2	2051-61-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 20	38444-84-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 200	52663-73-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 201	40186-71-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 202	2136-99-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 203	52663-76-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 204	74472-52-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 205	74472-53-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 206	40186-72-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 207	52663-79-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 208	52663-77-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 21	55702-46-0	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 22	38444-85-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 23	55720-44-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 24	55702-45-9	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 25	55712-37-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 26	38444-81-4	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 27	38444-76-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 28	7012-37-5	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 29	15862-07-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 3	2051-62-9	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 30	35693-92-6	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 31	16606-02-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 32	38444-77-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 33	38444-86-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 34	37680-68-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 35	37680-69-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 36	38444-87-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 37	38444-90-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 38	53555-66-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 39	38444-88-1	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 4	13029-08-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 40	38444-93-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 41	52663-59-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 42	36559-22-5	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 43	70362-46-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 44	41464-39-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 45	70362-45-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 46	41464-47-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 47	2437-79-8	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 48	70362-47-9	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 49	41464-40-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 5	16605-91-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 50	62796-65-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 51	68194-04-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 52	35693-99-3	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 53	41464-41-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 54	15968-05-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 55	74338-24-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 56	41464-43-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 57	70424-67-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 58	41464-49-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 59	74472-33-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 6	25569-80-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 60	33025-41-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 61	33284-53-6	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 62	54230-22-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 63	74472-34-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 64	52663-58-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 65	33284-54-7	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 66	32598-10-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 67	73575-53-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 68	73575-52-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 69	60233-24-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 7	33284-50-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 70	32598-11-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 71	41464-46-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 72	41464-42-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 73	74338-23-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 74	32690-93-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 75	32598-12-2	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 76	70362-48-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 77	32598-13-3	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 78	70362-49-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 79	41464-48-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 8	34883-43-7	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 80	33284-52-5	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 81	70362-50-4	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 82	52663-62-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 83	60145-20-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 84	52663-60-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 85	65510-45-4	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 86	55312-69-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 87	38380-02-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 88	55215-17-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 89	73575-57-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 9	34883-39-1	PCB,	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 90	68194-07-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 91	68194-05-8	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 92	52663-61-3	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 93	73575-56-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 94	73575-55-0	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 95	38379-99-6	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 96	73575-54-9	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 97	41464-51-1	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
PCB 98	60233-25-2	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
PCB 99	38380-01-7	PCB	1(2012)	Oral RfD Assessment: Message (Last revised: 06-01-1994), Carcinogenicity Assessment: Yes (Last revised: 10-01-1996)	cancer (1-Oct-89) developmental (1-Jan-91)
Pebulate	1114-71-2	Herbicide	-	-	-
Penconazole	66246-88-6	Fungicide,	-	-	-
Pendimethalin	40487-42-1	Herbicide,	-	-	-
Pentabromoethylbenzene	85-22-3	Flame Retardant	-	-	-
Pentabromotoluene	87-83-2	Flame Retardant	-	-	-
Pentachloroaniline	527-20-8	Fungicide,	-	-	-
Pentachloroanisole	1825-21-4	General Pesticide	-	-	-
Pentachlorobenzene	608-93-5	Fungicide, Insecticide, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987), Carcinogenicity Assessment: Yes (Last revised: 11-01-1992)	-
Pentachloronitrobenzene	82-68-8	Fungicide,	3(1987)	Oral RfD Assessment: Yes (Last revised: 09-30-1987),	-
Pentachlorophenol	87-86-5	Fungicide, Herbicide, Insecticide, Industrial, Pulp/Paper,		Oral RfD Assessment: Yes (Last revised: 09-30-2010), Inhalation RfC Assessment: Discussion (Last revised: 09-30-2010), Carcinogenicity Assessment: Yes (Last revised: 09-30-2010)	cancer (1-Jan-90)
Pentanochlor	2307-68-8	Herbicide	-	-	-
Permethrin	52645-53-1	Insecticide,	3(1991)	Oral RfD Assessment: Yes (Last revised: 03-31-1987),	-
Permethrin II	999046-03-6	Insecticide	-	-	-
Perthane	72-56-0	Insecticide	-	-	-
Phantolide	15323-35-0	PAH, Personal Care,	-	-	-
phenanthrene	85-01-8	PAH,	3(2010)	Carcinogenicity Assessment: Yes (Last revised: 12-01-1990)	-
Phenanthrene-1,4-dione	569-15-3	OPAH	-	-	-
Phenkapton	2275-14-1	Insecticide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Phenol	108-95-2	Pharmacological,	3(1999)	Oral RfD Assessment: Yes (Last revised: 09-30-2002). Inhalation RfC Assessment: Discussion (Last revised: 09-30-2002). Carcinogenicity Assessment: Yes (Last revised: 09-30-2002)	-
Phenothiazine	92-84-2	Fungicide, Insecticide, Pharmacological, Industrial	-	-	-
Phenothrin I	51186-88-0	Insecticide	-	-	-
Phenothrin II	26046-85-5	Insecticide	-	-	-
Phenoxyacetic acid	122-59-8	Pesticide Product	-	-	-
Phenthoate	2597-03-7	Insecticide,	-	-	-
Phorate	298-02-2	Insecticide	-	-	-
Phorate sulfone	2588-04-7	Insecticide	-	-	-
Phorate sulfoxide	2588-05-8	General Pesticide	-	-	-
Phorate-oxon	2600-69-3	General Pesticide	-	-	-
Phosalone	2310-17-0	Insecticide,	-	Oral RfD Assessment: Withdrawn (Last revised: 12-01-1988).	-
Phosfolan	947-02-4	Insecticide	-	-	-
Phosphamidon	13171-21-6	Insecticide	-	-	-
Phthalide	27355-22-2	Fungicide	-	-	-
Phthalimide	85-41-6	Fungicide, Industrial	-	-	-
Picloram methyl ester	14143-55-6	Herbicide	-	-	-
Picolinafen	137641-05-5	Herbicide	-	-	-
Picoxystrobin	117428-22-5	Fungicide	-	-	-
Pindone	83-26-1	Insecticide, Rodenticide	-	-	-
Piperalin	3478-94-2	Fungicide	-	-	-
Piperonyl butoxide	51-03-6	Insecticide,	3(1987)	-	-
Piperophos	24151-93-7	Herbicide,	-	-	-
Pirimicarb	23103-98-2	Insecticide,	-	-	cancer (1-Jul-08)
Pirimiphos-ethyl	23505-41-1	Insecticide	-	-	-
Pirimiphos-methyl	29232-93-7	Insecticide,	-	-	-
Plifenat	21757-82-4	Insecticide	-	-	-
Potasan	299-45-6	Insecticide	-	-	-
Prallethrin, cis-	23031-36-9	Insecticide	-	-	-
Prallethrin, trans-	999023-03-9	Insecticide	-	-	-
Pretilachlor	51218-49-6	Herbicide,	-	-	-
Probenazole	27605-76-1	Fungicide	-	-	-
Prochloraz	67747-09-5	Fungicide, Industrial,	-	Oral RfD Assessment: Yes (Last revised: 01-01-1989). Carcinogenicity Assessment: Yes (Last revised: 10-01-1989)	-
Procymidone	32809-16-8	Fungicide,	-	-	cancer (1-Oct-94)

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Prodiamine	29091-21-2	Herbicide,	-	-	-
Profenofos	41198-08-7	Insecticide,	-	-	-
Profenofos metabolite (4-Bromo-2-chlorophenol)	3964-56-5	General Pesticide,	-	-	-
Profluralin	26399-36-0	Herbicide	-	-	-
Prohydrojasmon I	158474-72-7	Herbicide	-	-	-
Prohydrojasmon II	999060-03-6	Herbicide	-	-	-
Promecarb	2631-37-0	Insecticide	-	-	-
Promecarb artifact [5-isopropyl-3-methylphenol]	3228-03-3	Insecticide,	-	-	-
Prometon	1610-18-0	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Prometryn	7287-19-6	Herbicide,	-	-	-
Propachlor	1918-16-7	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	cancer (27-Feb-01)
Propamocarb	24579-73-5	Fungicide,	-	-	-
Propanil	709-98-8	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 03-01-1988).	-
Propaphos	7292-16-2	Insecticide	-	-	-
Propargite	2312-35-8	General Pesticide	-	-	cancer (1-Oct-94) developmental (15-Jun-99)
Propargite metabolite [Cyclohexanol, 2-(4-tert-butylphenoxy)]	999004-03-4	Insecticide	-	-	-
Propazine	139-40-2	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 08-28-1987).	developmental, female (15-Jul-16)
propenyl guaethol	94-86-0	Personal Care,	-	-	-
Propetamphos	31218-83-4	Insecticide	-	-	-
Propham	122-42-9	Herbicide	3(1987)	Oral RfD Assessment: Yes (Last revised: 09-30-1987).	-
Propiconazole-II	999048-03-2	Fungicide	-	-	-
Propisochlor	86763-47-5	Herbicide	-	-	-
Propoxur	114-26-1	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 07-01-1992).	cancer (11-Aug-06)
Propyzamide	23950-58-5	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	cancer (1-May-96)
Prosulfocarb	52888-80-9	Herbicide	-	-	-
Prothioconazole-desthio	999007-03-3	General Pesticide	-	-	-
Prothiofos	34643-46-4	Insecticide,	-	-	-
Prothoate	2275-18-5	Insecticide	-	-	-
Pyracarbolid	24691-76-7	Fungicide	-	-	-
Pyraclofos	89784-60-1	Insecticide	-	-	-
Pyraflufen-ethyl	129630-19-9	Herbicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Pyrazon	1698-60-8	Herbicide	-	-	-
Pyrazophos	13457-18-6	Fungicide, Insecticide	-	-	-
Pyrazoxyfen	71561-11-0	Herbicide,	-	-	-
pyrene	129-00-0	PAH, Industrial,	3(2010)	Oral RfD Assessment: Yes (Last revised: 09-01-1990), Carcinogenicity Assessment: Yes (Last revised: 09-01-1990)	-
Pyrethrin I	121-21-1	Insecticide	-	-	-
Pyrethrin II	121-29-9	Insecticide	-	-	-
Pyributicarb	88678-67-5	Herbicide	-	-	-
Pyridaben	96489-71-3	Insecticide	-	-	-
Pyridaphenthion	119-12-0	Insecticide	-	-	-
Pyridate	55512-33-9	Herbicide,	-	-	-
Pyridinitril	1086-02-8	Fungicide	-	-	-
Pyrifenox I	88283-41-4	Fungicide,	-	-	-
Pyrifenox II	999049-03-5	Fungicide	-	-	-
Pyriftalid	135186-78-6	Herbicide	-	-	-
Pyrimethanil	53112-28-0	Fungicide,	-	-	-
Pyrimidifen	105779-78-0	Insecticide	-	-	-
Pyriminobac-methyl (E)	136191-64-5	Herbicide	-	-	-
Pyriminobac-methyl (Z)	999024-03-2	Herbicide	-	-	-
Pyriproxyfen	95737-68-1	Insecticide,	-	-	-
Pyroquilon	57369-32-1	Fungicide	-	-	-
Quinalphos	13593-03-8	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987),	-
Quinoclamine	2797-51-5	Fungicide, Herbicide, OPAH	-	-	-
Quinoline	91-22-5	Industrial,	-	Oral RfD Assessment: Discussion (Last revised: 09-27-2001), Inhalation RfC Assessment: Discussion (Last revised: 09-27-2001), Carcinogenicity Assessment: Yes (Last revised: 09-27-2001)	-
Quinoxyfen	124495-18-7	Fungicide,	-	-	-
Quintozene metabolite (pentachlorophenyl methyl sulfide)	1825-19-0	Fungicide	-	-	-
Quizalofop-ethyl	76578-14-8	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988), Carcinogenicity Assessment: Yes (Last revised: 06-01-1991)	male (24-Dec-99)
Rabenzazole	40341-04-6	Fungicide	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Resmethrin	10453-86-8	Insecticide,	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988),	cancer (1-Jul-08) developmental (6-Nov-98)
Resmethrine II	999025-03-5	Insecticide	-	-	-
retene	483-65-8	PAH,	-	-	-
Rotenone	83-79-4	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988),	-
S,S,S-Tributylphosphorotrithioate	78-48-8	Herbicide,	-	-	cancer (25-Feb-11)
Sebuthylazine	7286-69-3	Herbicide	-	-	-
Sebuthylazine-desethyl	37019-18-4	General Pesticide	-	-	-
Secbumeton	26259-45-0	Herbicide	-	-	-
Silafluofen	105024-66-6	Insecticide	-	-	-
Silthiopham	175217-20-6	Fungicide	-	-	-
Simazine	122-34-9	Herbicide,	3(1999)	Oral RfD Assessment: Yes (Last revised: 09-01-1993),	developmental, female (15-Jul-16)
Simeconazole	149508-90-7	Fungicide	-	-	-
Simetryn	1014-70-6	Herbicide	-	-	-
Spirodiclofen	148477-71-8	Insecticide	-	-	cancer (8-Oct-10)
Spiromesifen	283594-90-1	Insecticide	-	-	-
Spiroxamine I	118134-30-8	Fungicide, PAH	-	-	-
Spiroxamine II	999026-03-8	Fungicide	-	-	-
Spiroxamine metabolite (4-tert-butylcyclohexanone)	98-53-3	Pesticide Product	-	-	-
Sudan I	842-07-9	PAH, Industrial	3(1987)	-	cancer (15-May-98)
Sudan II	3118-97-6	PAH, Industrial	3(1987)	-	-
Sudan Red	1229-55-6	Industrial	-	-	-
Sulfallate	95-06-7	Herbicide	2B(1987)	-	cancer (1-Jan-88)
Sulfanilamide	63-74-1	Pharmacological	-	-	-
Sulfentrazone	122836-35-5	Herbicide	-	-	-
Sulfotep	3689-24-5	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 09-07-1988),	-
Sulfur (S8)	10544-50-0	Fungicide, Natural	-	-	-
Sulprofos	35400-43-2	Insecticide	-	-	-
Swep	1918-18-9	Fungicide, Herbicide	-	-	-
Tamoxifen	10540-29-1	Pharmacological	1(2012)	-	cancer (1-Sep-96)
TCEP	115-96-8	Flame Retardant	3(1999)	-	cancer (1-Apr-92)
TCMTB	21564-17-0	Fungicide, Industrial	-	-	-
TCPP	26248-87-3	Flame Retardant, Industrial, Pulp/Paper	-	-	-
Tebuconazole	107534-96-3	Fungicide,	-	-	-
Tebufenpyrad	119168-77-3	Insecticide	-	-	-
Tebupirimifos	96182-53-5	Insecticide	-	-	-
Tebutam	35256-85-0	Herbicide	-	-	-
Tebuthiuron	34014-18-1	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 08-22-1988),	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Tecnazene	117-18-0	Fungicide	-	-	-
Tefluthrin, cis-	79538-32-2	Insecticide	-	-	-
Temephos	3383-96-8	Insecticide	-	-	-
Terbacil	5902-51-2	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	developmental (18-May-99)
Terbucarb	1918-11-2	Herbicide	-	-	-
Terbufos	13071-79-9	Insecticide,	-	-	-
Terbufos-oxon-sulfone	999005-03-7	General Pesticide	-	-	-
Terbufos-sulfone	56070-16-7	General Pesticide	-	-	-
Terbumeton	33693-04-8	Herbicide	-	-	-
Terbuthylazine	5915-41-3	Herbicide	-	-	-
Terbuthylazine-desethyl	30125-63-4	General Pesticide	-	-	-
Terbutryn	886-50-0	Herbicide,	-	Oral RfD Assessment: Yes (Last revised: 09-26-1988).	-
Terrazole	2593-15-9	Fungicide,	-	-	cancer (1-Oct-94)
Tetrabromo-o-chlorotoluene	39569-21-6	Flame Retardant	-	-	-
Tetrabromophthalate diol	20566-35-2	Flame Retardant	-	-	-
Tetrachloroguaiacol	2539-17-5	Industrial, Pulp/Paper	-	-	-
Tetrachlorvinphos	961-11-5	Insecticide	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987).	-
Tetraconazole	112281-77-3	Fungicide	-	-	-
Tetradifon	116-29-0	General Pesticide	-	-	-
Tetraethyl pyrophosphate	107-49-3	Insecticide, Pharmacological	-	-	-
Tetrahydrophthalimide, cis-1,2,3,6-	27813-21-4	Fungicide	-	-	-
Tetramethrin I	7696-12-0	Insecticide,	-	-	-
Tetramethrin II	999050-03-2	Insecticide	-	-	-
Tetrapropyl thiodiphosphate	3244-90-4	Insecticide	-	-	-
Tetrasul	2227-13-6	General Pesticide	-	-	-
Thenylchlor	96491-05-3	Herbicide,	-	-	-
Theobromine	83-67-0	Pharmacological	3(1991)	-	-
Thiabendazole	148-79-8	Fungicide, Pharmacological	-	-	-
Thiazopyr	117718-60-2	Herbicide,	-	-	-
Thifluzamide	130000-40-7	Fungicide	-	-	-
Thiofanox	39196-18-4	Insecticide	-	-	-
Thiometon	640-15-3	Insecticide	-	-	-
Thionazin	297-97-2	Insecticide	-	-	-
Thymol	89-83-8	Pharmacological	-	-	-
Tilt	60207-90-1	Fungicide,	-	-	-
Tiocarbazil I	36756-79-3	Herbicide	-	-	-
Tiocarbazil II	999051-03-5	Herbicide	-	-	-
Tolclofos-methyl	57018-04-9	Fungicide,	-	-	-
Tolfenpyrad	129558-76-5	Insecticide	-	-	-
Tolylfluanid	731-27-1	Fungicide,	-	-	-
Tolylfluanid metabolite (DMST)	66840-71-9	General Pesticide	-	-	-
Tolyltriazole [1H-Benzotriazole, 4-methyl-]	29878-31-7	Industrial	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Tolyltriazole [1H-Benzotriazole, 5-methyl-]	136-85-6	Industrial	-	-	-
Tonalide	1506-02-1	Personal Care,	-	-	-
Toxaphene Parlar 26	142534-71-2	Insecticide	2B(2001)	Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	cancer (1-Jan-88)
Toxaphene Parlar 50	66860-80-8	Insecticide	2B(2001)	Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	cancer (1-Jan-88)
Toxaphene Parlar 62	154159-06-5	Insecticide	2B(2001)	Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	cancer (1-Jan-88)
TPP	115-86-6	Flame Retardant, Industrial,	-	-	-
trans-Nonachlor	39765-80-5	General Pesticide,	-	-	-
Transfluthrin	118712-89-3	Insecticide	-	-	-
Traseolide	68140-48-7	Personal Care,	-	-	-
Tri-p-tolyl phosphate	78-32-0	Industrial	-	-	-
Triadimefon	43121-43-3	Fungicide,	-	-	developmental, female, male (30-Mar-99)
Triadimenol	55219-65-3	Fungicide,	-	-	-
Triallate	2303-17-5	Herbicide	-	-	-
Triamiphos	1031-47-6	Fungicide, Insecticide	-	-	-
Triapenthenol	76608-88-3	General Pesticide	-	-	-
Triazamate	112143-82-5	Insecticide	-	-	-
Triazophos	24017-47-8	Insecticide	-	-	-
Tribromoneopentyl alcohol	1522-92-5	Flame Retardant	-	-	-
Tributyl phosphate	126-73-8	Flame Retardant, Industrial,	-	-	-
Trichlamide	70193-21-4	Fungicide	-	-	-
Trichlorfon	52-68-6	Insecticide,	3(1987)	-	-
Trichloronate	327-98-0	Insecticide	-	-	-
Trichlorosyringol	2539-26-6	Industrial, Pulp/Paper	-	-	-
Triclopyr methyl ester	60825-26-5	Pesticide Product	-	-	-
Triclosan	3380-34-5	Pharmacological, Personal Care, Industrial,	-	-	-
Triclosan-methyl	4640-01-1	General Pesticide	-	-	-
Tricresylphosphate, meta-	563-04-2	Flame Retardant, Industrial	-	-	-
Tricresylphosphate, ortho-	78-30-8	Flame Retardant, Industrial	-	-	-
Tricyclazole	41814-78-2	Fungicide,	-	-	-
Tridemorph , 4-tridecyl-	24602-86-6	Fungicide	-	-	-
Tridiphane	58138-08-2	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 01-31-1987).	-
Trietazine	1912-26-1	Herbicide	-	-	-
Triethyl phosphate	78-40-0	Insecticide, Flame Retardant, Industrial	-	-	-
Trifenmorph	1420-06-0	General Pesticide	-	-	-
Trifloxystrobin	141517-21-7	Fungicide	-	-	-
Triflumizole	68694-11-1	Fungicide,	-	-	-

Compound	CAS #	Classification	Risk		
			WHO IARC *	EPA IRIS**	Cal/EPA***
Trifluralin	1582-09-8	Herbicide,	3(1991)	Oral RfD Assessment: Yes (Last revised: 07-01-1989), Carcinogenicity Assessment: Yes (Last revised: 08-22-1988)	-
triphenylene	217-59-4	PAH	3(2010)	-	-
Tris(2-butoxyethyl) phosphate	78-51-3	Industrial,	-	-	-
Tris(2-ethylhexyl) phosphate	78-42-2	Flame Retardant, Industrial,	-	-	-
Triticonazole	131983-72-7	Fungicide	-	-	-
Tryclopyrbutoxyethyl	64470-88-8	Herbicide	-	-	-
Tycor (SMY 1500)	64529-56-2	Herbicide,	-	-	-
Uniconizole-P	83657-17-4	Fungicide	-	-	-
Vamidothion	2275-23-2	Fungicide, Insecticide	-	-	-
Vernolate	1929-77-7	Herbicide	-	Oral RfD Assessment: Yes (Last revised: 03-31-1987),	-
Vinclozolin	50471-44-8	Fungicide,	-	-	developmental (15-May-98) cancer (20-Aug-99)
XMC (3,4-Dimethylphenyl N-methylcarbamate)	2425-10-7	Insecticide	-	-	-
XMC (3,5-Dimethylphenyl N-methylcarbamate)	2655-14-3	Insecticide	-	-	-
Zinc diethyldithiocarbamate	14324-55-1	Personal Care, Industrial	-	-	-
Zoxamide	156052-68-5	Fungicide	-	-	-
Zoxamide decomposition product	999006-03-0	Fungicide	-	-	-

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Certificate of Analysis

Client Report For: EPA - José L. Zambrana, Jr., PhD
National Exposure Research Laboratory
US EPA Office of Research and Development

zambrana.jose@epa.gov

Project Name: EPA turf (wristbands) - Appendix 4
Project Number: F17-20
Report Date: February 23 2018

QC Review **Date**

FSES Director Approval: Kim A. Anderson **Date**

Methodology:

SOP 422.00 Determination of Volatile Organic Compounds (VOCs) using thermal desorption purge and trap interfaced with EI GC/MSs

Unit Conversions:
ppb = parts per billion
ppm = parts per million
ppt = parts per trillion
ng/g = ppb
ng/L = ppt
ng/mL = ppb
ng/µL = ppm
ng/g(Wristband) = ppb
pg/µL = ppb
µg/mL = ppm

Abbreviations:
J flag: Indicates lower precision in quantitation due to values near limits of detection or matrix effects.
B flag: The sample was background corrected.
< 123.45 U: Detection limit, indicates value was below limit of detection.

COA Notes:
Concentrations in ng/g wristband

Client Sample Name:	Site 1	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170977		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	8.36 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	1.92 B
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	< 0.24 U
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	2.39 B
Bromobenzene, 108-86-1	1.41	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	63.9 B
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	2.51 B		

Client Sample Name:	Site 1 - dup	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170979		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	13.5 B
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	8.62 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	< 0.24 U
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	1.07	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	< 2.42 U
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Site 2	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170981		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	1.33	n-Decane, 124-18-5	9.53 B
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	1.32 B	n-Octane, 111-65-9	< 0.24 U
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	2.57 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	3.43	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	50.6 B
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Site 3	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170983		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	5.56 B
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	0.43 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.32 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	0.83	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	< 2.42 U
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Site 4	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170985		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	< 0.24 U
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.54 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	1.59	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	< 2.42 U
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Site 5	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170987		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	0.79
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	6.03 B
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	0.32 B	n-Octane, 111-65-9	1.91 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	2.42 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	3.94	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	23.1 B
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Site 6	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170989		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	0.30 B
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	4.83 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	0.59 B
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.58 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	0.90 B
Bromobenzene, 108-86-1	1.22	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	76.9 B
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	1.47 B		

Client Sample Name:	Site 7	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A170991		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	2.43 B
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	9.83 B
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	0.90 B
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.34 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	0.78 B
Bromobenzene, 108-86-1	1.25	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	< 2.42 U
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	0.99 B		

Client Sample Name:	Trip blank 1	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A171394		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	< 0.24 U
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.34 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	< 0.24 U	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	< 2.42 U
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

Client Sample Name:	Trip blank 2	**Test Method:**	SOP 422.00 Determination of Volatile Organic Compo
FSES Sample ID:	A171395		
		Matrix:	Passive Sampling Device - Air

Chemical Name	Concentration (ng/g)	Chemical Name	Concentration (ng/g)
1,2,3-Trichlorobenzene, 87-61-6	< 2.42 U	n-Butylbenzene, 104-51-8	< 0.24 U
1,2,3-Trimethylbenzene, 526-73-8	< 0.24 U	n-Decane, 124-18-5	< 0.24 U
1,2,4-Trichlorobenzene, 120-82-1	< 2.42 U	n-Heptane, 142-82-5	< 0.24 U
1,2,4-Trimethylbenzene, 95-63-6	< 0.24 U	n-Nonane, 111-84-2	< 0.24 U
1,3,5-Trimethylbenzene, 108-67-8	< 0.24 U	n-Octane, 111-65-9	< 0.24 U
1,3-Dichlorobenzene, 541-73-1	< 0.24 U	n-Propylbenzene, 103-65-1	< 0.24 U
1,3-dimethylnaphthalene, 575-41-7	< 0.24 U	o-Dichlorobenzene, 95-50-1	< 0.24 U
1-methylnaphthalene, 90-12-0	< 0.24 U	o-Xylene, 95-47-6	< 0.24 U
2,6-dimethylnaphthalene, 581-42-0	< 0.24 U	p-Dichlorobenzene, 106-46-7	< 0.24 U
2-Chlorotoluene, 95-49-8	< 0.24 U	p-Isopropyltoluene, 99-87-6	0.94 B
2-methylnaphthalene, 91-57-6	< 0.24 U	sec-Butylbenzene, 135-98-8	< 0.24 U
4-Chlorotoluene, 106-43-4	< 0.24 U	Styrene, 100-42-5	< 0.24 U
Bromobenzene, 108-86-1	0.72	tert-Butylbenzene, 98-06-6	< 0.24 U
Chlorobenzene, 108-90-7	< 0.24 U	Toluene, 108-88-3	13.2 B
Cumene, 98-82-8	< 0.24 U	Tributyl phosphate, 126-73-8	< 2.42 U
Ethylbenzene, 100-41-4	< 0.24 U		

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