Research Protocol

Collections Related to Synthetic Turf Fields with Crumb Rubber Infill

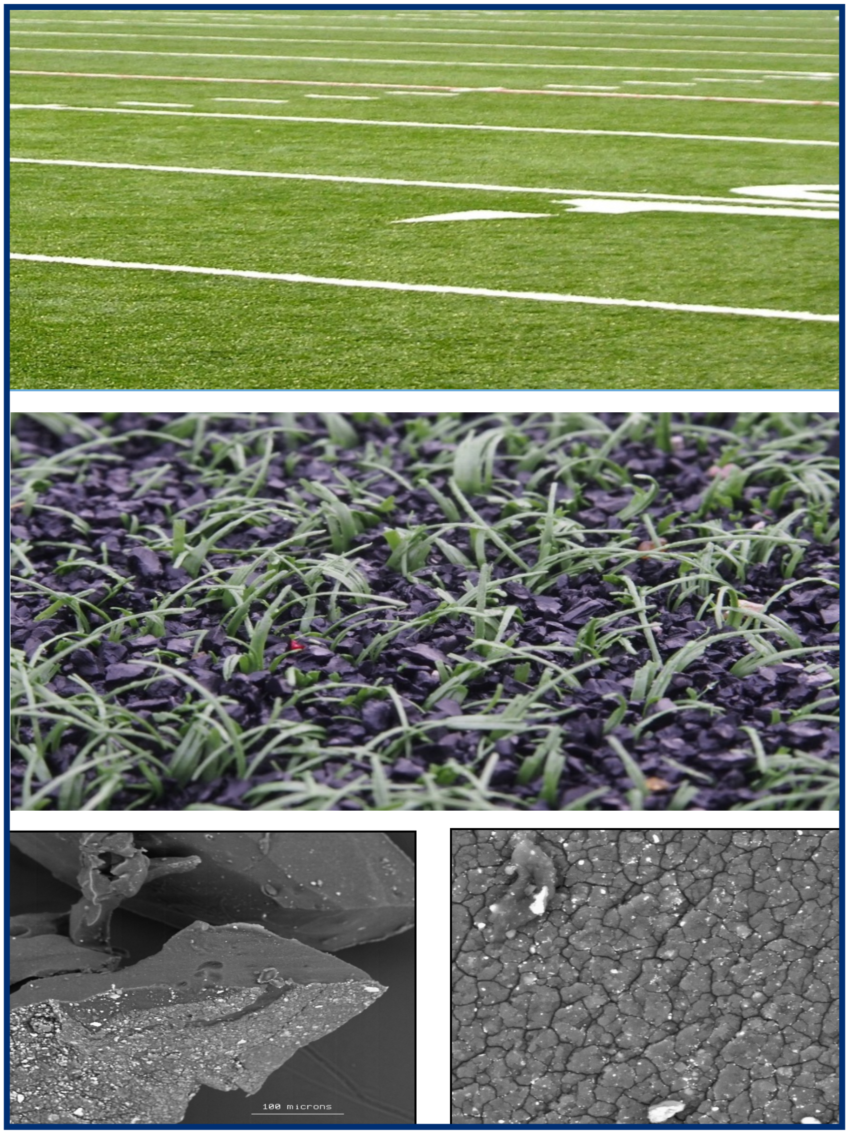

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Table of Contents

__Appendices__

Executive Summary

Concerns have been raised by the public about the safety of recycled tire crumb rubber used in synthetic turf fields and playgrounds in the United States. Several studies have been identified that examine exposure to tire crumb rubber infill in these settings. While, in general, these studies have not provided evidence for these health concerns, the existing studies do not comprehensively evaluate all aspects of exposure associated with these use scenarios. Additional research is needed to help fill important data gaps that will lead to improved exposure assessment and risk evaluation for children and adults using synthetic turf fields and playgrounds with tire crumb rubber. In response, the U.S. Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. Consumer Product Safety Commission (CPSC) launched a multi-agency federal action plan to study key environmental human health questions associated with tire crumb rubber on synthetic turf fields and playgrounds.

The "Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds" (referred to subsequently as the Federal Research Action Plan or FRAP) was finalized in February 2016. The U.S. EPA and CDC/ATSDR, in collaboration with CPSC, have prepared this research protocol to implement portions of the research activities outlined under the FRAP. Specifically, this research protocol is designed to implement three of the research elements described in the Federal Research Action Plan:

- Conduct a literature review and data gaps analysis;
- Perform tire crumb rubber characterization research;
- Perform human exposure characterization research.

<u>The literature review and data gaps analysis</u> is an important component of the Federal Research Action Plan and is needed to guide research in the near and longer terms. A number of previous research investigations have examined various aspects of the issues regarding tire crumb rubber, including the potential for human and ecological exposures and risks from recycled tire products used on synthetic fields and playgrounds. Literature identification and review is underway concurrently with development of this research protocol. Literature searches have been conducted through formal searches of multiple literature databases for published journal articles as well as internet searches and other approaches for identifying reports not available as published journal articles. Efforts are ongoing to finalize the information capture and consolidation, complete the data gaps analysis, and to prepare a white paper that will describe the review, summarize the literature, detail the gaps analysis, and provide conclusions.

<u>The tire crumb rubber characterization study</u> is a pilot-scale effort that will involve the collection of crumb rubber material from tire recycling plants and synthetic turf fields around the U.S., with laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs) (summarized in Figure ES-1). Laboratory analyses will include dynamic emission chamber measurements for VOCs and SVOCs under different temperature conditions and bioaccessibility measurements for metals and SVOCs. The emissions and bioaccessibility experiments will provide important information about the types and amounts of chemical constituents in the tire crumb rubber material available for human exposure through inhalation, dermal, and ingestion pathways. In addition to quantitative target chemical analyses, suspect screening and non-targeted analysis methods will be applied for VOCs and SVOCs in an attempt to identify whether there may be potential chemicals of interest that have not been identified or reported in previous research. The study will also collect tire crumb rubber infill from synthetic turf fields to assess microbial populations. A final piece of this research activity is to identify and collate extant toxicity reference data for selected chemical constituents and contaminants identified through the laboratory analyses.

The exposure characterization study is a pilot-scale effort to: (a) collect information on human activity parameters for synthetic turf field users that affect potential exposures to tire crumb rubber constituents; and (b) implement a human exposure measurement study to further develop and deploy appropriate sample collection methods and to generate data for improved exposure characterization (summarized in Figure ES-2). This data collection will use questionnaires administered to adults and youth (or the parents of children) who use synthetic turf fields with tire crumb rubber infill. Information will be collected to provide data about relevant parameters for characterizing and modeling exposures associated with the use of synthetic turf fields. A subset of participants will have video data collection performed during a physical activity on a synthetic turf field. In addition, publicly available videography of users engaged in activities on synthetic fields will, if feasible, be acquired to provide objective assessment of contact rates and types that are difficult to capture consistently using questionnaires. A subset of participants providing questionnaire responses will also be asked to participate in an exposure measurement study. A set of personal, biological, and field environment samples will be collected around a sport or training activity performed on a synthetic turf field. Personal and environmental samples will be analyzed for metal, VOC, and SVOC analytes, and a subset of SVOC samples will undergo suspect screening and non-targeted analysis. Biological samples will be held in a biorepository for future analysis once potential biomarker chemicals of interest are identified based on the tire crumb rubber and exposure characterization studies.

In summary, several of the key gaps and limitations identified in previous research will be addressed through the activities described in this research protocol. The results of the activities identified in the FRAP will be described in the key product, a report due in 2016. Hence, the research design is constrained by a number of factors most importantly including: the short timeline for initial research activity and reporting completion in 2016; and the resources available for implementing the research. By the end of 2016, the participating agencies anticipate releasing a draft status report that describes the preliminary findings of the research through that point in time. The draft status report will summarize the agencies' progress in: (1) Identifying key constituents of concern in recycled tire crumb used in artificial turf fields; (2) Assessing potential exposures to potentially harmful constituents; (3) Conducting an initial evaluation of potential cancer and non-cancer toxicity of key chemical constituents; and (4) Identifying follow-up activities that could be conducted to provide additional insights about potential risks. The report will also outline any additional research needs and next steps.

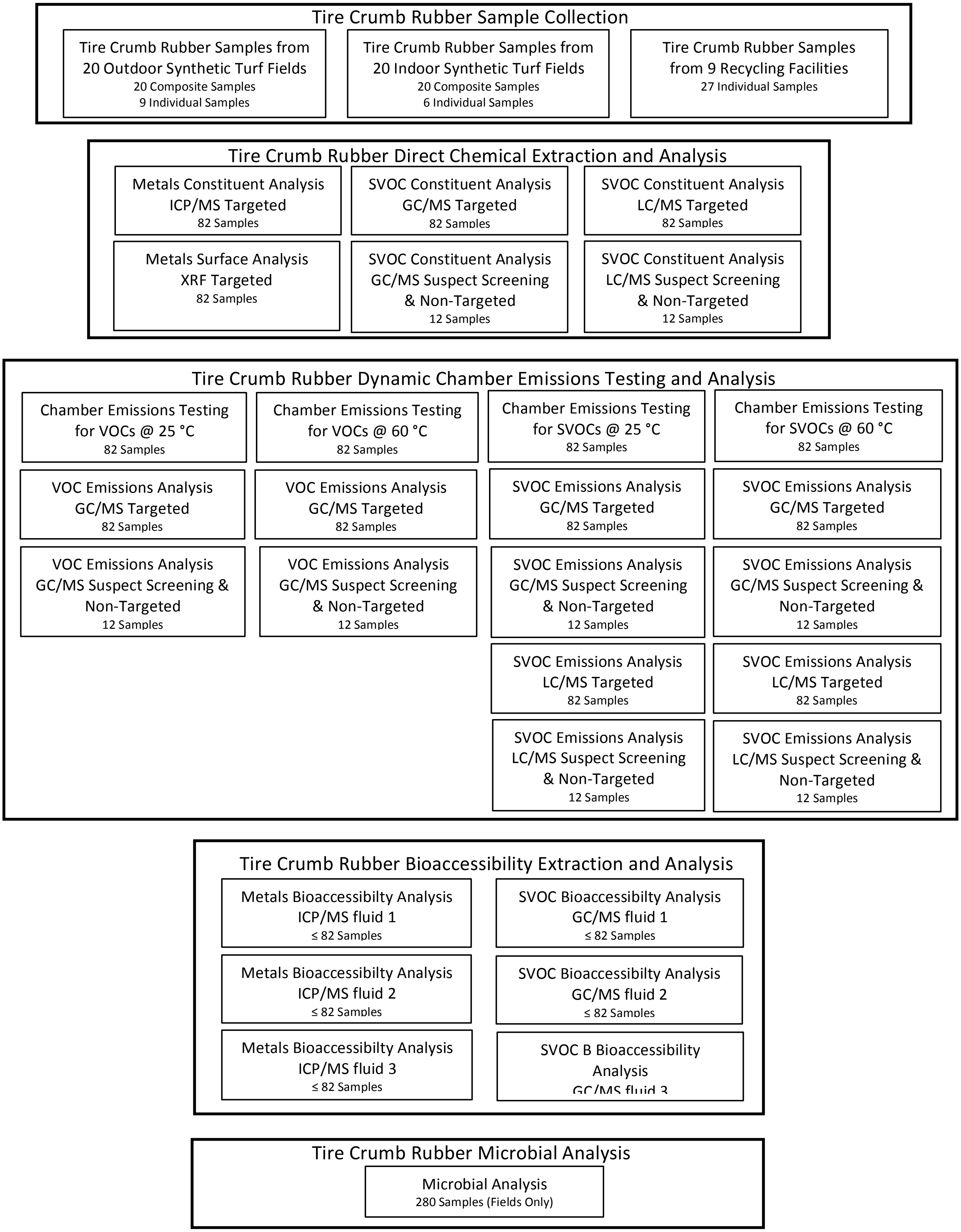

Figure ES-1. Tire crumb rubber characterization overview.

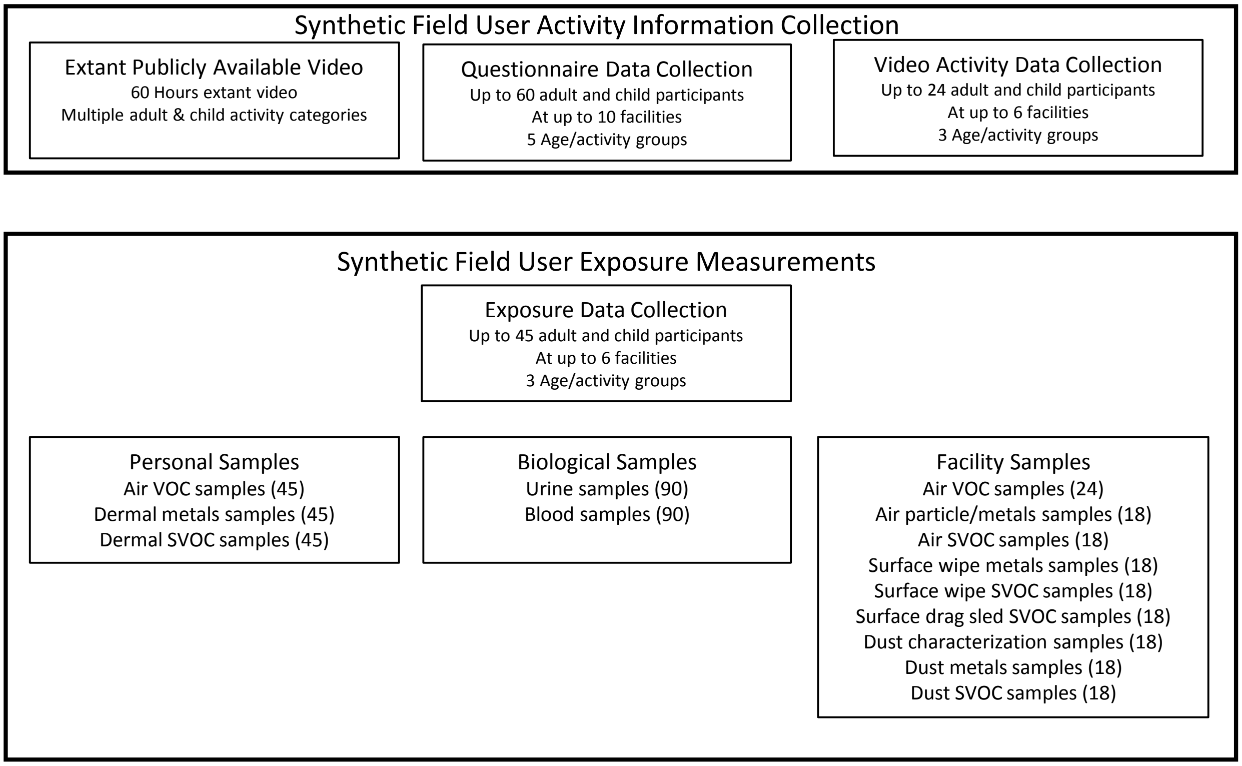

Figure ES-2. Exposure characterization overview.

List of Acronyms and Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ADQ	Audit of data quality
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
BEI	Biological exposure indices
CAI	Computer assisted interview
Cal-OEHHA	California Office of Environmental Health Hazard Assessment
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
CICAD	Concise International Chemical Assessment Documents
CPSC	Consumer Products Safety Commission
DNPH	Dinitrophenyl hydrazine
EPDM	Ethylene propylene diene monomer
FRAP	Federal Research Action Plan
GC/MS	Gas chromatography/mass spectrometry
HEAST	Health effects assessment summary table
IARC	International Agency for Research on Cancer
ICP/MS	Inductively coupled plasma/mass spectrometry
IPCS	WHO International Programme on Chemical Safety
IRIS	Integrated risk information system
ISO	International Standards Organization
IUR	Inhalation unit risk
LC/MS	Liquid chromatography/mass spectrometry
MRL	Minimum risk level
MRM	Multiple reaction monitoring
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
OSF	Oral slope factor
OSHA	Occupational Safety and Health Administration
PAH	Polyaromatic hydrocarbon
PCDL	Personal compound database list
PCR	Polymerase chain reaction
PE	Physical education
PID	Participant identification number
PPRTV	Provisional peer-reviewed toxicity value
PTFE	Polytetrafluoroethylene
PUF	Polyurethane foam
QA	Quality assurance
QAM	Quality assurance manager
QAPP	Quality assurance project plan
QC	Quality control

QMP	Quality management plan
QSA	Quality systems audit
REL	Recommended exposure limit
RfC	Reference concentration
RfD	Reference dose
RH	Relative humidity
RMA	Rubber Manufacturers Association
RNA	Ribonucleic acid
SHEDS	Stochastic human exposure and dose simulation
SIM	Selected ion monitoring
SOP	Standard operating procedure
STC	Synthetic Turf Council
SUV	Sport utility vehicle
SVOC	Semi-volatile organic compound
TC	Tire crumb rubber
TLV	Threshold limit value
TOFMS	Time of flight mass spectrometer
TPE	Thermoplastic elastomers
TSA	Technical systems audit
U.S.	United States of America
U.S. EPA	United States Environmental Protection Agency
UV	Ultraviolet
VID	Video identification number
VOC	Volatile organic compound
WHO	World Health Organization
XRF	X-ray fluorescence

1. Introduction

1.1 Background

Concerns have been raised by the public about the safety of recycled tire crumb rubber used for surfaces in playing fields and playgrounds in the United States (U.S.). Several studies that have examined potential human health risks have not shown an elevated risk from playing on fields with tire crumb rubber infill, but the existing studies do not comprehensively evaluate the concerns about health risks from exposure to tire crumb rubber. Additional research is needed to help fill important data gaps that will lead to improved exposure assessment and risk evaluation for children and adults using synthetic turf fields and playgrounds with tire crumb rubber. The U.S. EPA and CDC/ATSDR, in collaboration with CPSC, have prepared a research protocol that will implement three elements of the research outlined under the "Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds" (Appendix A). These include the literature review and data gaps analysis and the tire crumb rubber characterization and exposure characterization research efforts related to synthetic turf fields. Background information and descriptions of some of the research performed to date on this topic is provided below; the research design and methods are described in later sections.

1.1.1 Waste Tire Generation and Recovery Estimates

A large volume of used automobile and truck tires enters the waste stream in the U.S. each year. The U.S. EPA estimated that 4.77 million tons of waste tires were generated in 2013, and 40.5% or 1.93 million tons were recovered through recycling and production of retreaded tires (U.S. EPA, 2015). Much of the waste tire material not recovered was used as fuel in cement kilns, utility boilers, pulp and paper mills, industrial boilers, and dedicated scrap tire-to-energy facilities. In 2013, approximately 172 thousand tons of scrap tires were converted to tire shreds for use in road and landfill construction, septic tank leach fields, and other construction applications (RMA, 2016a). Approximately 975 thousand tons of scrap tires (representing approximately 59.5 million tires) were used in the ground rubber applications market, which includes the manufacture of new rubber products, rubber-modified asphalt, and playground and sports surfacing (RMA, 2014 and 2016a). The Rubber Manufacturers Association estimated that in 2013 about 590 million kg (1.3 billion pounds) of ground rubber was produced with 33% used in molded extruded products, 31% for playground mulch, 17% for sports surfaces, 7% in asphalt, 6% in automotive products, and 6% was exported (RMA, 2016b). Recycled rubber from tires is used in several types of recreational venues, including use as infill material in synthetic turf fields, on playgrounds as tire crumb rubber or rubber mats, for running surfaces, and in horse equestrian arenas. Recycled tire material may also be used in other applications such as tire-derived rubber flooring materials (CalRecycle, 2010).

1.1.2 Tire Crumb Rubber Manufacturing Process

Two tire recycling processes, ambient and cryogenic, are used to create tire crumb rubber in the 10 – 20 mesh (0.84 – 2.0 mm) size for use as synthetic turf infill. The ambient process uses interlocking knives to chop tires into successively smaller pieces with screening to separate pieces into specific mesh sizes. The cryogenic process uses liquid nitrogen to freeze partially shredded tires, which are then fed into a hammer mill to create tire crumb rubber. Fabric (including polyester, nylon, or other fibers) and steel belt materials are separated from the rubber using magnetic separators, air classifiers, or other separation equipment. Water may be used for cooling during the ambient process, otherwise no chemicals are added to the original rubber composition during either process. Seven rubber reprocessors produce approximately 95% or more of the recycled rubber used as infill in synthetic turf field applications (STC et al., 2016). Voluntary industry standards specify the use of only whole vulcanized auto, SUV, and light truck tires produced in North America that are less than seven years old from the date of production (STC et al., 2016). The extent of the use of large truck and bus tires as synthetic field infill is unclear, with at least one company describing an all-black crumb rubber infill product derived from truck tires that avoids fiber contamination that occurs from auto tires (Entech, 2016).

1.1.3 Synthetic Turf Fields

There are between 12,000 and 13,000 synthetic turf sports fields in the U.S., with approximately 1,200 – 1,500 new installations each year (STC et al., 2016). It is estimated that 95% of the fields utilize recycled rubber infill exclusively or in a mixture with sand or alternative infills (STC et al., 2016). Current generation synthetic turf fields are typically constructed using a gravel/stone base to allow drainage and a multi-layered polypropylene and urethane backing material with polyethylene fiber blades attached to the backing placed over the base. Sand or a sand/crumb rubber mix is often used as a lower layer infill material, with a top layer of infill material consisting of recycled tire crumb rubber, natural materials (such as ground coconut husk), ethylene propylene diene monomer (EPDM), or thermoplastic elastomers (TPE) granules (STC et al., 2016). Sand may also be used in a mixture with tire crumb rubber infill in top layers in some installations. Recycled tire crumb rubber synthetic turf infill serves as ballast, support for the synthetic grass blades, and as cushioning for field users. Infill material selection and installation may also be designed to aid water drainage. As many as 20,000 recycled tires are used to produce the rubber used in a field (STC et al., 2016). Routine synthetic turf field maintenance includes brushing for infill redistribution, raking or vacuuming for infill decompaction, and sweeping for debris removal (STC et al., 2016). New infill material is sometimes added to existing fields to refresh or replace existing tire crumb rubber.

Synthetic turf fields are installed at municipal and county parks; schools, colleges and universities; professional team stadiums and practice fields; and military installations. Both outdoor and indoor facilities have been widely constructed. Football, soccer, and baseball fields are among the most widespread types of fields. Synthetic turf fields are typically used for athletic, recreation, and physical education and physical training activities, although some fields may see multi-purpose uses, such as for concerts and ceremonies. No data were identified regarding the numbers of individuals using synthetic turf fields in the U.S.; however, given the large number of installed fields it can be reasonably anticipated that the number of users nationwide is in the millions. Users may include professional and college athletes, youth athletes in school and/or other athletic organizations, adult and youth recreational users, coaches, team and facility staff, referees, and fans and bystanders of all ages.

A Health Impact Assessment for the use of synthetic turf fields that examines evidence of benefits or harms has been prepared by Toronto Public Health (MacFarlane et al., 2015). Examples of benefits of synthetic turf fields with crumb rubber infill include reduced water use and increased number of playable days per year. Examples of concerns related to synthetic field use include high heat stress under some conditions, the potential for increased risk of skin abrasions, and the potential for adverse chemical exposure impacts for aquatic ecosystems resulting from release of metals in the tire crumb rubber or synthetic blade material. Public concerns have been raised about human exposures to chemicals associated with tire crumb rubber and synthetic turf fields. Recent health concern reports have centered on a number of cancer cases among young adults and youth soccer athletes, and particularly for soccer goal keepers likely to have relatively high contact rates with synthetic turf field materials. The Washington State Department of Health is working with the University of Washington's School of Public Health to try to determine if there is an increased rate of a specific cancer or cancers among soccer players (WA DOH, 2016).

1.1.4 Chemicals of Interest or Concern in Tires

Many of the concerns that have been raised are about the potential exposure to chemicals in tire crumb rubber infill used in synthetic turf fields. Tires are manufactured with a range of materials including rubber/elastomers; reinforcement filler material; curatives including vulcanizing agents, activators, accelerators, antoxidants and antiozonants, inhibitors and retarders; extender oils and softeners; phenolic resins, plasticizers; metal wire; polyester or nylon fabrics; and bonding agents (Dick and Rader, 2014; Cheng et al., 2014; ChemRisk, 2008; NHTSA, 2006). Chemicals of interest or concern range from polyaromatic hydrocarbons (PAHs) in carbon black, to ZnO which is used as a vulcanizing agent and may contain trace amounts of lead and cadmium oxides. Chemicals in many other classes may be used in tires including sulphenamides, guanidines, thiazoles, thiuams, dithiocarbamates, sulfur donors, phenolics, phenylenediamines, and other chemicals (ChemRisk, 2008). There is limited information to assess whether some of these chemicals may carry impurities or byproducts, or whether they may undergo chemical transformation over time. In addition, the rubber material may serve as a sorbent for chemicals in the air and in dust that falls onto the field. One laboratory reported irreversible adsorption of VOC and SVOC analytes spiked onto tire crumb rubber (NYDEC, 2009).

1.1.5 Exposure to Microbes in Synthetic Turf Fields

In addition to the potential for chemical exposures at synthetic turf fields, concerns have been raised about the potential for exposure to microbial pathogens. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) has caused outbreaks among athletic teams and artificial turf has been implicated as a fomite in transmission of MRSA among college athletes (Beigier et al., 2004). In this case a high-morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team was observed, facilitated by cosmetic body shaving and turf burns. Another study examined a MRSA outbreak among members of a professional football team (Kazakova et al., 2005). Likewise, synthetic turf fields could serve as a route of transmission for additional pathogens derived from body fluids deposited onto fields by athletes, coaches and spectators. To date, human pathogens have not been detected in samples of tire crumb rubber infill from artificial turf fields. However, very few studies have been conducted and few potential pathogens have been investigated. Furthermore, all studies reported to date have used traditional culture methods to detect and quantify total bacteria and pathogen densities. These methods can underestimate densities because culture media cannot support the growth of all bacteria and pathogens. Furthermore, bacteria can enter a viable but nonculturable state in some environments (Oliver, 2005), which prohibits their detection by culture methods. The use of molecular methods, like polymerase chain reaction (PCR) and high throughput sequencing are not hindered by these limitations and can provide a more thorough and robust analysis of bacteria and pathogens in tire crumb rubber infill.

1.1.6 Research Studies

Many studies have attempted to characterize chemical constituents of tire crumb rubber material through direct extraction or digestion (Marsili et al., 2014; Celeiro et al., 2014; Llompart et al., 2013; Simcox et al., 2011; Menichini et al., 2011; Highsmith et al., 2009; Mota et al., 2009), leaching experiments (Krüger et al, 2012; Rhodes et al., 2012; Li et al., 2010; NYDEC, 2009;) headspace or off-gassing analysis (Simcox et al., 2011; Nilsson et al., 2008; Incorvia et al., 2007), bioaccessibility testing (Pavilonis et al., 2014; Lioy and Weisel, 2011; Zhang et al., 2008; Cal-OEHHA, 2007; Highsmith et al., 2009), or through other techniques. Many of these studies have examined metal constituents, a modest number have measured VOCs, PAHs and benzothiazole, but relatively few studies have tried to measure or look for the presence or absence of many other organic chemicals potentially associated with tire materials.

Several studies have performed measurements at synthetic turf fields for selected metal or organic chemical analytes (Schiliro et al., 2013; Menchini et al., 2011; Shalat, 2011; Cal-OEHHA, 2010; Simcox et al., 2011; Van Rooij and Jongeneelen, 2010; Highsmith et al., 2009; NYDEC, 2009; Vetrano and Ritter, 2009; Castellano et al., 2008; Dye et al., 2006). Most of these measurements have been for particles, metals, or organics in air while only a few studies measured chemicals present on field surfaces using wipe samples (NYDEC, 2009; Highsmith et al., 2009; CPSC, 2008; Cal-OEHHA, 2007). Concentrations of chemicals in the air of indoor facilities have generally been found to be higher than those at outdoor facilities. Very few studies have reported biomonitoring data (Van Rooij and Jongeneelen, 2010; Castellano et al., 2008). In both cases, 1-hydroxypyrene was measured as a marker of exposure to pyrene, and no elevated levels were found following synthetic field sports use. Several studies collected personal air samples from people engaged in activities on synthetic turf fields (Menichini et al., 2011; Shalat, 2011; Simcox et al., 2011; Vetrano and Ritter, 2009; Moretto et al., 2007). No dermal sample collection reports have been identified. Only a few studies have examined microbiological populations at synthetic turf fields (Bass and Hintze, 2013; Keller, 2013; Cal-OEHHA, 2010; Vidair, 2010: McNitt et al., 2006).

Several studies have focused on assessing the toxicity of tire crumb rubber, or one or more of its constituents, either through testing or using available toxicity information (Dorsey et al., 2015; Schiliro et al., 2013; Ginsberg et al, 2011a; He et al., 2011; Gomes et al., 2010; Mota et al., 2009; Cal-OEHHA 2007; Birkholz et al., 2003). Several researchers and organizations have performed quantitative human health cancer and/or non-cancer risk evaluations or assessments using data from their measurement studies or data reported in the literature (Pavilonis et al., 2014; Ruffino et al., 2013; Cardno ChemRisk, 2013; Kim et al., 2012; Ginsberg et al., 2011b; NYDEC, 2009; Johns, 2008; Menichini et al., 2011; Denly et al., 2008; Cal-OEHHA, 2007). No significant human health risks from exposure to tire crumb rubber infill at synthetic turf fields have been identified in the studies listed above. Menichini et al. found that based on the 0.4 ng/m^3 of benzo(a)pyrene at an indoor facility, and using a conservative approach, there was the potential for an excess lifetime cancer risk of 1×10^{-6} for an athlete with an intense 30-year activity level. Marsili et al. (2014) found that the hazard indices and cumulative excess risk values for cancer were all below levels of concern for measured chemicals; however, based on laboratory tests of PAH releases at 60° C, estimated air concentrations at fields under hot conditions, and assuming long-term frequent exposures at the high temperature, they concluded cumulative PAH exposures under high heat conditions may be of concern. Kim et al. (2012) identified a potential risk for children with pica behavior through ingestion of crumb rubber material at playgrounds.

A few studies have investigated the bacterial loads and occurrence of select pathogens in synthetic turf athletic fields. These investigations did not focus directly on tire crumb rubber infill material; rather the samples were collected from the fields. Miller et al. (2002) investigated the presence of *Burkholderia cepacia*, an opportunistic pathogen that most often causes pneumonia, in a variety of soil types, including turf fields. While *B. cepacia* was present in soil, it was not detected in turf samples. An investigation of athletic fields in Pennsylvania used by athletes of all levels, from elementary to professional, revealed an average of 9×10^3 colony forming units (CFU) of total culturable bacteria per gram on non-selective media. The presence of the pathogen *Staphylococcus aureus*, was investigated, but was not detected in turf samples (McNitt et al., 2006). Bass and Hintze (2013) investigated a new (1 year old) and old (6 year old) field and found higher levels of culturable bacteria on older fields than new fields (1×10^8 compared to 2.5×10^5 CFU/g). Colonies that grew on selective media were presumed to be staphylococci, but no testing was performed to determine presence of *S. aureus*. Finally, five artificial turf fields of high school or colleges/universities in the San Francisco Bay Area were sampled for total culturable bacterial on non-selective media and *Staphylococcus* species on selective media. Total culturable bacteria ranged from $0 – 10^4$ CFU/g on the 5 artificial turf fields. While colonies of *Staphylococcus* were observed, none were identified as *S. aureus* (Vidair, 2010). In general, higher levels of culturable bacteria were found at natural grass fields than synthetic turf fields in the studies that performed comparative sampling.

While no significant human health risks have been identified in the research described above, no single systematic study has been performed with large numbers of fields or people. A limited number of potential tire crumb rubber related chemicals were measured in most studies, and there are gaps in exposure information and measurement data for dermal and ingestion pathways. Some of the gaps and limitations will be addressed through research described in this research protocol. The results of this research may be useful for designing and conducting larger scale exposure and biomonitoring studies, and for improving exposure and risk assessment.

There are other efforts in planning or ongoing to better understand this issue. A concerted effort will be made to understand what is being done in these studies to best leverage and contribute to reducing data gaps. Specifically, researchers at the California Office of Environmental Health Hazard Assessment (Cal-OEHHA) are designing a research study that is likely to have many parallels to the research described here. Consultation between the federal research team and Cal-OEHHA researchers will be used to identify and implement methods and approaches that may, where feasible, produce comparable data.

1.2 Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds

1.2.1 Federal Research

The Agency for Toxic Substances and Disease Registry (ATSDR), the United States Environmental Protection Agency (U.S. EPA), and the Consumer Product Safety Commission (CPSC) have drafted the "Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds" (Appendix A). Because of the need for additional information, these federal organizations are launching a multi-agency action plan to study key environmental health and human health questions. This coordinated federal action includes outreach to key stakeholders and seeks to fill important data and knowledge gaps, characterize constituents of recycled tire crumb rubber, and identify ways in which people may be exposed to tire crumb rubber based on their activities on the fields. While additional research questions may require evaluation beyond this year, the planned activities will help answer some of the key questions that have been raised.

1.2.2 Federal Research Action Plan Objectives

The specific objectives of research under the federal plan are to:

- Determine key knowledge gaps;

- Identify and characterize chemical compounds found in tire crumb rubber used in artificial turf fields and playgrounds;

- Characterize how people are exposed to these chemical compounds based on their activities on the fields;

- Identify follow-up activities that could be conducted to provide additional insights about potential risks.

The full Federal Research Action Plan on Recycled Tire Crumb Used on Playing Fields and Playgrounds is provided in Appendix A.

1.3 Research Protocol Scope

This research protocol is designed to implement three of the research elements described in the Federal Research Action Plan:

- Conduct a literature review and data gaps analysis;

- Perform tire crumb rubber characterization research;

- Perform human exposure characterization research.

The focus of the work under this research protocol is on tire crumb rubber infill used in synthetic turf fields. The literature review and gaps analysis will examine information available for playgrounds in addition to synthetic fields. The tire crumb rubber characterization and exposure characterization research will not be performed for playgrounds as part of this research protocol. The CPSC is developing research plans for playgrounds.

Important research design constraints include the short timeline for initial research activity and reporting completion in 2016 under the Federal Research Action Plan, and the resources available for implementing the research. By the end of 2016, the participating agencies anticipate releasing a draft status report that describes the preliminary findings and conclusions of the research through that point in time. The draft status report will summarize the agencies' progress in: (1) Identifying key constituents of concern in recycled tire crumb used in artificial turf fields; (2) Assessing potential exposures to potentially harmful constituents; (3) Conducting an initial evaluation of potential cancer and non-cancer toxicity of key chemical constituents; and (4) Identifying follow-up activities that could be conducted to provide additional insights about potential risks. The report will also outline any additional research needs and next steps.

2. Research Objectives

2.1 Objectives

The federal research described in this research protocol is intended to provide information needed to further characterize tire crumb rubber use in synthetic fields in the U.S. and to examine key factors that may affect human exposure to chemical and microbiological constituents. Specific research aims are described in the sections below.

2.2 Literature Review and Data Gap Analysis

Aim 1: Conduct a literature review and data gaps analysis of relevant literature addressing aspects of exposure to chemical and microbiological constituents for synthetic turf fields and playgrounds using recycled tire rubber products.

A substantial amount of research has been conducted to characterize tire crumb rubber constituents, and environmental concentrations of related chemicals. Less research has been performed to examine human exposures and potential risks to people using synthetic turf fields and playgrounds. It is important to examine the existing literature to collate and evaluate the currently available information and to identify key data gaps.

2.3 Tire Crumb Rubber Chemical and Microbiological Characterization

There are three primary aims for the pilot-scale tire crumb rubber characterization study.

Aim 1: Characterize a wide range of chemical, physical, and microbiological constituents and properties for tire crumb rubber infill material collected from tire recycling plants and synthetic turf fields around the U.S.

While a number of research studies have examined crumb rubber constituents, most studies have been relatively small, restricted to a few fields or material sources, and measured a limited number of constituents. Tire crumb rubber samples collected directly from tire recycling plants will provide information on constituents in unused material while samples collected from outdoor and indoor synthetic turf fields will provide a better understanding of constituents potentially available for exposure under different conditions of weathering and facility type. Characterization will include direct measurement of metal and SVOC constituents of tire crumb rubber, studies of VOC and SVOC emissions and emission rates from tire crumb rubber, and bioaccessibility testing of metal and SVOC constituents. Multiple analytical methods will be used provide information on a wide range of metals and organic chemicals. A combination of targeted quantitative analysis, suspect screening, and non-targeted approaches will be applied for VOCs and SVOCs. The research will help fill data gaps regarding the types and concentrations of the chemical constituents in crumb rubber material and their potential availability for human exposure. Physical characteristics such as particle size will be examined to better understand potential exposures. The research will also address gaps in our knowledge regarding microbial pathogens associated with tire crumb rubber on synthetic turf fields.

Aim 2: Collect information from facilities around the U.S. to better understand how synthetic turf fields with tire crumb rubber infill are operated, maintained, and used with regard to characteristics potentially impacting human exposure to tire crumb rubber constituents.

Questionnaires will be administered to facility owners/managers to obtain information about potential factors that may affect exposures, including source materials, material age, tire crumb rubber addition or replacement frequencies, maintenance procedures, facility operations, and how people use the facilities.

Aim 3: Identify and collate existing toxicity reference information for selected chemical constituents identified through the tire crumb rubber characterization measurements.

Toxicity reference information will be identified and collated from existing on-line databases and literature sources for selected chemical constituents identified as part of the tire crumb rubber chemical characterization research. Selection of chemicals for toxicity reference information gathering will be based on a combination of factors that may include presence/absence, frequency of detection, relative concentration magnitude, and other information.

2.4 Exposure Characterization

There are two primary aims of the pilot-scale human exposure characterization study.

Aim 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.

There are important data gaps in human activity parameters for various synthetic turf field users that are needed for estimating exposures and evaluating risks from contact with tire crumb rubber constituents. While the potential for inhalation exposures has been characterized for some constituents there is far less information for characterizing dermal and ingestion exposure pathways. Improved exposure factor information is needed for estimating and modeling exposures from the inhalation, dermal, and ingestion pathways. This study is intended to collect information using questionnaires from adults and youth who use synthetic turf fields with crumb rubber infill for several types of active uses including athletics and possibly physical education or physical training. Video data collection for a subset of participants engaged in activity on synthetic fields will be used to obtain objective information about important dermal and ingestion contact rates. In addition, extant videography of users engaged in activities on synthetic fields will, if feasible, be acquired to provide additional data on contact rates for a wider group of people and activities that are difficult to capture consistently using questionnaires. The human activity information will provide data for parameters used in characterizing and modeling exposures associated with the use of synthetic turf fields and is likely to substantially improve the information available for dermal and ingestion exposure pathways.

Aim 2: Conduct an exposure measurement sub-study for people using synthetic turf fields with tire crumb rubber infill, in what are likely to be among the higher exposure scenarios to improve understanding of potential exposures, particularly for the dermal and ingestion exposure pathways.

Human exposure measurement data for synthetic turf field users are limited. Important data gaps exist, particularly for potential dermal and ingestion exposures to synthetic turf field and tire crumb rubber chemical constituents. There are also important limitations in the types of methods that have been developed and used for human exposure measurements during activities on synthetic fields. Challenges include collecting relevant surface, dust, and personal air samples. Few studies have performed measurements of dermal exposures. In addition, few studies have collected urine or blood samples that might be used for measuring biomarkers of exposures to chemicals in crumb rubber infill. As a pilot scale effort, this study will implement a human exposure measurement study to further develop and deploy appropriate sample collection methods and to generate data for improved exposure characterization. The study will be aimed at generating data for field use scenarios anticipated to be among those with relatively high potential exposures with regard not only to frequency and duration of time spent on synthetic fields, but also based on the potential for contact with synthetic field materials.

3. Research Design

3.1 Research Design Overview

As part of the Federal Research Action Plan, three specific research study components are described in this research protocol. The first is a literature review and data gaps analysis. The second study component is a tire crumb rubber characterization effort aimed at obtaining information about synthetic turf field facilities and operations, collection of recycled tire crumb rubber infill samples from tire recycling plants and synthetic turf fields, and chemical and microbiological constituent analyses. The third study component is a pilot-scale exposure characterization effort aimed at obtaining synthetic field facility user activity information and data, with a subset of respondents taking part in an exposure measurement study. The exposure characterization study is intended to provide information on human activity and exposures for synthetic turf field use scenarios that are likely to be associated with higher exposures.

Important research design constraints include the short timeline for initial research activity and reporting completion in 2016 under the Federal Research Action Plan, and the resources available for implementing the research. Therefore, a convenience sample will be used for both the tire crumb rubber characterization and exposure characterization studies. Because the studies will not involve probability-based sampling from the entire population of interest, the research will not provide data suitable for nationwide generalizations. However, the research is anticipated to provide more information than is currently available, fill key data gaps, and improve exposure characterization estimates needed for designing and implementing future studies which could include biomonitoring studies, epidemiologic investigations, and risk assessments. The study is being designed to include more fields and field users than any previous single study in the U.S. It will apply a wide range of analytical approaches for identifying and characterizing important chemical and microbiological constituents and the potential for human exposure. By the end of 2016, the participating agencies anticipate releasing a draft status report that describes the findings of the study to that date.

3.2 Literature Review and Gaps Analysis

The literature review and data gaps analysis is an important component of the Federal Research Action Plan and is needed to guide research in the near and longer terms. A number of previous research investigations have examined various aspects of the potential for human and ecological exposures and risks from recycled tire products used on synthetic fields and playgrounds. While the studies have typically been small in scope and often limited in the number and types of chemical or microbiological agents considered, they cumulatively offer insight on the current state of the science and data in our understanding about the potential risks.

Literature identification and review is underway concurrently with development of this research protocol. Literature searches have been conducted through formal searches of multiple literature databases for published journal articles as well as internet searches and other approaches for identifying reports not available as published journal articles. Using these approaches a preliminary list of 90 journal articles and reports have been identified that are most relevant for characterizing chemical constituents and microbiological populations and for understanding human and ecological exposures and risks from recycled tire products used on synthetic fields and playgrounds (Appendix B). Literature review efforts to date include preparing summaries of key articles or reports, performing a summary classification of information and key conclusions across all articles and reports, and extracting and summarizing chemical reporting information from studies where available. This draft information has informed development of this research protocol with regard to early identification of some data gaps (e.g., limited information on dermal and ingestion exposures and exposure pathways) and selection of proposed target chemical analytes. Further efforts are ongoing to finalize the information capture and consolidation, complete the data gaps analysis, and to prepare a white paper describing the review, summarizing the literature, performing the gaps analysis, and providing conclusions.

3.3 Tire Crumb Rubber Characterization

3.3.1 Overview

The tire crumb rubber characterization study will involve the collection of crumb rubber material from tire recycling plants and synthetic turf fields around the U.S., with laboratory analysis for a wide range of metals, volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs) (Figure 1). Laboratory analyses will include dynamic emission chamber measurements for VOCs and SVOCs under different temperature conditions and bioaccessibility measurements for metals and SVOCs. The emissions and bioaccessibility experiments will provide important information about the types and amounts of tire crumb rubber chemical constituents available for human exposure through inhalation, dermal, and inhalation pathways. In addition to quantitative target chemical analyses, suspect screening and non-targeted analysis methods will be applied for VOCs and SVOCs in an attempt to identify whether there may be potential chemicals of interest that have not been identified or widely reported in previous research. The study will also collect tire crumb rubber infill from synthetic turf fields to assess microbial populations; however, microbial assessments will not be conducted for tire crumb rubber collected at tire recycling plants. A final piece of this research activity is to identify and collate extant toxicity reference data for selected chemical constituents identified through laboratory analysis.

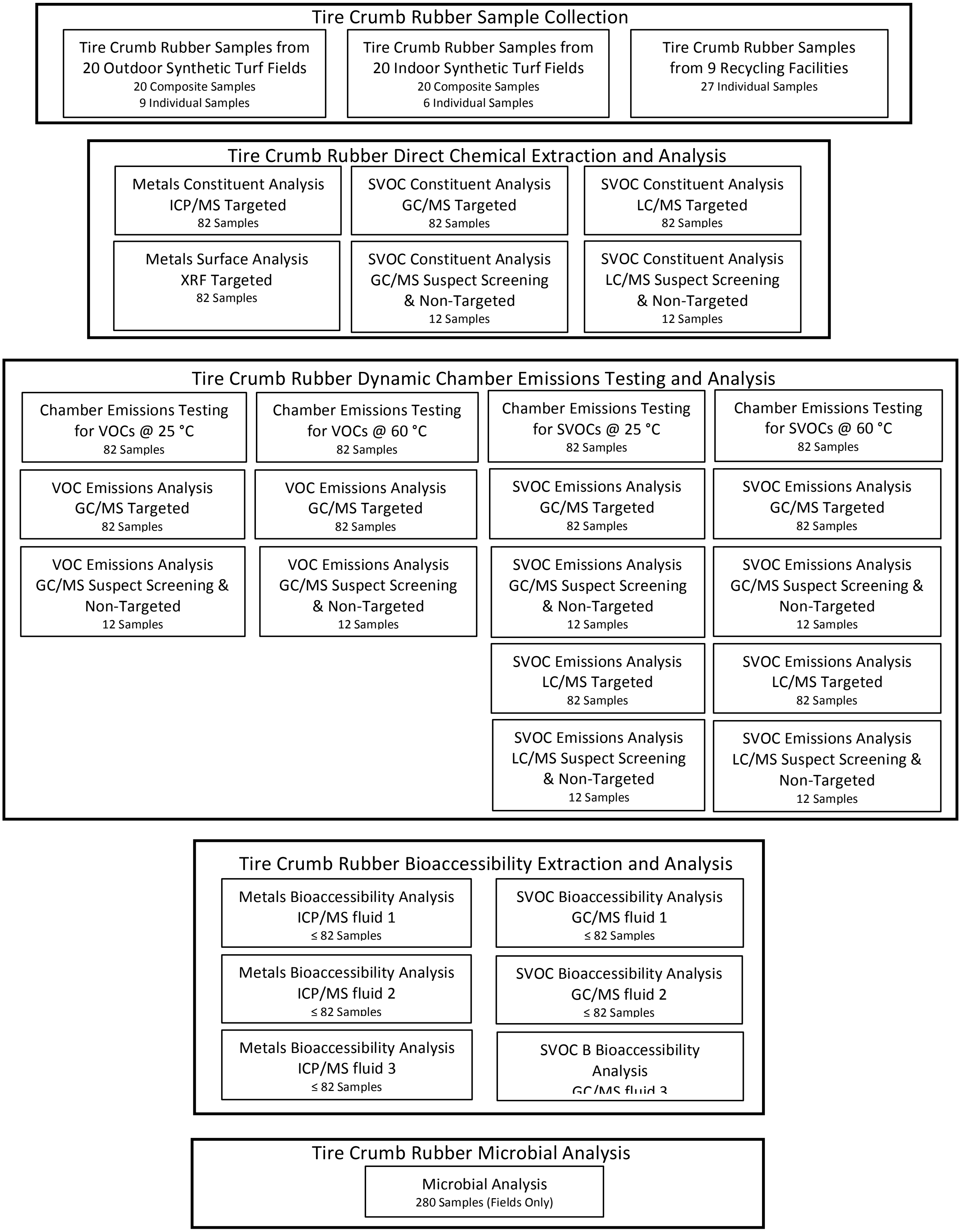

Figure 1. Tire crumb rubber characterization overview

3.3.2 Tire Recycling Plant and Synthetic Field Recruitment

Up to nine tire recycling plants that produce tire crumb rubber for use on synthetic turf fields will be identified and asked to provide tire crumb rubber material samples. Outreach to the Recycled Rubber Council, the Synthetic Turf Council, and individual recycling companies will be used to gauge general interest in providing recycled tire crumb rubber samples. Following outreach discussions, specific companies will be contacted to determine interest in research participation and scheduling availability using a telephone script (Appendix C) with agreements finalized using a participation agreement form (Appendix D). Tire crumb rubber from recycling plants will represent 'new' material that has not undergone weathering at synthetic fields for comparison with material from fields that has undergone weathering and active play. Three samples from different production batches and/or storage containers will be collected from each plant by research staff. If possible, about half of the samples from recycling plants will be from the ambient production process and about half from the cryogenic production process. The actual numbers will depend on plant participation.

Forty facilities with synthetic turf fields with tire crumb rubber infill will be recruited across the four U.S. census regions. If feasible, additional fields may be recruited and sampled; however, the samples will not be analyzed for the current program but will be stored for future analysis. By studying unused material from recycling plants, and material that has from synthetic turf fields, we will get some insight as to whether organic chemical decreases as reported by Zhang et al. (2008) are common among outdoor and indoor fields. We anticipate that we will collect samples from field materials that have a range of ages, and to the extent feasible, we will assess whether there may be trends in chemical content and particle size distribution with age. The geographical diversity is likely to provide a range of material weathering conditions for outdoor fields and may include differences in tire crumb rubber source material. Stratification by facility type (indoor vs. outdoor) will be attempted at the facility identification and recruitment stage. Higher indoor air concentrations of organic chemicals potentially associated with tire crumb rubber have been measured in some studies as compared to levels measured at outdoor fields. Stratification of tire crumb rubber characterization by outdoor and indoor facilities will help determine whether the higher potential inhalation exposures are due to differential weathering effects on the amounts and types of chemicals available for exposure or a function of ventilation rates at indoor facilities. Chemical constituents from outdoor and indoor synthetic field samples will also be compared with unused samples from recycling plants to better understand the impact of weathering and facility use on the types and amounts of constituents available for human exposure. The recycling plant and synthetic turf field facilities sampling design goals are shown in Table 1.

Table 1. Goals for the number and types of tire recycling plants and synthetic turf fields

U.S. Census Region	Outdoor Fields	Indoor Fields	Total Number of Fields or Recycling Plants
Synthetic Turf Fields			
Northeast	5	5	10
South	5	5	10
Midwest	5	5	10
West	5	5	10
Total Number of Fields	20	20	40
Tire Recycling Plants			
Ambient Recycling Process			5
Cryogenic Recycling Process			4
Total Number of Facilities			9[a]

[a]Samples from three different batches or containers per recycling plant are planned for collection, so the total number of samples is expected to be 3 x 9 = 27 samples. The proportion of facilities using different processes is a goal; actual proportion will depend on availability and participation.

Multiple outreach mechanisms will be used to identify and recruit synthetic turf field facilities. The Department of Defense or specific military branches may be interested in participation of facilities at military installations. Specifically, the U.S. Army Medical Command may be able to facilitate access to fields at military installations and has expressed interest in a possible research collaboration. Federal contacts and outreach with state government organizations, including state and local departments of health, may identify state and local facilities, or serve as an intermediary for introduction to other state and local government organizations. Consideration will be given to including fields offered by local municipalities that are interested in participating. Professional and college athletic organizations may be contacted. Additionally, individual institutions or municipalities may be contacted directly in our recruitment efforts.

Contacts will be made with facility owners/managers to determine their level of interest, potential eligibility (facilities with fields in Table 1 categories), and availability during the research implementation time frame for answering a questionnaire and providing or allowing collection of tire crumb rubber material samples. Up to 70 facilities may be contacted for eligibility determination using a structured eligibility assessment screening form (Appendix E) with agreements finalized using a participation agreement form (Appendix D).

A series of fact sheets designed for the different respondent groups have been developed that describe the research effort. These include one for tire recycling/rubber manufacturing plants, synthetic turf field facilities and synthetic turf field facility users (Appendix M).

3.3.3 Statistical Design Considerations

The research is not being conducted under a representative sampling design due to time and resource constraints. A convenience sample of tire recycling plants and synthetic turf field facilities will be used. Statistical power was considered in the stratified facility design (Table 1). The statistical power for assessing differences in tire crumb rubber chemical constituents, emissions, and bioaccessibility is of interest to determine if there are likely to be meaningful differences in exposures based on tire crumb rubber age and weathering (unused recycling plant tire crumb rubber vs. synthetic field tire crumb rubber) and based on the synthetic field facility type (indoor vs. outdoor location). Although the potential difference in chemical concentrations between and among fields in different U.S. regions is of interest, this study is likely to be underpowered for assessing those differences.

Measurement data for two chemicals of possible interest, lead and benzo(a)pyrene (BaP), were obtained from the literature. Using reported means and standard deviations, a range of powers for detecting significant differences in group means was calculated for sample sizes of 20 in each group (Table 2).

These estimates suggest that for measurements of chemicals in tire crumb rubber materials with relatively low variability (low coefficients of variation or CV) differences in means below 20% for 20 in each group may be detected with reasonable statistical power (power ≥ 0.8). For chemicals with higher variability among fields, statistically significant differences may only be detected for differences in means above 100% when group sizes are 20. While larger samples sizes would be preferred, the proposed sample size offers the opportunity to assess whether there are likely to be important differences that may affect human exposure.

Beyond statistical tests of differences of data between groups, the proposed data collection across a diverse range of synthetic turf field facilities in the U.S. will provide important information for characterizing exposures to tire crumb rubber constituents:

a) facility installation and operation information and data,
b) the spectrum of user groups, activity types, use durations and frequencies,
c) the concentrations and bioaccessibility of selected tire crumb rubber chemical constituents,
d) emission rates of selected tire crumb rubber constituents under different conditions,
e) potential identification of tire crumb rubber constituents not previously measured or identified,
f) frequencies of tire crumb rubber constituents found across all analyses, and,
g) toxicological information for identified tire crumb rubber constituents of interest.

The information and data will be made available for human exposure screening assessments and more detailed exposure modeling.

Table 2. Power of the t-test to detect differences between two groups (α=0.05)

	N = 20/facility group	
	Lead	BaP
	CV1 = 0.18	CV1 = 1.13
Difference	CV2 = 0.18	CV2 = 1.13
20%	0.925	0.085
50%	>0.99	0.278
100%	>0.99	0.782
200%	>0.99	>0.99

[a] Lead measured in tire crumb rubber from 5 fields, mean 26.6 ± 4.1 μg/g; Highsmith R., Thomas K.W., Williams R.W. (2009). A Scoping-Level Field Monitoring Study of Synthetic Turf and Playgrounds; EPA/600/R-09/135. National Exposure Research Laboratory, U.S. Environmental Protection Agency.
[b] Benzo(a)pyrene measured in uncoated tire crumb rubber from four fields, mean 4.1 ± 4.5 μg/g ; Menichini et al. (2011). Artificial-turf Playing Fields: Contents of Metals, PAHs, PCBs, PCDDs and PCDFs, Inhalation Exposure to PAHs and Related Preliminary Risk Assessment. Sci Total Environ. 409(23):4950-7.

3.3.4 Tire Crumb Rubber Characterization Data Collection

The numbers and types of questionnaires and samples scheduled for collection at recycling plants and synthetic turf facilities are shown in Table 3. Synthetic turf field facility owners/managers agreeing to participate will be asked to complete a questionnaire (Appendix F) administered by trained research staff in-person or over the phone using a Computer Assisted Interview. Samples will be collected following methods described in Section 4. Trained research study staff will collect most of the samples following specific protocols. Some organizations may have staff members with skills and knowledge (e.g. industrial hygiene or environmental assessment) required to implement standard protocols. In order to reduce research costs, in some cases consideration will be given to training facility organization staff to collect samples following standard protocols and using research study supplied materials.

Table 3. Number and types of facilities, questionnaires, and tire crumb rubber samples

	Number of Plants, Fields or Questionnaires	Number of Individual Crumb Rubber Samples[a]	Number of Composite Crumb Rubber Samples[b]
Plants and Fields			
Tire recycling plants	9		
Synthetic turf fields	40		
Questionnaires			
Synthetic Field facility owner/manager	40		
Recycling Plant Sample Collection			
For metals analysis (3 per plant)		27	
For organics analysis (3 per plant)		27	
Synthetic Field Sample Collection			
For metals analysis		15	40
For organics analysis		15	40
For microbial analysis		280	

[a]For tire recycling plants individual samples will be collected from separate batches or storage containers, if available. For synthetic fields a subset of three individual samples collected at five fields will be analyzed to assess within-field variability for metal and organic chemical analytes. Seven individual samples from each field will be collected for microbial analysis.

[b]Composite samples prepared from seven individual samples collected at each field will be used for metal and organic chemical analysis.

3.3.5 Tire Crumb Rubber Chemical and Microbiological Analysis

The numbers and types of sample analyses scheduled for tire crumb rubber characterization are described in Table 4. Tire crumb rubber material will be analyzed by laboratories for a wide range of volatile and semi-volatile organic (VOC and SVOC) and metals constituents. SVOC analyses will be performed using both GC/MS and LC/MS methods to capture a wide potential range of chemicals with differing chemical and physical properties. Quantitative analyses will be performed for some target analyte chemicals (Tables 5 - 7). Suspect screening and non-targeted analysis methods will be applied to a subset of SVOC constituent analyses. Suspect screening analyses for SVOCs may result in semi-quantitative estimates of concentrations. Sample analysis methods are described in more detail in Section 4.

Table 4. Number and types of samples and analyses for tire crumb rubber characterization

Sample Type	Number of Samples/ Analyses[a]	Additional Information
Direct Constituent Analysis		
Samples for metals constituent ICP/MS analyses	82[b]	
Samples for metals constituent XRF analyses	82	
Samples for targeted SVOC constituent LC/MS analyses	82	
Samples for non-targeted SVOC constituent LC/MS analyses	12	subset of samples
Samples for targeted SVOC constituent GC/MS analyses	82	
Samples for non-targeted SVOC constituent GC/MS analyses	12	subset of samples
Bioaccessibility Analysis		
Samples for metals bioaccessibility analyses	246	Maximum of 82 samples with 3 simulated fluids/sample
Samples for SVOC bioaccessibility analyses	246	Maximum of 82 samples with 3 simulated fluids/sample
Dynamic Chamber Emissions Experiments		
Chamber experiments for VOCs in TC[c]	82	at 25 °C
Chamber experiments for VOCs in TC	82	at 60 °C
Chamber experiments for SVOCs in TC	82	at 25 °C
Chamber experiments for SVOCs in TC	82	at 60 °C
Emissions Sample Analyses		
Samples for targeted VOC emissions analyses	164	
Samples for non-targeted VOC emissions analyses	12	subset of 60 °C samples
Samples for targeted SVOC emissions LC/MS analyses	164	
Samples for non-targeted SVOC emissions LC/MS analyses	12	subset of 60 °C samples
Samples for targeted SVOC emissions GC/MS analyses	164	
Samples for non-targeted SVOC emissions GC/MS analyses	12	subset of 60 °C samples
Microbial Sample Analysis		
Samples for TC microbial analyses	280	

[a]Does not include quality control/quality assurance samples or analyses.

[b]The total of 82 samples is based on 40 synthetic field composite samples, 15 synthetic field individual samples, and 27 individual recycling plant samples.

[c]TC = tire crumb rubber samples.

Both bioaccessibility testing and dynamic chamber emission experiments will be used to generate measurement data useful for gaining a better understanding of the potential for human exposure through inhalation, dermal, and ingestion pathways. Samples will be analyzed for assessing bioaccessibility of selected metals and SVOCs using three different simulated biological fluids. For the in vitro analysis, metals and SVOCs will be selected based on existing data and on data from the current activities, if available. Bioaccessibility testing results will only be reported for the analytes with concentrations above the limit of quantitation.

Tire crumb rubber samples will be placed in dynamic emission chambers under controlled conditions of ventilation, temperature, and humidity. Laboratory chamber emission experiments will be made using two temperature conditions, including a temperature that may represent a warm indoor facility (25 °C) and an upper temperature that approaches what has been reported for synthetic field surfaces under hot ambient conditions (60 °C). Emission rates will be measured for selected VOCs and SVOCs for which quantitative analyses are performed. Suspect screening and non-targeted chemical analysis techniques will also be applied to a subset of VOC and SVOC emission samples.

There is interest in how silicone wristbands might be used in future exposure measurement studies for synthetic field users. As a first step towards determining feasibility, it is important to understand how to measure the relevant chemicals in wristbands and to assess the sorption of chemicals when exposed to tire crumb rubber materials. Exploratory tests are intended to provide the initial assessment and demonstration, which can then inform decisions about using the wristbands in future exposure studies. If time and resources permit, a small number (≤ 5) of exploratory chamber experiments will be performed to evaluate the uptake of selected chemical constituents by silicone wristbands, one buried in tire crumb rubber material and one suspended in the chamber air. The results are intended to inform evaluation of the potential utility in future facility and personal monitoring research studies.

Tire crumb rubber samples will also be analyzed to assess the presence and densities of *Staphylococcus* species, and specifically, *S. aureus* using droplet digital PCR (ddPCR). In addition, the Panton-Valentine leucocidin cytotoxin virulence gene of *S. aureus* and antibiotic resistance genes will be quantified in samples. To investigate the presence of potential pathogens and relative contribution of human-associated microbes to the artificial turf field microbiome, non-targeted analysis of the bacteria in samples will be conducted using high throughput genomic sequencing.

3.3.6 Proposed Target Analytes

An important goal of this research study is to apply a range of sensitive and specific analytical methods that are likely to provide quantitative measurement or presence/absence data for a wide range of chemicals potentially associated with tire crumb rubber. Proposed metal, VOC, and SVOC target analytes are shown in Tables 5 - 7. Target analyte selection was based on a combination of information from previous tire crumb rubber research studies, information on potential tire manufacturing chemical ingredients, and analytical laboratory and method capabilities. The table includes reference ID numbers linking to the preliminary literature review citations in Appendix B. Many of the citations include measurement results for the listed chemical in tire crumb rubber or playground surface rubber, rubber leachate, headspace analysis, or an environmental measurement. In some cases the study reported only presence without quantitative results. Some chemicals are included because they were reported through the literature or other sources to be potential tire manufacturing component or process chemicals.

Many of the chemicals are proposed as target chemicals for quantitative analysis as noted in Tables 5 - 7. Other chemicals are proposed for suspect screening where standards and mass spectra may be available to identify the presence of the chemical with some degree of confidence. In some cases where standards can be obtained and analyzed, it may be possible to provide semi-quantitative or relative amount estimates. A subset of VOC and SVOC samples will also be analyzed using non-targeted approaches, which will generate characteristic mass spectra that can be explored to tentatively identify or propose chemical presence for further investigation. Non-targeted measurement data analysis is very time consuming and initial efforts will likely focus on chromatographic peaks and spectra for unidentified chemicals appearing in relatively large amounts.

The proposed target analyte list may change during the study as experience is gained through implementation of the methods and the analytical processes. We may find that some chemicals included for suspect screening can be part of the quantitative analysis. For other chemicals we may find that the proposed methods do not provide adequate sensitivity or there are degradation or chromatography problems under the established conditions that will prevent acceptable analysis under defined QA/QC specifications.

3.3.7 Extant Toxicological Reference Information

Extant toxicological reference information will be compiled for selected tire crumb rubber chemical constituents of interest identified in the tire crumb rubber characterization and exposure characterization studies. Selection criteria may include frequency and magnitude of detection, detection in multiple media, and other factors including measurement reports from other studies as identified in the literature review. Multiple sources of toxicity reference information will be used to identify and compile values for chemicals where available, and to demonstrate gaps where not available. Identification and compilation of other extant toxicity data from primary sources may also be considered for some chemicals.

Table 5. Proposed metals for analysis in tire crumb rubber samples

Metal Name	CAS Number	Literature Review Reference ID (see Appendix B)
Aluminum	7429-90-5	6, 7, 36, 49, 63, 66, 71
Antimony	7440-36-0	6, 7, 49
Arsenic	7440-38-2	6, 7, 17, 36, 45, 49, 51, 60, 63, 66, 71, 79
Barium	7440-39-3	6, 7, 17, 36, 49, 51, 57, 63, 71, 78
Beryllium	7440-41-7	6, 45, 49, 60
Cadmium	7440-43-9	6, 7, 17, 28, 34, 45, 47, 49, 51, 60, 63, 66, 71, 79, 89
Chromium	7440-47-3	6, 7, 17, 28, 32, 36, 45, 47, 49, 51, 57, 60, 63, 66, 71, 76, 78, 79, 89
Cobalt	7440-48-4	6, 7, 49, 63
Copper	7440-50-8	6, 7, 17, 36, 45, 47, 49, 51, 57, 60, 63, 66, 71
Iron	7439-89-6	6, 7, 36, 47, 49, 57, 63, 66, 71
Lead	7439-92-1	6, 7, 16, 17, 20, 28, 32, 34, 36, 45, 47, 49, 51, 57, 60, 63, 66, 71, 78, 79, 89
Magnesium	7439-95-4	6, 7, 36, 45, 49, 60, 66
Manganese	7439-96-5	6, 17, 36, 49, 57, 63, 66, 71
Mercury	7439-97-6	6, 7, 28, 49, 51, 78, 89, 71
Molybdenum[a]	7439-98-7	6, 7, 49, 66
Nickel	7440-02-0	6, 7, 17, 47, 49, 51, 57, 63, 66, 71
Rubidium[a]	7440-17-7	6, 36, 49
Selenium	7782-49-2	6, 7, 34, 45, 49, 51, 60, 66, 71
Strontium	7440-24-6	6, 36, 49
Tin[a]	7440-31-5	6, 28, 49, 63, 71, 89
Vanadium	7440-62-2	6, 7, 45, 49, 60, 71
Zinc	7440-66-6	6, 7, 17, 28, 32, 34, 36, 47, 49, 51, 54, 57, 61, 63, 66, 71, 72, 79, 89

[a]These metals are not listed in EPA methods 3051A and/or 6020B for digestion and ICP-MS analysis.

Table 6. Proposed VOCs for targeted and suspect screening analysis in tire crumb rubber samples

VOC Name	CAS Number	Target Analyte[a]	Suspect Screening[b]	Literature Review Reference ID (see Appendix B)
Acetone	67-64-1	Yes		15, 16, 55, 57, 76
Aniline	62-53-3	Yes		7, 54, 57
Benzene	71-43-2	Yes		2, 10, 11, 12, 15, 16, 32, 55, 57, 63, 65, 71
Benzothiazole	95-16-9	Yes		7, 15, 16, 17, 34, 3646, 51, 54, 55, 57, 82
t-Butylamine	75-64-9	Yes		
Carbon disulfide	75-15-0	Yes		12, 15, 16, 71, 78
Carbon tetrachloride	56-23-5	Yes		16, 32, 57
Chlorobenzene	108-90-7	Yes		16
Chloroform	67-66-3	Yes		76
Chloromethane	74-87-3	Yes		15, 16, 32, 76
Ethyl benzene	100-41-4	Yes		10, 11, 15, 16, 57, 61
Formaldehyde	50-00-0	Yes		94
Hexane	110-54-3	Yes		2, 11, 12, 15, 16, 32, 57, 76
Methyl ethyl ketone	78-93-3	Yes		12, 15, 16, 32, 76, 78,
Methylene chloride	75-09-2	Yes		2, 15, 16, 32, 57, 76
Methyl isobutyl ketone	108-10-1	Yes		15, 16, 32, 54, 55, 57, 71
Naphthalene	91-20-3	Yes		7, 10, 12, 15, 17, 23, 28, 45, 46, 47, 57, 61, 72, 79, 82,
Styrene	100-42-5	Yes		11, 12, 15, 16, 55
Toluene	108-88-3	Yes		8, 10, 11, 12, 15, 16, 32, 55, 57, 61, 63, 65, 71, 76, 78
Tetrachloroethylene	127-18-4	Yes		16, 57
Trichloroethylene	79-01-6	Yes		16
m-Xylene	108-38-3	Yes		8, 10, 11, 12, 15, 16, 32, 55, 57, 61, 63, 65
p-Xylene	106-42-3	Yes		8, 10, 11, 12, 15, 16, 32, 55, 57, 61, 63, 65
o-Xylene	95-47-6	Yes		16, 55, 57, 61
1,1,1-Trichloroethane	71-55-6	Yes		12
1,3-Butadiene	106-99-0	Yes		
2-Butene (cis and/or trans)	590-18-1; 624-64-6	Yes		
cis-1,2-Dichloroethene	156-59-2	Yes		61
1,1,2-Trichloro-1,2,2-trifluoroethane	76-13-1	Yes		16, 32
1,2,4-Trimethyl benzene	95-63-6	Yes		8, 11, 16, 57, 61
1,3,5-Trimethyl Benzene	108-67-8	Yes		16, 61
4-Ethyltoluene	622-96-8	Yes		
Acetonitrile	75-05-8		Yes	2, 16
Acrolein	107-02-8		Yes	16
Biphenyl	92-52-4		Yes	23
Butylbenzene	104-51-8		Yes	55, 61
Chloromethane	74-87-3		Yes	15, 16, 32, 76
Decane	124-18-5		Yes	11
Ethanol	64-17-5		Yes	16, 76
Heptane	142-82-5		Yes	11, 12, 15, 16
Isopropylbenzene	98-82-8		Yes	8, 61
Isopropyltoluene	527-84-4		Yes	61
Methyl Alcohol	67-56-1		Yes	16
Nonane	111-84-2		Yes	11
Octane	111-65-9		Yes	11
Pentane	109-66-0		Yes	16
Phenol	108-95-2		Yes	7, 12, 71, 57, 78
Propylbenzene	103-65-1		Yes	11, 61
1,2,3-Trimethyl benzene	526-73-8		Yes	71
2-Methylbutane	78-78-4		Yes	16
3-Methylpentane	96-14-0		Yes	16

[a]These VOCs are proposed for quantitative analysis through the use of calibration standards. Some analytes have not yet been tested for quantitative analysis using the proposed methods and the list is subject to change.
[b]These VOCs are proposed for suspect screening or non-targeted analysis for presence/absence.

Table 7. Proposed SVOCs for targeted and suspect screening analysis in tire crumb rubber samples

SVOC Name	CAS Number	Target Analyte[a]	Suspect Screening[b]	Literature Review Reference ID (see Appendix B)
PAHs				
Acenaphthene	83-32-9	Yes		15, 23, 28, 45, 46, 47, 61, 79, 82, 89
Acenaphthylene	208-96-8	Yes		15, 23, 28, 45, 46, 61, 82, 89
Anthracene	120-12-7	Yes		23, 45, 46, 47, 61, 79, 82, 82, 89
Benz[a]anthracene	56-55-3	Yes		15, 23, 28, 45, 46, 47, 49, 63, 65, 79, 82, 89
Benzo(b)fluoranthene	205-99-2	Yes		7, 15, 28, 45, 46, 47, 49, 63, 65, 79, 82, 89
Benzo(k)fluoranthene	207-08-9	Yes		15, 28, 45, 46, 47, 63, 79, 82, 89
Benzo[a]pyrene	50-32-8	Yes		15, 23, 28, 45, 46, 47, 49, 63, 65, 79, 82, 89
Benzo[b]fluoranthene	205-99-2	Yes		7, 15, 28, 45, 46, 47, 49, 63, 65, 79, 82, 89
Benzo[ghi]perylene	191-24-2	Yes		15, 23, 28, 46, 47, 49, 63, 6579, 89
Chrysene	218-01-9	Yes		7, 15, 23, 28, 45, 46, 47, 49, 63, 65, 79, 82, 89
Dibenz[a,h]anthracene	53-70-3	Yes		28, 45, 47, 63, 65, 79, 89
Fluoranthene	206-44-0	Yes		7, 10, 15, 17, 23, 28, 45, 46, 47, 61, 65, 79, 82, 89
Fluorene	86-73-7	Yes		7, 15, 23, 28, 45, 46, 47, 61, 65, 79, 82, 89
Indeno[1,2,3-cd]pyrene	193-39-5	Yes		23, 28, 46, 49, 65, 79, 89
Naphthalene	91-20-3	Yes		7, 10, 15, 17, 23, 28, 45, 46, 47, 57, 61, 65, 79, 82, 89
Phenanthrene	85-01-8	Yes		7, 10, 15, 17, 23, 28, 45, 46, 47, 61, 65, 79, 82, 89
Pyrene	129-00-0	Yes		7, 10, 15, 17, 23, 28, 45, 46, 47, 49, 61, 63, 65, 79, 82, 89
1-Hydroxypyrene	5315-79-7		Yes	10, 82
1-Methyl naphthalene	90-12-0		Yes	15, 17, 23
1-Methyl phenanthrene	832-69-9		Yes	15, 17, 23
2-Bromomethylnaphthalene	939-26-4		Yes	36
2-Methyl naphthalene	91-57-6		Yes	15
2-Methyl phenanthrene	2531-84-2		Yes	23
3-Methyl phenanthrene	832-71-3		Yes	23
9-Methylphenanthrene	883-20-5		Yes	23
Benzo(e)pyrene	192-97-2		Yes	15, 23
Coronene	191-07-1		Yes	23
Phthalates				
Benzylbutyl phthalate	85-68-7	Yes		10, 82
Di(2-ethylhexyl)adipate	103-23-1	Yes		15, 17, 23
Di(2-ethylhexyl)phthalate	117-81-7	Yes		15, 17, 23
Dibutyl phthalate	84-74-2	Yes		36
Diethyl phthalate	84-66-2	Yes		15
Diisobutyl phthalate	84-69-5	Yes		23
Diisononyl phthalate	28553-12-0	Yes		23
Dimethyl phthalate	131-11-3	Yes		23
Di-n-octyl phthalate	117-84-0	Yes		15, 23
Diisodecyl phthalate	26761-40-0		Yes	23

[a]These SVOCs are proposed for quantitative analysis through the use of calibration standards. Some analytes have not yet been tested for quantitative analysis using the proposed methods and the list is subject to change.
[b]These SVOCs are proposed for suspect screening or non-targeted analysis for presence/absence.

Table 7. Proposed SVOCs for targeted and suspect screening analysis in tire crumb rubber samples (continued)

SVOC Name	CAS Number	Target Analyte[a]	Suspect Screening[b]	Literature Review Reference ID (see Appendix B)
Potential Turf Field Biocides[c]				
Alkylbenzyldimethyl ammonium chloride	68424-85-1	Yes		
Alcohol Ethoxylate 6	68439-45-2	Yes		
Dioctyldimethyl-ammonium chloride	5538-94-3	Yes		
Didecyl dimethyl ammonium chloride	7173-51-5	Yes		
Alkyl dimethyl benzyl ammonium chloride	68391-01-5	Yes		
Alkyl dimethyl ethyl benzyl ammonium chloride	68956-79-6	Yes		
Potential Rubber Curatives, Antioxidants/Antizonants, and Other Chemicals Reported in Literature				
2-Mercaptobenzothiazole (MBT)	149-30-4	Yes		46, 57
4-tert-(octyl)-phenol	140-66-9	Yes		16, 17, 34, 51
Aniline	62-53-3	Yes		7, 54, 57
Benzothiazole	95-16-9	Yes		7, 15, 16, 17, 34, 3646, 51, 54, 55, 57, 82
Benzothiazolone	934-34-9	Yes		54, 57
Bis-(2,2,6,6-tetramethyl-4-piperidinyl)sebacate	52829-07-9	Yes		54
Butylated hydroxytoluene	128-37-0	Yes		15, 16, 17, 34, 46, 54, 82
Butylbenzene	104-51-8	Yes		55, 61
Cyclohexamine, N-cyclohexyl-	101-83-7	Yes		7, 54
Cyclohexanamine	108-91-8	Yes		54
Cyclohexanamine, N-cyclohexyl-N-methyl-	7560-83-0	Yes		54, 57
Cyclohexane, isothiocyanato-	1122-82-3	Yes		54, 57
Dibenzothiophene	132-65-0	Yes		23, 46
Hexadecane	544-76-3	Yes		17, 34
Phthalimide	85-41-6	Yes		7, 57
Resorcinol	108-46-3	Yes		94
1,3-Dicyclohexylurea	2387-23-7		Yes	54
1,4-Benzenediamine, N,N0-diphenyl-	74-31-7		Yes	36, 94
1-Phenanthrenecarboxylic acid, 1,2,3,4,4	1740-19-8		Yes	36
2-(1-phenylethyl)-phenol	26857-99-8		Yes	54
2-(Methylthio)benzothiazole	615-22-5		Yes	54
2-(4-morpholinothio)benzothiazole (MBS)	102-77-2		Yes	94
2,2,4-Trimethyl-1,2-dihydroquinoline (TMQ)	147-47-7		Yes	94
2,2'-Methylene-bis-(4-methyl-6-tert-butylphenol)	119-47-1		Yes	94
2,4-Dimethylphenol	105-67-9		Yes	57
2,6-Di-tert-butyl-4-methylphenol (BHT)	128-37-0		Yes	94
2,2’Dithiobis(benzothiazole) (MBTS) (MBTS)	120-78-5		Yes	
2-Ethylanthracene-9,10-dione	84-51-5		Yes	36
o-cresol	95-48-7		Yes	57
2-Morpholinodithiobenzothiazole (MBSS)	95-32-9		Yes	
2-Phenylbenzimidazole	716-79-0		Yes	36
2-Phenylbenzothiazole	883-93-2		Yes	36

[a]These SVOCs are proposed for quantitative analysis through the use of calibration standards. Some analytes have not yet been tested for quantitative analysis using the proposed methods and the list is subject to change.
[b]These SVOCs are proposed for suspect screening or non-targeted analysis for presence/absence.
[c]Reported by the California Office of Environmental Health Hazard Assessment as potential turf biocides. These compounds are not amenable to gas chromatographic analysis. It is not clear yet whether they can be successfully analyzed using the proposed liquid chromatography procedures.

Table 7. Proposed SVOCs for targeted and suspect screening analysis in tire crumb rubber samples (continued)

SVOC Name	CAS Number	Suspect Screening[a]	Literature Review ID (see Appendix B)
<u>Potential Rubber Curatives, Antioxidants/Antiozonants, and Other Chemicals Reported in Literature</u>			
3,5-Di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	Yes	54
p-cresol	106-44-5	Yes	57
4-Nonylphenol	104-40-5	Yes	54, 61
4-tert-butylphenol	98-54-4	Yes	46
5-Methyl-2-hexanone	110-12-3	Yes	54
Acetophenone	98-86-2	Yes	54, 57
Benzene, isocyanato-	103-71-9	Yes	54
Benzoic acid	65-85-0	Yes	55, 57
Benzyl alcohol	100-51-6	Yes	57
Biphenyl	92-52-4	Yes	55
Butylated hydroxyanisole (isomeric mixture)	25013-16-5	Yes	15, 17, 82
Caprolactam disulfide (CLD)	23847-08-7	Yes	94
Carbazole	86-74-8	Yes	45, 57
Cyclohexane, isocyanato	3173-53-3	Yes	54
Cyclohexanone	108-94-1	Yes	7, 54
Cyclohexylthiophthalimide (CTP)	17796-82-6	Yes	
Di-(2-ethyl)hexylphosphorylpolysulfide (SDT)	Not Found	Yes	94
Dibenzofurane	132-64-9	Yes	23
Dicyclohexylamine	101-83-7	Yes	
Dimethyldiphenylthiuram disulfide (MPTD)	53880-86-7	Yes	94
Di-ortho-tolylguanidine (DOTG)	97-39-2	Yes	94
Dipentamethylenethiuram tetrasulfide (DPTT)	120-54-7	Yes	94
Diphenylamine	122-39-4	Yes	2, 36
Dithiodimorpholine (DTDM)	103-34-4	Yes	94
Docosanoic acid	112-85-6	Yes	36
Dodecanoic acid	143-07-7	Yes	54
Dotriacontane	544-85-4	Yes	36
Drometrizol	2440-22-4	Yes	54
Eicosane	112-95-8	Yes	36
Erucylamide	112-84-5	Yes	54
Ethanol, 1-(2-butoxyethoxy)	54446-78-5	Yes	54
Ethanol, 2-butoxy-	111-76-2	Yes	54
Ethanone, 1,1'-(1,3-phenylene)bis-	6781-42-6	Yes	54
Ethanone, 1,1'-(1,4-phenylene)bis-	1009-61-6	Yes	54
Ethanone, 1-[4-(1-methylethenyl)phenyl]-	5359-04-6	Yes	54
Ethylenethiourea (ETU)	96-45-7	Yes	94
Formamide, N-cyclohexyl	766-93-8	Yes	54
Heptadecane	629-78-7	Yes	36
Hexa(methoxymethyl)melamine	3089-11-0	Yes	54
Hexacosane	630-01-3	Yes	36
Hexanoic acid, 2-ethyl-	149-57-5	Yes	54
iso-Nonylphenol	11066-49-2	Yes	61
Isophorone	78-59-1	Yes	57
N,N'-Bis(1,4-dimethylpentyl)phenylendiamine (77PD)	3081-14-9	Yes	94
N,N-Dicyclohexyl-2-benzothiazolesulfenamide (DCBS)	4979-32-2	Yes	94

[a]These SVOCs are proposed for suspect screening or non-targeted analysis for presence/absence.

Table 7. Proposed SVOCs for targeted and suspect screening analysis in tire crumb rubber samples (continued)

SVOC Name	CAS Number	Suspect Screening[a]	Literature Review ID (see Appendix B)
Potential Rubber Curatives, Antioxidants/Antiozonants, and Other Chemicals Reported in Literature			
N,N'-Diethylthiourea (DETU)	105-55-5	Yes	94
N,N'-Diphenylguanidine (DPG)	102-06-7	Yes	94
N,N'-Diphenyl-p-phenylenediamine (DPPD)	74-31-7	Yes	94
N,N'-Ditolyl-p-phenylenediamine (DTPD)	27417-40-9	Yes	94
N-1,3-(dimethyl-butyl)-N'-phenyl-p-phenylenediamine (6PPD)	793-24-8	Yes	94
N-Cyclohexyl-2-benzothiazolesulfenamide (CBS)	95-33-0	Yes	94
n-Isopropyl-n'-phenylparaphenylenediamine (IPPD)	101-72-4	Yes	
N-Methyl-2-pyrrolidone	872-50-4	Yes	54
n-Nitrosodiphenylamine	86-30-6	Yes	57
Nonadecane	629-92-5	Yes	36
N-Oxydiethylenedithiocarbamyl-N`-oxydiethylenesulfenamide (OTOS)	13752-51-7	Yes	94
N-tert-Butyl-2-benzothiazolesulfenamide (TBBS)	95-31-8	Yes	94
Octadecane	593-45-3	Yes	36
Octadecanoic acid, methyl ester	112-61-8	Yes	36
o-Cyanobenzotic acid	3839-22-3	Yes	7, 36
Pentacosane	629-99-2	Yes	36
Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	Yes	54
Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	2772-45-4	Yes	36, 54
Phenol, m-tert-butyl-	585-34-2	Yes	54
p-phenylenediamine (PPD)	106-50-3	Yes	
Pyrazole	288-13-1	Yes	36
Pyrimidine, 2-(4-pentylphenyl)-5-propyl-	94320-32-8	Yes	36
Tetrabenzylthiuram disulfide (TBZTD)	10591-85-2	Yes	94
Tetrabutylthiuram disulfide (TBTD)	1634-02-2	Yes	94
Tetracosane	646-31-1	Yes	36
Tetramethylthiuram disulfide (TMTD)	137-26-8	Yes	94
Tetramethylthiuram monosulfide (TMTM)	97-74-5	Yes	94
Tricosane	638-67-5	Yes	36
Zn-Dibenzyldithiocarbamate (ZBEC)[b]	14726-36-4	Yes	94
Zn-dibutyldithiocarbamate (ZDBC)	136-23-2	Yes	94
Zn-diethyldithiocarbamate (ZDEC)	136-94-7	Yes	94
Zn-dimethyldithiocarbamate (ZDMC)	137-30-4	Yes	94

[a]These SVOCs are proposed for suspect screening or non-targeted analysis for presence/absence.
[b]Some of the dithiocarbamates of these Zn salts may be volatile chemicals in samples.

3.4 Exposure Characterization

3.4.1 Overview

The exposure characterization study is a pilot-scale effort to: (a) collect information on human activity parameters for synthetic turf field users that affect potential exposures to tire crumb rubber constituents; and (b) implement a human exposure measurement study to further develop and deploy appropriate sample collection methods and to generate data for improved exposure characterization (Figure 2).

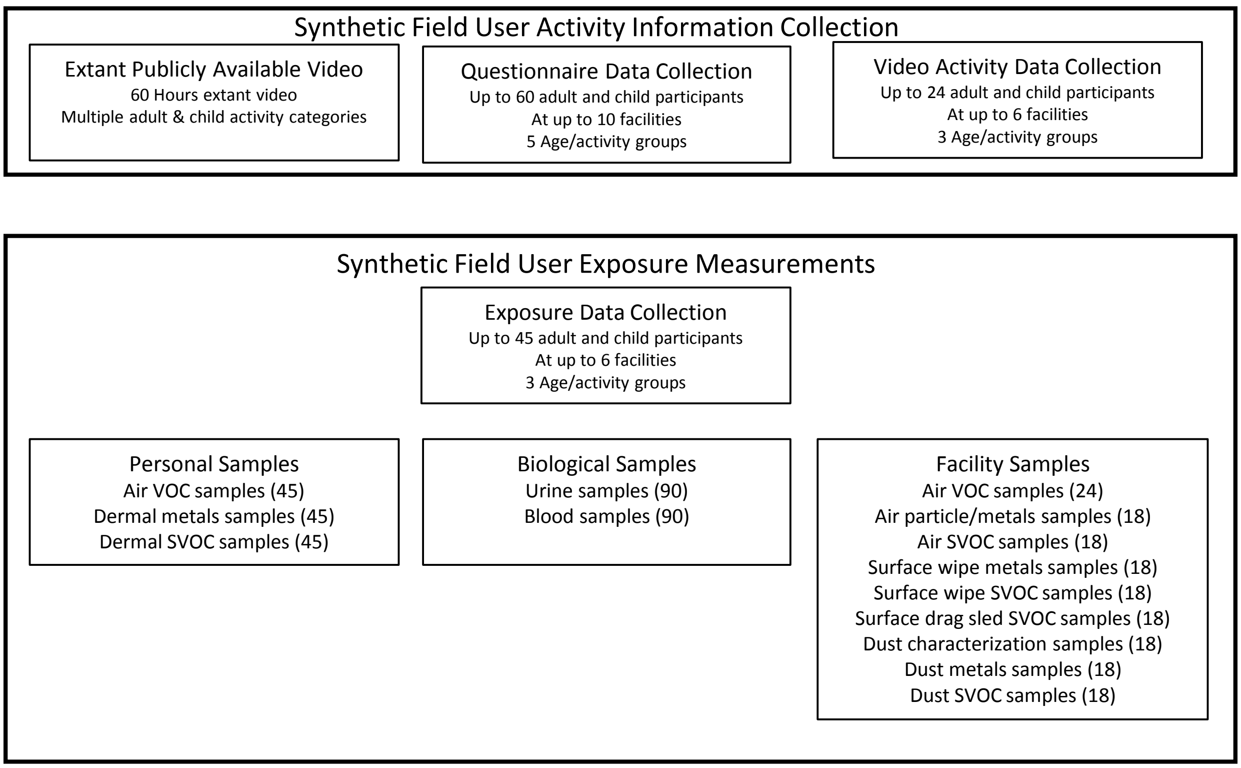

Figure 2. Exposure characterization overview

Several different age groups are included in the research described below; for this research the age group definitions include ≥ 18 years old (adults), 7 to 9 years old (child), 10 to 12 years old (youth), and 13 to 17 years old (adolescent).

The human activity data collection will use questionnaires administered to adults and adolescents; and to the parents of youth and child participants who use synthetic turf fields with tire crumb rubber infill for several types of activities, including athletics and possibly physical education or physical training. Information will be collected to provide data about relevant parameters for characterizing and modeling exposures associated with the use of synthetic turf fields. A subset of participants in the exposure measurement study will have video data collection performed during a physical activity on a synthetic turf field. In addition, publicly available videography of users engaged in activities on synthetic fields will, if feasible, be acquired to provide objective assessment of contact rates and types that are difficult to capture consistently using questionnaires.

A subset of participants providing questionnaire responses will be asked to participate in an exposure measurement study. A set of personal, biological, and field environment samples will be collected around a sport or training activity performed on a synthetic turf field with tire crumb rubber infill. The personal and field environment samples will be analyzed for a range of target metal, VOC, and SVOC analytes, and a subset of SVOC samples will undergo suspect screening and non-targeted analysis (see Tables 5 – 7). It is possible that the exposure measurement study samples will not be collected in time for analyses that will allow inclusion of results in the 2016 status report. The biological specimens collected from the exposure measurements study participants will be stored indefinitely in a biorepository.

The biological samples will be held in a biorepository for future analysis. The decision to delay analysis of biological samples is intentional. The tire crumb rubber characterization and exposure characterization measurements and analyses need to be completed prior to analyzing biological samples. It will be important to apply biological specimen analysis for chemicals that have a high likelihood of demonstrating exposure to tire crumb rubber constituents and not simply reflect exposures to chemicals from other sources in people's lives. At this time, we do not know what the most relevant chemicals might be for the synthetic turf field exposure scenarios. The research may identify a need to develop or assess analysis methods not currently used as part of the routine CDC biomonitoring analysis program prior to analyzing the biological samples.

The human activity and exposure measurements study is intended to provide information and fill key data gaps on routes of exposure to synthetic field and tire crumb rubber chemical constituents, particularly for the dermal and ingestion pathways where key data gaps exist. While the study is aimed at collecting data that will improve exposure assessment and risk assessment across all exposure pathways, another purpose is the development and application of information collection and measurement approaches that can be applied in future studies. For example, dust associated with the fields may be an important source of inhalation, dermal absorption, and ingestion exposure, yet dust collection methods have been tested in only a small number of studies to date (NYDEC, 2009; Shalat, 2011). Dust sample collection and analysis method development will be attempted as part of this effort. In another example, future biomonitoring studies may be designed to assess differential exposures between synthetic turf field users and those not using synthetic turf fields. Before that can be done effectively, it is important to determine which chemicals are most relevant. Data and samples that will allow further development and application of biomonitoring approaches for this exposure scenario will be collected.

3.4.2 Activity and Exposure Measurement Participant Recruitment

Up to 75 people who engage in physical activities at facilities with synthetic turf fields with tire crumb rubber infill will be contacted for recruitment across several use-type categories. We anticipate up to 60 people will agree to participate in providing questionnaire responses. The categories will include activity types anticipated to be among those resulting in higher exposure scenarios either because of the intensity and frequency of field use or because of potentially inherent differences in activity factors (e.g., soccer goalkeepers expected to have repeated contact with turf materials or children age 12 or younger that are likely to have higher hand-to-mouth contact rates). Examples of user types and categories and number of respondents of interest for data collection are shown in Table 8.

Table 8. Number and types of facility users to be recruited for questionnaire and videography data collection

	Total Number	Total Number	Questionnaires		
Example Activity Types[a]	Of Users Recruited	Of Users Participating [b]	Indoor Facility	Outdoor Facility	Total Number Of Facilities
Professional athletics (Group A)	15	12	6	6	2
College athletics (Group B)	15	12	6	6	2
High school P.E. or athletics (Group C)	15	12	6	6	2
Youth ages 10 – 12 athletics (Group D)	15	12	6	6	2
Children ages 7 – 9 athletics (Group E)	15	12	6	6	2
Total Number of Users	75	60	30	30	10

[a]These are examples of activity types of potential interest; the final categories will depend on recruitment success. Different activity types of interest for higher exposure scenarios may be identified through the facility information gathering process.

[b]It is anticipated that up to 60 of the 75 people recruited will participate.

Participants will be recruited from users of a subset of non-military facilities recruited for participation in the tire crumb rubber characterization study described in Section 3.3. As part of the contact with facility owners and managers (identified and contacted as part of the tire crumb rubber characterization study), the respondents will be asked whether they can assist in identifying and providing contact information for facility users. A fact sheet has been developed to describe the research to potential participants (Appendix M). We anticipate recruiting facility users in a specific activity type category from only two different facilities (ideally, one indoor and one outdoor facility, Table 8) to minimize time and cost. Facility users will be contacted to determine eligibility using an eligibility screening form (Appendix G) and to request participation in the questionnaire and exposure measurement research activities (for a subset of questionnaire respondents). Based on a priori decisions regarding activities that may be among higher exposure scenarios, the recruitment may focus on specific types of users among a larger group. For example, more soccer goalies than field players may be recruited from a soccer team or league based on their likely higher contact rates with field materials.

Up to 45 people who engage in physical activities at facilities with synthetic turf fields with tire crumb rubber infill will be recruited across one to three use categories for participation in the exposure measurement portion of the research study (Table 9). These participants will be a subset of those who respond to the questionnaire administration. The category or categories will include activity types expected to be among those resulting in higher exposures either because of the intensity and frequency of field use or because of differences in activity factors and likely contact rates. Examples of user types and categories and number of respondents of interest for data collection are shown in Table 9. We will recruit facility users in a given activity type category from only two different facilities (ideally, one indoor and one outdoor facility, Table 9) to minimize time and cost. Specifically, we will first attempt to recruit participants from Groups A, B, and E (consent and permission forms H2, H12, and H13 in Appendix H). If the participant sample size is not reached within those three groups, we will recruit in Groups C and D until the sample size requirement is met. It is important to recognize that the activity type categories shown in Table 9 may change based on interest and availability of potential participants.

3.3.4 Questionnaire and Video Data Collection

Facility users who agree to participate will be administered a questionnaire (Appendix I) by trained research staff in person or over the phone using a Computer Assisted Interview program. All consented participants will be asked to complete the questionnaire component of the study. For youth and child participants (Groups D and E), a parent or guardian will be asked to complete the questionnaire. For all other groups, the participant will complete the questionnaire. The questionnaire will collect Information about characteristics and activity parameters that may affect the magnitude of exposure to tire crumb rubber infill constituents, including:

a) demographic characteristics,
b) frequency of field use across a range of activity types,
c) duration of field use across a range of activity types,
d) levels of physical exertion that affect breathing rates,
e) contact rates for different types of activities,
f) clothing types and uses, and,
g) hygiene practices.

Table 9. Number and types of facility users to be recruited for exposure measurements

Example Activity Types[a]	Exposure measurement Indoor Facility[b]	Exposure measurement Outdoor Facility[b]	Total Number of Users	Videography Indoor Facility[c]	Videography Outdoor Facility[c]	Total Number of Facilities
Professional athletics (Group A)	8	7	15	4	4	2
College athletics (Group B)	8	7	15	4	4	2
Children 7 – 9 athletics (Group E)	8	7	15	4	4	2
Total Number of Users			45	12	12	6

[a]These are examples of activity types of potential interest; the final categories will depend on recruitment success. Different activity types of interest for higher exposure scenarios may be identified through the facility information gathering process.
[b]It is assumed that all of the people recruited for questionnaire administration in selected activity categories will participate in the exposure measurement portion of the study. Up to two facilities for each type of activity; the facilities are likely to be different for each activity type.
[c]Videography will be done for a subset of participants who complete the questionnaire and participate in the exposure measurements (see recruitment description below).

Two types of videography data collection are proposed for this study. Publicly available videography of physical activities for adult and youth sports participating on synthetic turf fields will be identified. A range of activities, including those in team practices and in games, may be considered for video information collection and analysis. Information about types and frequencies of various contact rates, along with clothing and protective equipment usage, will be collected. A subset of participants in the exposure measurement study will also have videography performed for a specific sports or training activity on a synthetic turf field with tire crumb rubber. We anticipate enrolling 24 participants in the videography component of the study. Enrollment in the videography component will be co-initiated with the exposure measurement sub-study until the minimum sample size has been reached (consent and permission form addenda H3, H10, H11, H14, H15 in Appendix H). Once the videography target sample size has been reached, the videography consent addendum will be removed from the consent form package. Video data collection will include simple counts of specific activity types, including but not limited to hand-to-mouth, diving on turf, falling on turf, laying on turf, sitting on turf, and hand contact with turf. Questionnaire and video data collection methods are described in Section 5.

Questionnaire and video data will be organized into a database suitable for exposure characterization purposes, including exposure screening calculations and exposure modeling. Although time and cost constraints limit participant numbers across different use categories, differences among user groups will be explored to assess whether differences in activity types, durations, and frequencies occur that may affect exposure to tire crumb rubber constituents.

3.3.5 Exposure Measurement Data Collection

Several types of personal, biological and facility samples will be collected (Table 10). Specific sample collection and analysis methods are described in Section 5. Some methods, including collection of dust from synthetic turf fields, will require method development in advance of conducting the exposure measurement study. For collection of SVOCs in facility air, a low-volume collection method is proposed to facilitate sampling at fields. However, if adequate detection limits cannot be achieved, a high-volume sample collection method will be considered. For both metals and SVOC facility air samples, two on-field samples are proposed, with a third sample collected upwind off of the field to serve as a background sample. No background samples will be collected for field wipe and dust samples due to the difficulty in interpreting results collected from other types of surfaces.

Personal air and dermal samples will be collected during or immediately following one sports or training activity on the synthetic turf field. Although it would be ideal to collect personal air samples for metals and SVOC analysis, the size of the pumps and filters able to achieve desired detection limits for short activity time periods make them unsuitable for participants engaged in vigorous physical activity, and could pose a safety hazard. Personal air sampling for VOCs will be attempted using a passive sorbent device that will be attached to participant clothing for the duration of the participant's time on the field before, during, and following a sports or training activity. The personal passive VOC sample is collected using a 6.5 x 1.6 cm Radiello^{R} tube mounted on a triangular back-plate with 8.5 cm edge lengths. The entire assembly weighs just 23 g, and is unlikely to impede activities and has a low likelihood of causing injury even during falls or collisions. Both metal and dermal SVOC samples will be collected from participants following their sports or training activity. Separate wipe samples will be collected for metals analysis from the hand, forearm, and leg on the participant's left side, while separate wipe samples for SVOC analysis will be collected from the hand, forearm, and leg on the participant's right side.

Urine samples will be collected by participants at three time points around the monitored sports or training activity. One sample will be collected prior to the activity, a second sample will be collected following the activity, and a third sample will be collected 24 hours later (Sample Collection SOPs, Appendix J). Participants will be asked to provide blood samples at two time points around the monitored sports or training activity. For participants agreeing to provide blood samples, one sample (15mL) will be collected prior to the activity and a second sample (15mL) will be collected 24 hours later (Sample Collection SOPs, Appendix J). Blood samples will be collected by qualified phlebotomists or nurses. For participants weighing less than 110 pounds, study staff will ensure no more than 3mL per kg are collected. Participants declining to provide urine or blood samples can still participate in other study activities.

Table 10. Number and types of samples for exposure characterization measurements[a]

Sample Type	Number of Users	Number of Facilities[c]	Number of Locations or Samples	Analytes[d]	Total Samples or Analyses
Personal Samples					
Air	45		1	VOCs	45
Dermal	45		3	SVOCs	135
Dermal	45		3	Metals	135
Urine	45		2	PAH metabolites	90
Urine	45		2	Metals	90
Urine	45		2	Creatinine	90
Urine	45		2	VOC metabolites	90
Blood	45		3	Metals	90
Serum	45		2	Metals	90
Facility Samples[b]					
Air		6	3[e]	VOCs	18
Air		6	3	SVOCs	18
Air		6	3	Particulates/Metals	18
Surface wipe (drag sled)		6	3	SVOCs	18
Surface wipe (by hand)		6	3	SVOCs	18
Surface wipe (by hand)		6	3	Metals	18
Dust[f]		6	3	SVOCs	18
Dust[f]		6	3	Metals	18
Dust[f]		6	3	Characterization	18

[a]These are anticipated types and numbers of samples. Final decisions will be based on method availability, resources, participant burden, and participant safety considerations
[b]Samples of tire crumb rubber infill analyzed for constituents as part of Study 1.
[c]Includes one indoor and one outdoor facility for each activity type
[d]Each analyte type will require a separate sample
[e]Includes one-off field background location for each field.
[f]Dust sample collection method development is required.

Exposure measurement data will be organized into a database suitable for exposure characterization purposes, including exposure screening calculations and exposure modeling. Although a statistical design is not being implemented, differences among user groups will be explored to assess whether differences in activity and/or facility types result in differences in exposure to tire crumb rubber constituents. Estimation of population distributions of exposures will not be possible using these data; however, if the scenarios do represent those leading to higher exposures then the data will the assessment of exposures that are likely to be in the upper end of the distribution.

3.5 Limitations

There are important limitations in the research described in this research protocol. Several key limitations are described below.

3.5.1 Research on Playgrounds

This research protocol is directed primarily at characterizing tire crumb rubber material and exposure characterization for synthetic turf field users. It will not directly study playgrounds or playground users. However, some of the information collected as part of the literature review and gaps assessment will be based on research for playground settings and materials. In addition, the tire crumb rubber chemical characterization may be informative with regard to recycled tire products used on playgrounds.

3.5.2 Assessing the Safety of Synthetic Fields with Tire Crumb Rubber Infill

There is continuing public concern about the safety of synthetic turf fields with tire crumb rubber infill. Public expectations for the federal research effort center on the question of whether these facilities are safe for use by children and adults, posing no short-term or long-term health risks. It is important to communicate to the public and other stakeholders that the study activities that can be completed in 2016 are not designed to, and will not be sufficient by themselves to directly answer the public's question about safety, but will implement research necessary to achieve that goal in the longer term. From a science perspective, we consider the question of safety from a risk assessment framework, which requires information on both exposure and hazard (toxicity). This research will provide more information on the types of chemical and microbiological agents of potential interest or concern, will improve our understanding of the potential for exposure to these agents (particularly for the dermal and ingestion exposure pathways), and will organize available information from this and other research studies in a way suitable for incorporation in exposure modeling and risk assessment. This work will also provide information for assessing risks and designing meaningful studies, including biomonitoring studies and epidemiology studies.

3.5.3 Design Constraints

The requirement to complete work under the Federal Research Action Plan in 2016 and research funding limitations place important constraints on the research that are reflected in design choices. For example, the tire crumb rubber characterization study is limited to 40 synthetic turf fields and 9 recycling plants, with modest power for detecting difference between strata. A representative sampling design was considered, but the time and costs required to develop and implement a study based on a national sampling frame of synthetic turf fields are well beyond the scope of time and funds available. Likewise, the exposure characterization study is not based on a representative sampling design, and is likely underpowered for assessing differences among potential exposure factors. However, the exposure characterization study is intended as a pilot-scale effort to further develop measures and approaches suitable for providing relevant exposure information in larger studies. Another design constraint was a decision to focus characterization research on the tire crumb rubber infill and not to include other synthetic turf field materials (synthetic grass blades and backing material) because there is not currently sufficient time and resources for a high-quality characterization of all three types of materials. However, to the extent that permission is provided by synthetic field facilities, we will attempt to separate and store for potential future analysis the synthetic field blades that are inadvertently collected as a by-product of tire crumb rubber sample collection.

3.5.4 No Characterization of Other Types of Fields

The research described in this research protocol is exclusively aimed at synthetic turf fields with tire crumb rubber. . While it may be desirable for reasons noted below to include other types of fields, current constraints do not allow for sufficient time and resources to investigate other types of fields (e.g. natural grass, synthetic fields with natural product infill, synthetic fields with EPDM or TPE infill). However, people use different types of fields, and communities are faced with having to make choices among several field types with incomplete information regarding chemical exposure and risk. While there is concern about chemical exposures resulting from the use of recycled tire and other materials in synthetic fields, it is important to recognize that chemicals are present in other types of fields, including natural grass fields. Metals (including lead) and PAHs (including benzo(a)pyrene) of concern at synthetic fields with tire crumb rubber infill are also often found in surface soil in the U.S. and are likely to be present at natural grass playing fields. In addition, insecticides and herbicides may be used on some natural grass fields, leading to exposures that may not be experienced by synthetic turf field users. Because many recreational and sports field users spend time on both natural grass and synthetic fields (either concurrently or during different life stages) an understanding of relative exposures across different field types is important for risk assessment and epidemiological investigations. An ideal research investigation would perhaps characterize chemical and microbiological agents at all relevant types of fields to enable improved risk and safety assessments and to allow communities to make more informed choices when selecting fields for their schools and communities.

3.5.5 Multi-source and Pathway Exposure Characterization

People are exposed to many of the chemicals of interest at synthetic turf fields (e.g., metals, PAHs, phthalates, VOCs) from other sources and environmental media, including ambient and indoor air, soil, house dust, food, and water. Other types of chemicals (e.g., rubber vulcanization agents or accelerators, antioxidants) may have relatively unique exposure relationships with the use of synthetic turf fields with tire crumb rubber infill, although people are likely exposed to tire wear particles in the environment as well and many rubber products are used in buildings and transportation systems. Field users and athletes may also experience chemical exposures from uniforms and equipment (e.g., goalie gloves, uniforms, mouth guards) that will not be directly assessed as part of this research.

In any risk assessment or epidemiological investigation, it would be important to try to understand the relative exposures from all sources and pathways, including synthetic turf fields. This study will provide some information that could be used in multi-source and multi-pathway modeling assessments for some chemicals with sufficient data, but doing those assessments is beyond the current time and resources available for this research. Design limitations will not allow for control of exposures that occur for off-field activities and/or exposures when urine, blood, and dermal wipe sampling take place. Therefore, relating these measures to measures of exposure from tire crumb field samples will involve significant uncertainty.

3.5.6 Other Limitations

Data will not be collected to directly address the potential for ecological exposure and risks beyond performing chemical characterization of tire crumb rubber material. The study will not address potential heat exposure concerns and injury. The focus of this research will be on the potential for human exposures to chemicals and microbes at synthetic turf fields. In the dermal measurements as part of the exposure characterization study, it would be ideal to collect pre- and post-activity samples; however, given the time and complexity for collecting two types of samples from six body areas, we have judged that the participant burden and time to complete make this not feasible in the current assessment and there are insufficient resources to analyze the large numbers of additional samples that would be generated.

4. Tire Crumb Rubber Characterization Methods

4.1 Tire Crumb Rubber Sample Collection at Synthetic Turf Fields

4.1.1 Sample Collection for Semi-Volatile Organic Compound and Metal Analysis

Tire crumb rubber samples will be collected from tire crumb processing facilities and synthetic turf fields to support characterization of chemical constituents. Characterization includes analysis of SVOC and metal analytes in the tire crumb rubber, bioaccessibility analysis of SVOCs and metals from tire crumb rubber, and emissions testing of VOCs and SVOCs from tire crumb rubber. Substantial variability in tire crumb rubber chemical concentrations have been reported. Therefore, a composite sample collection approach will be used at synthetic turf fields. Individual samples will be collected from seven locations at each field (Figure 3) for SVOC analysis, and another seven samples will be collected at the same locations for metals analysis. Additional samples will be collected from each field to support particle characterization analysis. Different sampling locations may be identified if different types of fields (e.g., baseball fields) are recruited into the study. In this case, a chart will be prepared for each field showing the seven selected sampling locations.

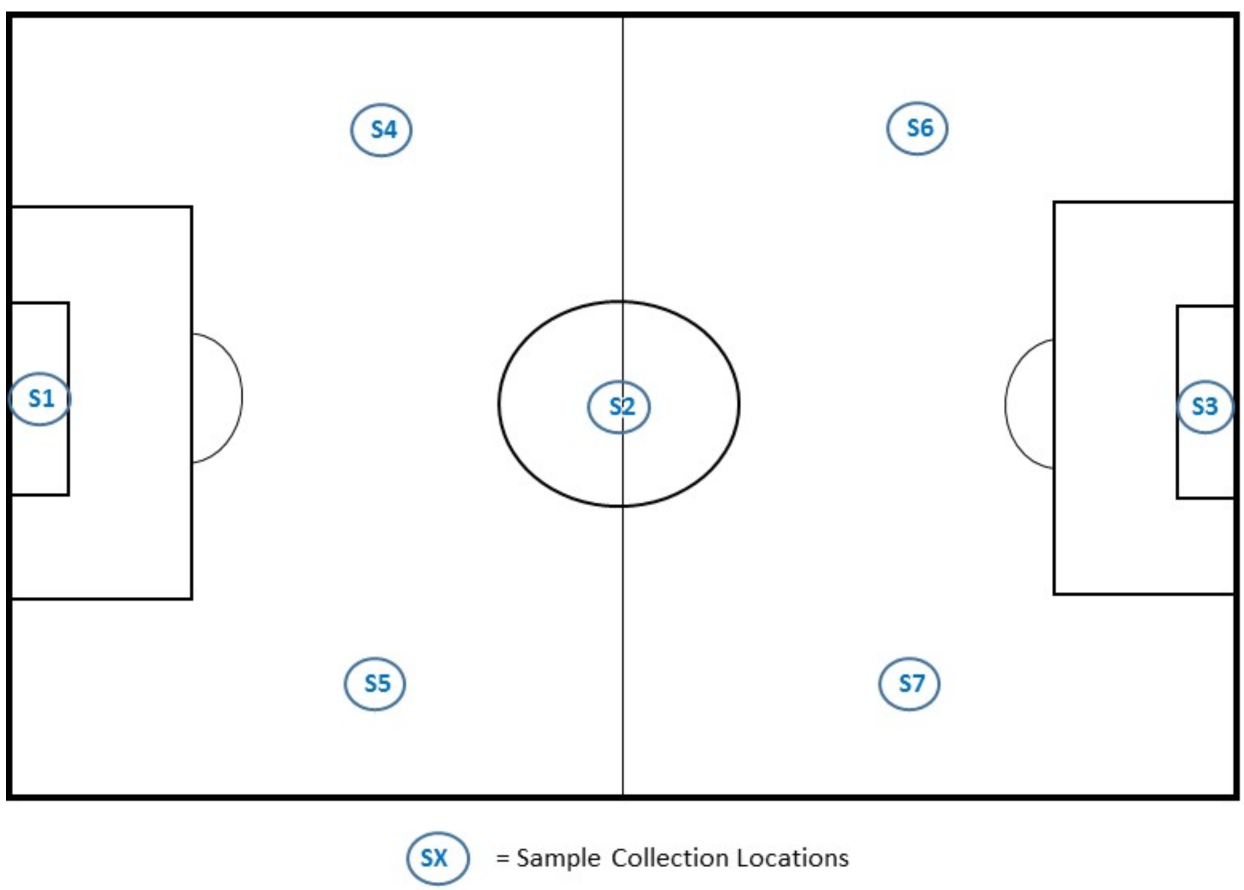

Figure 3. Locations for collecting tire crumb rubber samples at synthetic turf fields.

For SVOC samples, a small handheld metal rake will be used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field will be no more than about 3 cm from the surface. Collected tire crumb rubber will be placed into certified pre-cleaned 250-mL amber glass wide-mouth containers with Teflon-lined lids. The containers will be completely filled with tire crumb rubber material. For metals samples, a small handheld plastic rake will be used to pull tire crumb rubber from the field at each location. Ideally, the collection depth in the field will be no more than about 3 cm from the surface. Collected tire crumb rubber will be placed into certified pre-cleaned 250-mL polyethylene wide-mouth containers. The containers will be completely filled with tire crumb rubber material. Additional tire crumb material will be collected across the seven field locations to fill two 1-L certified pre-cleaned polyethylene wide-mouth containers to support particles size characterization analysis. Samples will be shipped overnight to a central processing laboratory.

A single composite sample will be prepared from the seven SVOC samples at the central processing laboratory. Approximately 35 g of the tire crumb rubber material from each of the seven individual location samples will be added to a single certified pre-cleaned 500-mL amber wide mouth glass container with Teflon-lined lid. The same weight or volume of each individual sample will be added to the larger container to obtain a representative composite sample. The composite sample will be thoroughly mixed in the sealed 500-mL container through rotation and shaking. The remainder of the individual samples will be retained in their sealed containers, and all samples will be placed in a freezer at -20 °C. The same procedure will be used to prepare composite samples for metals analysis using certified pre-cleaned HPDE containers. The remaining sample from each individual field location (approx. 90g each) will be used to support analyses from individual field locations for a subset of three field locations (location numbers 1, 2, and 3 in Figure 3) at five fields.

4.1.2 Sample Collection for Microbiome Analysis

Tire crumb rubber samples will also be collected from synthetic turf fields to support microbiome analysis. Samples collected from tire recycling plants will not be analyzed for microbes. Individual samples will be collected from each field at all seven as shown in Figure 3. Aseptic techniques must be employed while collecting and handling samples or sampling equipment. Nitrile (or appropriate alternative) gloves must be worn at all times when handling the sample or sampling equipment. A clean disposable lab coat will be worn during sample collection. A sterile polypropylene spatula will be used to collect each sample. To collect samples, the sterile spatula will be inserted into the athletic field surface at an approximate 30 degree angle to maximum depth of 3 cm from the surface and moved forward to collect tire crumb material. The tire crumb rubber will be added to a sterile 50 mL polypropylene container with volumetric lines. The container will be filled with tire crumb rubber material to the 25 ml line. Once samples are collected, samples will immediately be placed in a cooler with ice packs. Samples will be shipped the same day as they are collected, in a container with ice packs, to the appropriate laboratory.

4.2 Synthetic Turf Field Facility Owner/Manager Questionnaire

A questionnaire will be used to collect information from managers or owners of athletics facilities that have synthetic turf fields with tire crumb rubber infill (Appendix F). The purpose of the questionnaire is to collect information on general facility operations, turf history and maintenance, and public use at the facilities. The questionnaire will record specific types of data including type of fields (e.g., indoor or outdoor), age of fields, sports/activities played on fields, frequency and hours of field use, and turf maintenance, cleaning, and replacement practices. The questionnaire will be administered by trained study staff members in person or over the phone using a computer assisted interview (CAI), and responses will be entered directly into the computer interview form. Skip patterns and several response range limits are built into the CAI software instrument. All questionnaires and responses will be reviewed by the field study leader prior to incorporation in the study database. In addition to the questionnaire, the location of the field facility will be geo-located using either exact address or, if necessary, through GPS coordinate identification.

4.3 Tire Crumb Rubber Sample Collection from Recycling Plants

Recycled tire crumb rubber samples of the size category used in synthetic turf fields (typically 10 – 20 mesh) will be collected from tire recycling plants by research staff members. Six samples will be collected from each plant for metals analysis, six samples will be collected for organics analysis, and three samples will be collected for particle characterization. Ideally, the samples will come from three different manufacturing batches, storage containers, and/or the production lines. For each manufacturing batch, storage container, or production line sample, one 1-L HPDE jars will be filled for metals analysis, two 1-L amber glass jars will be filled for organic chemical analysis, and two 1-L HPDE jar will be filled for particle characterization.

Pre-cleaned stainless steel scoops will be used to gather tire crumb rubber into pre-cleaned and certified 1-L amber glass wide mouth jars with Teflon-lined lids for organics analysis. The jars will be completely filled. The same scoop may be used to gather samples into all sample containers. Pre-cleaned plastic scoops will be used to gather tire crumb rubber into pre-cleaned and certified 1-L polyethylene wide mouth jars for metals analysis. The jars will be completely filled. The same scoop may be used to gather samples into all three sample containers. Each sealed sample container will be placed into a zip-lock plastic bag, sealed, bubble wrapped, and shipped to the laboratory. Upon arrival at the laboratory, the samples will be stored in a freezer (at -20 °C).

4.4 Analysis of Metal Constituents in Tire Crumb Rubber

Tire crumb rubber samples will be extracted using EPA Method 3051A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils" (U.S. EPA, 2007a). It is anticipated that 1-g samples of tire crumb rubber will be used in the digestion method, but this amount may change based on experience with initial samples. This method is a rapid multi-element microwave assisted digestion method that does not intend to accomplish total decomposition of the sample. The method is applicable to the microwave-assisted acid extraction of 26 metals (aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdemum, nickel, potassium, selenium, silver, sodium, strontium, thallium, vanadium, zinc). The sample is placed in a fluorocarbon polymer or quartz vessel with concentrated nitric acid or a combination of concentrated nitric acid and concentrated hydrochloric acid. The vessel is sealed and heated in the microwave unit for a specified period of time. Once the extraction time is completed, the vessel is allowed to cool down and its contents are filtered, centrifuged, or allowed to settle. The extract is then diluted to volume and analyzed. The extracted samples will be analyzed by EPA Method 6020B "Inductively Couple Plasma-Mass Spectrometry" (U.S. EPA, 2014a). The method is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests. The performance acceptability for the ICP-MS method has been determined for the following analytes: aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, silver sodium, thallium, vanadium and zinc. The analyst will monitor potential sources of interferences and evaluate the performance of the method for the tire crumb rubber matrix.

4.5 Analysis of Semi-Volatile Organic Compounds (SVOC) Compounds in Tire Crumb Rubber

4.5.1 Gas Chromatography/Mass Spectrometry Targeted Analysis

The semi-volatile organics in tire crumb rubber consist primarily of the constituents present from tire manufacturing, but may also include chemicals that the tire material has been exposed to in significant quantities since production. As semi-volatile compounds, these components may exist both in the gas phase and as residues in the condensed state with vapor pressures typically between 10^{-14} and 10^{-4} atm. The SVOC residues will be extracted using dispersive solvent extraction or through sonication using 1:1 acetone:hexane using available test methods (U.S. EPA 3500C, 2007b; U.S. EPA 3550C, 2007c; with modification as needed for the tire crumb rubber matrix). It is anticipated that 1-g samples of tire crumb rubber will be used in the extraction method, but this amount may change based on experience with initial samples. Reference materials consisting of isotopically labelled analogs of representative chemicals will be used as internal standards and will be added during sample preparation to enable quantitative analysis. Interferences that may be present in sample extracts will be monitored and addressed through sample clean-up if necessary through filtration or use of procedures listed in U.S. EPA 3500C (2007b). Extracts may also be split or solvent exchanged in order to analyze using other analytical conditions or instruments.

After extraction, samples will be analyzed by gas chromatography with mass spectrometry (GC/MS) using either an Agilent model 5973 single quadrupole instrument in SIM (selected ion monitoring) mode or an Agilent model 7010 triple quadrupole instrument in MRM (multiple reaction monitoring) mode. Instruments will be standardized using autotune parameters built into the instrument software. Component-specific calibration will be performed using a least squares regression model generated from the area and nominal concentration ratios from a series of calibration standards and their associated internal standards. Component data for each sample will be calculated using the corresponding regression equation. Measured concentrations that exceed the highest calibration level by more than 30% will require the sample to be diluted to a concentration within calibration limits.

4.5.2 Gas Chromatography/Mass Spectrometry Suspect Screening and Non-Targeted Analysis
Non-targeted screening by GC/MS will be performed using the extracts from the targeted analysis if possible, otherwise a similar approach will be taken for extraction with minimal processing. Data will be acquired using an Agilent 5973 mass spectrometer with electron impact (EI) fragmentation in scan mode (50-550 m/z). Chromatographic separation will be performed using an Agilent 6890 gas chromatograph with a 60m capillary column running a thermal gradient from 40ºC to 340ºC at 5ºC per minute. Those data will be deconvoluted and compared to a screening database and the NIST.11 spectral database for tentative identification using AMDIS or Agilent Unknowns Analysis software packages.

The screening database will be populated with chemicals where standards have been prepared and analyzed using the same instrument and conditions in order to have confirmed presence/absence. Components not identified within the screening database will be ranked by abundance and match score for further analysis. It is likely that the time-consuming data reduction and analysis of non-targeted GC/MS spectra will not be completed in time for inclusion in a 2016 report.

4.5.3 Liquid Chromatography/Mass Spectrometry Targeted Analysis
The aforementioned extracts described in section 4.5.1 will be solvent exchanged from acetone:hexane in to a solvent amenable for LC/MS analysis (methanol and/or acetonitrile). Back-calculation of the portion of the total SVOC extract will be recorded for LC/MS analysis to estimate concentrations. A personal compound database list (PCDL) of all suspect analytes will be used to screen for presence of target analytes in SVOC extracts using an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS). The PCDL will consist of neutral monoisotopic mass, CAS# and molecular formula for suspect screening. Select compounds where standards are available and are amenable for LC/MS analysis will be quantitated. Compounds where standards are not available will be estimated in concentration based on relative response to the most similar analyte a standard is available for.

4.5.4 Liquid Chromatography/Mass Spectrometry Suspect Screening and Non-Targeted Analysis
Suspect screening and nontargeted screening of tire crumb rubber will be performed by sonic extraction of the media of interest in an organic solvent amenable to HPLC-MS analysis. This will be with in acetonitrile and/or methanol depending on the performance on test samples. After extraction samples with be filtered for particle removal and reduced in volume for preparation for analysis. Samples with be injected onto an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS) for suspect screening and non-targeted screening analysis.

Samples with be processed according to the procedures of Rager et al. (2015). In brief after running of samples in both positive and negative mode samples with be subjected to a molecular feature extraction (MFE) algorithm to identify peaks for further exploration. Features identified for suspect screening purposes will be compared to the US EPA's DSS-TOX database (~33,000 chemicals). Chemicals matching within 5ppm of the suspect chemical according to accurate mass and scoring >90% will be deemed as a provisional match. Features not matching will be subjected to a nontargeted screening workflow where by features will be prioritized based on occurrence and abundance into discrete data packets. Further work on these peaks may include compound discovery, verification with authentic standards and comparison to outside databases (Chemspider, Scifinder) for provisional matching. Suspect and non-targeted screening features will be summarized with descriptive statistics. It is likely that the time-consuming data reduction and analysis of non-targeted LC/MS spectra will not be completed in time for inclusion in the 2016 report.

4.6 Characterization of Dust Associated with Tire Crumb Rubber

Dust often consists of atmospherically-deposited particles that are derived from various sources such as soil, pollen, and pollution. In the indoor environment, dust may also contain small amounts of dander, hair, textile fibers, paper fibers, minerals from outdoor soil, and human skin cells. In cases where tire crumb rubber is used on sports fields not only is there dust as previously described but there is also likely to be dust-sized particles that are created due to the breakdown/weathering of the tire crumb rubber. All these dust sources can be trapped in the mass of tire crumb rubber particles and within the plastic "blades of grass" that make up the sports field. Due to the smaller particle sizes associated with dust particles, exposures to dust occurs through inhalation, ingestion, and through dermal contact as the dust can cling to skin and clothes.

To investigate the potential for exposure to chemicals attached to or released from dust particles, we propose analyses that include particle size characterization (% sand, silt, and clay), nano-particle size analysis, and metals analyses via X-ray fluorescence. Determination of the particle-size distribution will be performed following a method derived from Miller and Miller (1987), which employs a micro-pipetting method. This method allows for the use of very small sample sizes that we anticipate receiving for this study versus the 50 to 100 gram samples used in standard soil-based particle-size determinations. Associated with the determination of the clay fraction (particles with mean diameters < 0.002 mm) will be the determination of the nano-sized fraction (particles between about 0.3 and 10 nm mean diameter). The nano fraction analyses will be performed using the Malvern Nanosizer which employs dynamic light scattering as the detection method. X-ray fluorescence will be used to determine metal contents following the premises in SW-846 Method 6200. A field-portable X-ray fluorescence device was selected for laboratory analyses due to the advantages that a single run detects multiple elements simultaneously and the analyses are non-destructive such that dust mass can be conserved and used for other analyses.

4.7 Bacterial Community Composition and Antibiotic Resistance Genes in Tire Crumb Rubber

A preliminary literature review revealed very limited data on the microbiology of tire crumb rubber infill used on artificial turf athletic fields. Therefore, to assess the possibility of microbial risks to individuals exposed to tire crumb rubber infill, a study to examine the composition of the microbial communities and occurrence of pathogens and select antibiotic resistance genes is proposed. Microbial communities will be sampled at each of the seven sample collection locations on every field. Once at the laboratory, five grams of each sample will be processed to dissociate microbes from tire crumb rubber using surfactants and physical disruption. Microbial genomes will be extracted from dislodged communities using commercial kits. The purified genomes will be interrogated by deep sequencing techniques targeting the 16S small subunit ribosomal RNA gene to taxonomically classify the community members and identify potential pathogens. In addition, the concentration of *Staphylococcus* species, *S. aureus* and antibiotic resistance genes within the community will be determined by droplet digital PCR.

4.8 Chamber Testing for Tire Crumb Rubber Constituent Emissions

Constituents such as volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) can be released to the environment from tire crumb rubber under different environmental conditions. Laboratory chamber tests will be performed to characterize the emissions of VOCs and SVOCs under two different chamber conditions (e.g. 25 °C and 50% relative humidity (RH); 60 °C and approx. 9% RH) and defined air change rates. Emissions of VOCs from tire crumb rubber samples will be investigated using 53-L electro-polished stainless steel small chambers and emissions of SVOCs will be investigated using micro chambers. Test conditions are described below and may be adjusted as experience is gained during sampling and analysis. For example, the 24-hour equilibration period may be reduced if timed experiments demonstrate that a quasi-steady state for analyte concentrations in chamber air is reached in a shorter time period.

53-L small chambers are housed in temperature-controlled incubators. Nominal dimensions of the chambers are 51 cm (width) by 25 cm (height) by 41 cm (depth). In a dynamic chamber test, clean, VOC-free air will be supplied to the chamber through a dedicated system consisting of an air compressor, dryer, pure air generator, and gross particle filters. The chambers are equipped with inlet and outlet manifolds for the air supply, as well as with temperature and RH sensors. The RH of the air supply to the chamber will be controlled by blending dry air with humidified air. All air transfer and sampling lines are composed of glass, stainless steel, or Teflon. An OPTO 22 data acquisition system (DAS) continuously records mass flow controllers' outputs, temperature, and RH in the chamber and inlet air. A 1 ½" computer cooling fan will be placed in the chamber to provide mixing for tests. During tests, clean air flow will be supplied to the test chamber where a test sample is placed with a ventilation rate equivalent to one air change per hour. A total of 15 g of tire crumb rubber sample material will be placed in the center of the chamber. Air samples for analysis of most VOCs will be collected on pre-cleaned Carpopack X tubes at a flow rate of 100 mL/min for 60 min following a 24-hr equilibration period. Samples for formaldehyde analysis will be collected using dinitrophenyl hydrazine (DNPH) sampling cartridges. The small chamber tests will be consistent with the methods described in the ASTM Standard Guide D5116-10 (ASTM, 2010).

Two micro chamber systems, μ-CTE and M-CTE250, will be used for the project. The systems consist of four (M-CTE250) or six (μ-CTE) micro chambers that allow surface or bulk emissions to be tested simultaneously from up to six samples at the same temperature and flow rate. Each μ-CTE micro chamber consists of an open-ended cup constructed of silicone-coated stainless steel measuring 30-mm deep with a diameter of 45 mm and a volume of 44 mL. Each M-CTE250 micro chamber consists of an open-ended cup constructed of Silicosteel® measuring 36-mm deep with a diameter of 64 mm and a volume of 114 mL. Both systems have temperature and humidity control that allows the tests to be conducted at 50% RH and at ambient temperature or at elevated temperatures up to 120 °C (μ-CTE) or

250 °C (M-CTE250). The chamber's flow distribution system maintains a constant flow of air through each sample chamber, independent of sorbent-tube impedance and whether or not a sorbent tube is attached. The flow rate is controlled by the source air pressure and the flow distribution device in the unit. During tests, clean air flow will be supplied to the chambers. A total of 10 g of tire crumb rubber sample material will be placed in the chamber. Air samples will be collected on pre-cleaned polyurethane foam (PUF) tubes at a flow rate of 60 mL/min for 3 hours following a 24-hr equilibration period. The micro chamber tests will conform to ASTM D7706-11 (2010) and ISO- 16000-25 (2011).

4.9 Analysis of Volatile Organic Compound (VOC) Emissions from Tire Crumb Rubber

4.9.1 GC/MS Analysis of Target Analyte VOCs in Tire Crumb Rubber Emissions Samples

VOC chamber emission samples will be collected on reusable industry standard PerkinElmer-style ceramic coated stainless steel tubes containing Carbopack X sorbent and analyzed by thermal desorption/GC/MS. Carbopack X is a graphitized carbon sorbent suitable for the collection and quantitative analysis of hydrocarbons and chlorinated compounds containing 3 to 9 carbons. The PerkinElmer-style tube configuration allows the collection of samples by either a diffusion (passive) process or an active process whereby a measured volume of air is pumped through the tube. The tire crumb rubber chamber emission samples will be sampled using the active process as described above in Section 4.8. The sampling tubes are reusable and will be prepared for sample collection prior to each use by thermally conditioning for a minimum 1 hour at 350 °C while purging with research grade helium. This conditioning removes most contaminants from the sorbent tube remaining after analysis. It should be noted that some small residuals of benzene remain on the tubes that cannot be completely eliminated due to the nature of the sorbent; however, backgrounds can be reduced to minimal levels that can be corrected using background subtraction techniques.

The system used to analyze collected tube samples will be a PerkinElmer TurboMatrix ATD interface attached to an Agilent 6890 gas chromatograph and Agilent 5975 mass spectrometer. The analytical process is described as follows. Sample tubes for analysis are loaded into an automated carousel and the system is programmed to individually retrieve and thermally desorb each tube. Compounds desorbed during this process are then trapped and concentrated on a Peltier cooled sorbent trap containing sorbents of different retention strengths. The trap is then rapidly heated and backflushed with helium to transfer the compounds to the chromatographic column for separation and the mass spectrometer for detection and response. Quantitation is based on comparison of sample responses to a multipoint calibration curve. The thermal desorption, gas chromatography, and mass spectrometry conditions will be optimized for the target compounds of interest. Samples are proposed to be analyzed in SIM/Scan mode. The advantage of SIM/Scan mode is that in addition to the lower sensitivity possible with SIM monitoring, full scan data is also available for non-targeted compound identification. Appropriate QA/QC will be implemented to assure quality data.

4.9.2 Non-Targeted GC/MS Screening of VOCs in Tire Crumb Rubber Emissions Samples

As described above in Section 4.9.1, PerkinElmer-style tubes containing Carbopack X sorbent are proposed for sample collection. Selected samples will be analyzed on a Markes TOF (time-of-flight) GC/MS to provide detailed compound identifications and approximate quantitation of non-targeted compounds observed in the tire crumb rubber emission samples. Analytical instrumentation used for this analysis will include a Markes Ultra 2 thermal desorption auto sample/Unity 2 thermal desorber coupled to an Agilent 7890B GC and Markes BenchTOF-Select time-of-flight mass spectrometer.

The principle of sample introduction to this system is described as follows. Sample tubes for analysis are loaded into the Ultra 2 thermal desorption autosampler and the system is programmed to individually retrieve and thermally desorb each tube. Compounds desorbed during this process are trapped and concentrated on an internal sorbent trap containing sorbents of different retention strengths. The trap is then rapidly heated and backflushed with helium to transfer the compounds to the chromatographic column for separation and the TOF mass spectrometer for detection and response.

Standards for targeted compounds will be prepared and analyzed from Carbopack X tubes. A multipoint calibration curve will be developed. Standards will not be available for the non-targeted compounds so estimates of compound concentrations will be determined based on relative concentrations to known compounds. Compound identifications will be determined based on comparisons to the NIST spectral database. Appropriate QA/QC will be implemented to assure quality data.

4.9.3 Formaldehyde Analysis

Small chamber air samples collected on DNPH cartridges will be analyzed for formaldehyde by high performance liquid chromatography using an ultraviolet detector (HPLC-UV) following EPA Method TO-11A. A subset of samples will be analyzed by LC/MS for analyte identity confirmation.

4.10 Analysis of Semi-Volatile Organic Compound (SVOC) Emissions from Tire Crumb Rubber

4.10.1 GC/MS Analysis of Target Analyte SVOCs in Tire Crumb Rubber Emissions Samples

SVOC emissions from tire crumb rubber will be measured from components in air captured on polyurethane foam (PUF). The collection media will be solvent extracted by either Soxhlet or sonication using 1:1 acetone:hexane (EPA 3540C, 2007d; EPA 3550C, 2007c) and the extract volume will be reduced as necessary. Internal standards consisting of isotopically labelled analogs of representative chemicals will be added during sample preparation to enable quantification. Interferences will be monitored and samples will be cleaned-up as necessary using an appropriate procedure as prescribed in EPA 3500C (U.S. EPA 2007b). Extracts may also be split or solvent exchanged in order to analyze using other analytical conditions or instruments.

After extraction, samples will be analyzed by gas chromatography with mass spectrometry (GC/MS) using either an Agilent model 5973 single quadrupole instrument in SIM (selected ion monitoring) mode or an Agilent model 7010 triple quadrupole instrument in MRM (multiple reaction monitoring) mode. Instruments will be standardized using autotune parameters built into the instrument software. Component-specific calibration will be performed using a least squares regression model generated from the area and nominal concentration ratios from a series of calibration standards and their associated internal standards. Component data for each sample will be calculated using the corresponding regression equation. Measured concentrations that exceed the highest calibration level by more than 30% will require the sample to be diluted to a concentration within calibration limits.

4.10.2 Non-Targeted GC/MS Screening of SVOCs in Tire Crumb Rubber Emission Samples

Non-targeted screening by GC/MS will be performed using the extracts from the targeted analysis if possible, otherwise a similar approach will be taken for extraction with minimal processing. Data will be acquired using an Agilent 5973 mass spectrometer with electron impact (EI) fragmentation in scan mode (50-550 m/z). Chromatographic separation will be performed using an Agilent 6890 gas chromatograph with a 60m capillary column running a thermal gradient from 40ºC to 340ºC at 5ºC per minute. Those data will be deconvoluted and compared to a screening database and the NIST.11 spectral database for tentative identification using AMDIS or Agilent Unknowns Analysis software packages.

The screening database will be populated with chemicals where standards have been prepared and analyzed using the same instrument and conditions in order to have confirmed presence/absence. Components not identified within the screening database will be ranked by abundance and match score for further analysis.

4.10.3 LC/MS Analysis of Target Analyte SVOCs in Tire Crumb Rubber Emission Samples

The extracts described in section 4.10.1 will be solvent exchanged from acetone:hexane into a solvent amenable for LC/MS analysis (methanol, acetonitrile). Back-calculation of the portion of the total SVOC extract will be recorded for LC/MS analysis to estimate concentrations. A personal compound database list (PCDL) of all suspect analytes will be used to screen for presence of target analytes in SVOC extracts using an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS). The PCDL will consist of neutral monoisotopic mass, CAS# and molecular formula for suspect screening. Select compounds where standards are available and are amenable for LC/MS analysis will be quantitated. Compounds where standards are not available will be estimated in concentration based on relative response to the most similar analyte a standard is available for.

4.10.4 Non-Targeted LC/MS Screening of SVOCs in Tire Crumb Rubber Emission Samples

Suspect screening and nontargeted screening of tire crumb rubber will be performed by sonic extraction of the media of interest in an organic solvent amenable to HPLC-MS analysis. This will be with in acetonitrile or methanol depending on the performance on test samples. After extraction samples with be filtered for particle removal and reduced in volume for preparation for analysis. Samples with be injected onto an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS) for suspect screening and non-targeted screening analysis.

Samples with be processed according to the procedures of Rager et al. (2015). In brief after running of samples in both positive and negative mode samples with be subjected to a molecular feature extraction (MFE) algorithm to identify peaks for further exploration. Features identified for suspect screening purposes will be compared to the US EPAs DSS-TOX database (~33,000 chemicals). Chemicals matching within 5ppm of the suspect chemical according to accurate mass and scoring >90% will be deemed as a provisional match. Features not matching will be subjected to a nontargeted screening workflow where by features will be prioritized based on occurrence and abundance into discrete data packets. Further work on these peaks may include compound discovery, verification with authentic standards and comparison to outside databases (Chemspider, Scifinder) for provisional matching. Suspect and non-targeted screening features will be summarized with descriptive statistics.

4.11 Bioaccessibility Analysis of Metals and SVOCs in Tire Crumb Rubber

Reliable assessment of potential hazard to human from exposure to chemicals in tire crumb rubber materials depends on accurate information on a number of key parameters, including (1) chemicals and their concentrations in tire crumb rubber; (2) toxicity of the chemicals; (3) exposure pathways, including dermal contact, ingestion, and inhalation of vapors and particulates; (4) pathway-specific exposure dose of the tire crumb rubber materials or the chemical constituents in the materials; and (5) the extent of chemical absorption from the tire crumb rubber ("bioavailability") from each pathway. The amount of chemicals that actually enters the blood and body tissues from an exposure depends on the physical-chemical properties of the chemicals, as well as the composition of the body fluids. Therefore, knowledge of bioavailability is important in assessing potential health effect of exposure to chemicals in tire crumb rubber. In the case of tire crumb rubber, exposure potential will likely be influenced by additional factors, such as the age and wear of the materials, manufacture-related factors (e.g., manufactures and manufacturing processes), and environmental conditions (e.g., indoor or outdoor, ambient temperature and humidity). Therefore, this project will investigate tire crumb rubber samples taken from various manufacturers and up to 40 fields that are currently in-use.

For the purposes of this study design document, the term *bioavailability* refers to the fraction of dose that can cross biological barriers and become available for distribution to internal target tissues and organs. The term *bioaccessibility* refers to an *in vitro* measure of the physiological solubility of chemicals that may be available for absorption into the body. Bioaccessibility data can be used to estimate bioavailability using different established models. Since solubilization is usually required for absorption across membranes, poorly soluble forms of constituents with low bioaccessibility (such as metals) may also have low bioavailability. In certain circumstances (such as lead in soil), if solubility is the major determinant of absorption at the portal of entry, bioaccessibility may be a main predictor of bioavailability.

Metals and SVOCs selected for bioaccessibility testing will be based on existing data on chemical concentrations and on data from current and future laboratory analyses. For metals, it is likely that lead and zinc are required, although other metals may be identified and measured in the tests. For SVOCs, target analytes likely include polycyclic aromatic hydrocarbons (PAHs), phthalates, benzothiazole, etc. Bioaccessibility testing results will only be reported for the analytes that are present in the specific tire crumb rubber materials with concentrations above the limit of quantitation. We propose a tiered approach for project implementation and laboratory analyses:

- Phase 1: Optimization of the Methods. This phase will be devoted to method optimization and validation using a research tire crumb rubber material. Both constituent analysis methods and *in vitro* bioaccessibility methods will be finalized. At the end of Phase 1, the contractor will prepare and submit a Standard Operation Procedure of the final methods.
    - Phase 1a. Optimization of the Methods for constituent analysis and bioaccessibility testing of metals in tire crumb rubber
    - Phase 1b. Optimization of the Methods for constituent analysis and bioaccessibility testing of SVOCs in tire crumb rubber

- Phase 2: Determination of the *in vitro* Bioaccessibility. In this phase, the methods optimized in Phase 1 will be applied on up to 67 “unknown” tire crumb rubber samples (up to 27 manufacturer-provided samples and up to 40 composite field samples). At the end of Phase 2, the contractor will report the results (constituent analyses and *in vitro* bioaccessibility testing) on all “unknown” tire crumb rubber samples.
    - Phase 2a: Determination of the *in vitro* bioaccessibility of metals in tire crumb rubber samples
    - Phase 2b: Determination of the *in vitro* bioaccessibility of SVOCs in tire crumb rubber samples

- Phase 3: Assessment of the Bioavailability. In this phase, the bioaccessibility data obtained in Phase 2 will be used to estimate bioavailability using established models. At the end of Phase 3, the contractor will report the bioavailability assessment of chemicals in all “unknown” tire crumb rubber samples.
    - Phase 3a: Assessment of the bioavailability of metals in tire crumb rubber samples
    - Phase 3b: Assessment of the bioavailability of SVOCs in tire crumb rubber samples

The purpose of this work is to provide *in vitro* bioaccessibility testing of metals and SVOCs in tire crumb rubber materials to simulate ingestion and dermal absorption exposures. Artificial bio-fluids (e.g. gastric, saliva, and sweat) will be prepared using established recipes from reputable sources, such as ATSM, ISO, EPA, CDC/NIOSH, and CSPC. For the method to prepare artificial sweat, it is desirable to include an appropriate component, e.g., artificial sebum, to simulate the skin oil that can be present in humans sweat and affect the dermal absorption of target analytes. Methodologies for formulation of artificial saliva, artificial gastric fluid, and artificial sweat and sebum are provided in Appendix K; however, the specific composition can change after optimization of laboratory procedures.

The method for extraction of metals and SVOCs in tire crumb rubber by artificial biofluids can be adopted from established bioaccessibility method for lead in soil, e.g., EPA Method 1340, *In Vitro* Bioaccessibility Assay for Lead in Soil (U.S. EPA, 2013), and/or, EPA Method 9200.2-86, Standard Operating Procedure for an *In Vitro* Bioaccessibility Assay for Lead in Soil (U.S. EPA, 2007e). An example procedure is described as follows. All test tire crumb rubber samples should be dried (<40°C) and thoroughly mixed prior to use to ensure homogenization. After drying, 1 g of tire crumb rubber sample is rotated (30±2 rpm) with 100 mL of buffered extraction fluid (artificial gastric fluid, saliva, and sweat) at 37 °C for one hour. The supernatant is separated from the sample by filtration.

For metal analysis, the supernatant analyzed by an appropriate analytical method to measure multiple metals, e.g., EPA Method 6020B (EPA, 2014a) “Inductively coupled plasma—mass spectrometry” (This analytical method can measure 26 metals including zinc and lead).

To determine the concentration of SVOCs, the supernatant will first need to undergo sample preparation steps (e.g., solvent extraction, clean-up, and evaporation), before the analytical measurement. The sample preparation method can be referenced from EPA methods measuring SVOCs in soil using ultrasonic extraction (Method 3550C; U.S. EPA, 2007c), microwave extraction (Method

3546; U.S. EPA, 2007f), or pressurized fluid extraction (Method 3545A; U.S. EPA, 1998). The analytical method to quantify SVOCs in the extracts can be referenced from an appropriate analytical method, e.g., EPA Method 8270D, "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)" (U.S. EPA, 2014b).

4.12 Identifying and Collating Extant Toxicity Reference Data for Chemical Constituents

Selection of chemicals for toxicity reference information identification and compilation will be based primarily on measurement results produced in both the tire crumb rubber characterization and the exposure characterization studies. Selection criteria may include a combination of measurement detection frequency, magnitude of concentrations or estimated levels, and presence in multiple media. Consideration may also be given to chemicals that have been measured in other studies of tire crumb rubber and synthetic turf fields. We will develop a list of chemicals (including Chemical Abstract Services registry numbers) and compile extant toxicity reference data using the following sources of information shown below, and others, as deemed appropriate. Identification and compilation of other toxicity data from primary sources may also be considered for some chemicals. A summary of high-throughput toxicity screening data that are available on EPA's ToxCast database for the identified list of chemicals will be provided.

- EPA Integrated Risk Information System (IRIS)
- EPA Provisional Peer-reviewed Toxicity Value (PPRTV)
- EPA Health Effects Assessment Summary Table (HEAST)
- Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs)
- World Health Organization (WHO) International Programme on Chemical Safety (IPCS) Concise International Chemical Assessment Documents (CICAD)
- International Agency for Research on Cancer (IARC) Monographs
- California Environmental Protection Agency (CalEPA) Toxicity Criteria Database
- Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs)
- California Division of Occupational Safety and Health (Cal/OSHA) Permissible Exposure Limits (PELs).
- National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Limits (RELs).
- American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs).

EPA IRIS provides toxicity values for health effects resulting from chronic exposure to chemicals, including cancer and noncancer hazard characterization and oral reference doses (RfDs), inhalation reference concentrations (RfCs), oral slope factors (OSFs) and inhalation unit risk (IURs). An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime, while RfCs similarly represent an inhalation exposure. An OSF is an upper-bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime oral exposure to an agent. This estimate, usually expressed in terms of the proportion (of a population) affected per mg/kg-day, is generally reserved for use in the low-dose region of the dose-response relationship; that is, for exposures corresponding to risks less than 1 in 100, while IURs are similarly an estimate of the increased cancer risk from inhalation exposure. OSFs and IURs can be multiplied by estimated lifetime exposures to estimate the lifetime cancer risk. EPA PPRTVs have been developed for EPA's Superfund program and can also include provisional RfDs and RfCs for non-cancer effects, and provisional OSFs and IURs for cancer. HEAST also provides oral and inhalation toxicity values developed for EPA's Superfund program.

Like RfDs, ATSDR oral and inhalation MRLs represent estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable adverse non-cancer health effects over a specified duration of exposure. WHO CICAD provides summaries of potential health effects of chemicals on human health and the environment, and IARC Monographs provide summary information on chemicals that can increase the risk of human cancer. CalEPA provides no Significant Risk Levels (NSRLs) for carcinogens and Maximum Allowable Dose Levels (MADLs) for chemicals causing reproductive toxicity, and Reference Exposure Levels (RELs) which represent air concentrations at or below which no adverse health effects are anticipated to occur in human populations, including sensitive subgroups. While not directly applicable to all populations that may be exposed to tire crumb rubber, occupational limits developed by OSHA, CalOSHA, NIOSH, and ACGIH will also be reviewed for tire crumb rubber constituents. Typically, these values represent recommended levels of chemicals in workplace air that should not be exceeded over an 8- or 10-hour workday.

A database will be developed that cross-references chemicals in the tire crumb rubber constituent list with toxicity reference data from the sources described above. The database will be useful for informing future screening level health risk assessments, and for identifying toxicity information data gaps.

5. Exposure Characterization Methods

5.1 Exposure Characterization Overview

As described in the Federal Research Action Plan, this portion of the research is a pilot-scale effort aimed at providing information and data for characterizing exposures to chemical constituents for users of synthetic turf fields with tire crumb rubber infill. Participants will be recruited from among those thought to be in one or more higher-exposure scenarios based on the frequency and duration of synthetic turf field use, as well as specific activities that may be involved in higher levels of contact with synthetic turf field materials including tire crumb rubber. We anticipate that the facilities used by the participants will be a subset of the facilities participating in the tire crumb rubber characterization portion of the study (Section 4). There are two components to the exposure characterization research, a) activity information collection and b) exposure measurement. Methods for activity information collection and exposure measurements, including both facility and personal sample collection and analysis, are described in this section.

5.2 Activity Data Collection for Synthetic Field Users

5.2.1 Collection and Use of Extant Publicly-Available Video

Using publicly available videos (e.g., YouTube), videography will be used to collect activity pattern data on adults, adolescents, youth, and/or children playing and/or practicing on artificial turf fields that contain tire crumb rubber 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 tire crumb rubber infill that are difficult to capture consistently using questionnaires. The videography will record specific types of data about the participants and their activities, including: type of activity or sport; type of field (e.g., indoor or outdoor); participant's age group; durations of rest or low, moderate, and high activity; and mouthing and contact rates on turf for different types of activities. In total, up to 60 hours of videotaped footage will be collected on adults, adolescents, youth, and/or children actively engaged in sporting activities on artificial turf fields.

Selection criteria for the types of specific activities for video assessment will be informed by initial review of the types of extant videos available from the internet to determine which players (soccer goalies, football linemen etc.) and which activities (games, practices, training) are likely be in higher contact-rate scenarios based on activity patterns. These criteria have not been pre-defined as some flexibility is needed in gaining a better understanding of the types of public video information available.

Acceptance criteria for downloading video data for activity characterization include activity on a synthetic turf field, resolution (i.e., must be high enough to discern an athlete's contact with field), continuity (whether one athlete can be tracked for 30 continuous minutes of activity), and applicability for research goals (user and activity must correspond with the groups and activities targeted for high-end exposure characterization).

EPA staff and/or contractors will download public video recordings that meet the study's acceptance criteria from YouTube and similar publically available video-hosting Web sites onto a non-networked, password-protected computer at an EPA facility. The EPA research leader or designee will maintain custody of this computer and require staff and/or contractors to sign out the computer for the purpose of downloading or viewing extant video. Video downloading will only occur on-site at an EPA facility with information technology firewall protections in place.

The staff and/or contractors will assign each downloaded video with a video identification number (VID) and save each video onto a computer folder labeled "Tire Crumb Extant Video," exclusively using VIDs in lieu of filenames and descriptions. The EPA research leader will also copy the downloaded extant video files onto a portable USB drive or external hard drive to be kept in a locked cabinet. In order to minimize bias, staff and/or contractors who shall review, extract, and analyze the downloaded extant videos must not have participated in selecting or downloading any of those videos. Activity pattern data (e.g., hand-to-mouth and object-to-mouth, skin-to-surface contact rates, etc.) will be recorded using a paper template by the reviewer/analyst. Upon completing data tabulation from the videos, the EPA staff and/or contractors will enter the handwritten data into a spreadsheet or database in Microsoft Excel to be input into exposure modeling software such as SHEDS-Multimedia. The exact format of database entry is to be specified by the EPA-internal model developers. A subset of videos will be subjected to data extraction and entry by a second trained staff member or contractor to assess comparability and inter-reviewer consistency.

The videography will collect 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 data extraction will be performed for non-participants. No organization name, team name, or location information will be collected in the information extraction. 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 as part of the research effort and research reporting. The video recordings will be destroyed no later than five years following downloading.

5.2.2 Synthetic Turf Field Facility User Questionnaire

A questionnaire will be used to collect data from participants who routinely play on artificial turf fields that contain tire crumb rubber infill at athletic facilities (Appendix I). The purpose of the questionnaire is to collect information on the participants' activity patterns that may impact the magnitude, frequency, and duration of their exposures to chemicals found in tire crumb rubber infill. The questionnaire is intended to produce information that will improve exposure screening and modeling of inhalation, dermal, and ingestion pathways of exposures to constituents of synthetic turf fields with tire crumb rubber infill. The questionnaire will 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. The questionnaire will be administered by trained study staff members in person or over the phone using a computer assisted interview (CAI) and responses will be entered directly into the computer interview form. Two versions of the facility user questionnaires will be used, one for adults and adolescents and another for youth and child. For youth and child (Groups D and E), the parents/guardians will be asked to complete the questionnaire for his/her child. Skip patterns and several response range limits are built into the CAI software instrument. All questionnaires and responses will be reviewed by the field study leader prior to incorporation in the study database. No names or other personally identifiable data will be collected in the questionnaire. Only a study ID number will be used in the questionnaire for linking participants to other study data sets. The questionnaire data will be managed and secured to allow access and use only by trained study staff for the intended study purposes.

5.2.3 Videography of Exposure Measurement Study Participants

Videography will be used to collect activity pattern data on participants who routinely play on artificial turf fields that contain tire crumb rubber infill at athletic facilities. The purpose of the video data collection is to supplement the information obtained from the facility user questionnaire on the participants' activity patterns that may impact the magnitude and frequency of their exposures to chemicals found in tire crumb rubber infill. About 24 participants will have their activities videotaped for up to 1 hour while playing or practicing sports at the facilities.

Trained EPA staff or contractors will assign each participant with a participant identification number (PID). The EPA staff or contractor shall record the activities of the participants on artificial turf fields for 1 hour using a video camera that contains a video data card. The staff or contractor shall make two copies of all the participants' video data cards. The staff or contractor shall send the EPA research leader via certified mail the original and one copy of the video data cards of all study participants (with PIDs, only). Also, the staff or contractor shall keep the second copy of the video data cards in a secured location (e.g., locked cabinet in an office). After receiving the video data cards, the EPA research leader or designee will also keep the video data cards in a locked cabinet in his/her office. Trained EPA staff and/or contractors shall sign out the video data cards from the EPA research leader and view them using a non-networked computer that is password protected. Activity pattern data (e.g., hand-to-mouth and object-to-mouth, skin-to-field contact rates) will be recorded using a paper template. Upon completing data tabulation from the videos, the EPA staff and/or contractors will enter the handwritten data into a spreadsheet or database in Microsoft Excel to be input into exposure modeling software such as SHEDS-Multimedia. A subset of videos will be subjected to data extraction and entry by a second trained staff member or contractor to assess comparability and inter-reviewer consistency.

The videography will collect images that are 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. The videographer will 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 publically released. Video recordings will be destroyed no later than five years after collection.

5.3 Collection of Exposure Measurement Synthetic Turf Field Facility Samples

5.3.1 Air Samples for Particulate and Metals

PM_{10} is defined as airborne particulate matter with an aerodynamic size less than 10 µm. A total of three samples will be collected simultaneously at each synthetic turf field. Air samples will be collected from two points at each synthetic field as close as possible to where activities occur without posing an obstruction or safety hazard. A third sample will be collected upwind and at a sufficient distance from the field to represent background. In the case of indoor fields, the background sample will be collected outside of the facility building and in an upwind direction. Selection of locations for background samples will consider and attempt to avoid other potential exposure sources such as parking lots and roads. Air sample inlets will be located 1 meter above the field or ground surface.

Samples will be collected at a nominal flow rate of 20 L/min using metered, direct-current-supplied active samplers (SKC-HV-30 air pumps or equivalent) and size-selective impactor inlets (Harvard Impactor Inlets, Air Diagnostic and Engineering or equivalent), enabling PM_{10} mass loading on 47-mm Teflo filter media (Williams et al., 2008). Air sampling will be initiated for all monitors in quick order on their setup and calibration and continued without interruption through the monitoring event. It is anticipated that sample collection durations will be approximately three hours to represent an exposure period that includes time spent at the field prior to an athletic activity, the athletic activity period ranging up to two hours, and a short time spent at the field following the athletic activity. Sampler flow rates will be measured and recorded, along with the start and stop times at the beginning and completion of the sampling period. At the conclusion of the sampling event, filter samples will be recovered, stored in sealed transportation containers, and returned to the laboratory under ambient temperatures.

5.3.2 Air Samples for VOCs

Two types of VOC samples will be collected at the synthetic turf fields. Air samples will be collected from two points at each synthetic fields as close as possible to where activities occur without posing an obstruction or safety hazard. A third sample will be collected upwind and at a sufficient distance from the field to represent background. The primary sampling approach will employ passive samplers (Radiello™ passive diffusive bodies containing Carbopack X sorbent, SigmaAldrich) deployed at the same three locations at each field described in section 5.3.1. The Radiello™ samplers were selected due to their relatively high effective sampling rates which is anticipated to provide improved limits of detection for short duration sampling events. The on field use of the Radiello™ passive samplers will be used to provide comparability to the proposed personal sample collection approach (Section 5.4.1) and to reduce the amount of equipment and set-up time for sample collection. In addition, another sample for VOC analysis will be collected using an active pumping system and Carbopack X sampling tubes. One active sampling system will be deployed at each field and will be located at one of the two on-field locations. This active VOC sampler will be used to help calibrate effective sampling rates of analytes on the Radiello™ passive samplers.

It is anticipated that sample collection durations will be approximately three hours to represent an exposure period that includes time spent at the field prior to an athletic activity, the athletic activity period ranging up to two hours, and a short time spent at the field following the athletic activity. Active sampler flow rates will be measured and recorded at the start and completion of the sampling period. Passive samplers will be removed from their storage containers to start sampling, and will be returned to the storage containers immediately at the end of the sampling period. All sampling start and stop times will be recorded. Air sample inlets will be located 1 meter above the field or ground surface. At the conclusion of the sampling event, filter samples will be recovered, stored in sealed transportation containers, and returned to the laboratory with ice packs at 4 °C or lower. Following receipt at the laboratory, samples will be stored at -20 °C until analysis.

5.3.3 Air Samples for SVOCs

SVOCs comprise many potential chemical analytes with large ranges of vapor pressures and physical/chemical properties. Some SVOCs with higher vapor pressures will be found primarily in the vapor phase in air, while SVOCs with low vapor pressures will be found primarily on airborne particles. In this study, air samples will be collected without a size-selective particle inlet and will simultaneously collect both vapor- and particle-phase SVOCs.

Air samples will be collected from two points at each synthetic fields as close as possible to where activities occur without posing an obstruction or safety hazard. A third sample will be collected upwind and at a sufficient distance from the field to represent background. Samples will be collected on pre-cleaned open-cell polyurethane foam (PUF) filters in 30-mm × 70-mm tubes. Pre-cleaned total suspended particle quartz filters will be used as part of the sample filter assembly; however, the filter and the PUF will be extracted and analyzed together as a single sample. Separate particle- and gas-phase air concentrations will not be measured. A total of three samples will be collected simultaneously at each synthetic turf field and will be collocated with the particulate sample locations described in 5.3.1. Air sample inlets will be located 1 meter above the field or ground surface.

Samples will be collected at a nominal flow rate of 10 L/min using metered, direct-current-supplied active samplers (SKC-HV-30 air pumps or equivalent). Air sampling will be initiated for all monitors in quick order on their setup and calibration and continued without interruption through the monitoring event (day). It is anticipated that sample collection durations will be approximately three hours to represent an exposure period that includes time spent at the field prior to an athletic activity, the athletic activity period ranging up to two hours, and a short time spent at the field following the athletic activity. Sampler flow rates will be measured and recorded, along with the start and stop times at the beginning and completion of the sampling period. At the conclusion of the sampling event, filter samples will be recovered, stored in sealed transportation containers, and returned to the laboratory with ice packs at 4 °C or lower. Following receipt at the laboratory, samples will be stored at -20 °C until extraction.

[If preliminary laboratory preparation suggests that adequate detection limits will not be achieved using the low-volume sample collection method proposed for this study, a hi-volume method such as EPA

Method TO-13A (U.S. EPA, 1999) will be considered for collecting adequate amounts of target analytes for analysis.]

5.3.4 Field Surface Wipe Samples for Metals

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. Samples will be collected 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. Samples will be collected at positions #1, #2, and#3 as shown in Figure 3, for a total of three separate samples. No background sampling location wipe sample will be collected.

Samples will be collected following the ASTM E1728 method (ASTM 1728-16, 2016b), a standard wet-wipe method for collecting dust from indoor floor surfaces that used water as the wetting agent. Specifically, a 929 cm^2 (1-ft^2) template is placed on the surface of the field. Using clean, powderless plastic gloves, the field sampling technician remove the wet wipe from the foil packet. Using one side of the wipe, the turf surface is wiped in a U-shaped pattern within the template area. After folding the wipe in half to get a fresh wipe surface, the area is wiped again in a U-shaped pattern perpendicular to the first wipe pattern. The wipe is then folded in half again and the edges near the interior portion of the template were wiped. Prior to placing the wipe in a storage tube, plastic forceps are used to remove full size tire crumb rubber infill granules, synthetic grass blades, and other large debris or litter. Finally, the wipe is folded and placed in a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage. The tube is tightly capped and transported at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

5.3.5 Field Surface Wipe Samples for SVOCs

Two types of field surface wipe samples will be collected for SVOCs analysis. One method uses a dry wipe material attached to a heavy sledge. The second uses an isopropanol-wetted wipe.

Surface wipe aledge samples for SVOC analysis will be collected at synthetic turf field sites using a dry wipe material (Texwipe TX312 Cleanroom Twill, 30.5 cm × 30.5 cm, cotton) that is cleaned by pre-extraction using a series of solvents including acetone and hexane prior to use. Samples will be collected 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. Samples will be collected at positions #1, #2, and#3 as shown in Figure 3, for a total of three separate samples. Samples will be collected from different areas than the areas used for metals wipe sample collection. No background sampling location wipe sample will be collected. Using clean, powderless nitrile gloves, the field sampling technician remove the wipe material from its storage container and clamps it to a wipe sampling push sledge device. 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. The wipe material is secured so that the bottom face of the block with an area of 645 cm^2 is completely covered by the wipe material. Using a tape measure and marking tape, a 4 m x 1 m area (4 m^2) will be marked on the synthetic turf field. With the wipe sampler starting in one corner of the marked area, six wipe sampling passes will be made in one direction to cover the entire area one time. The sampler will then be moved to another corner, and six more wipe sampling passes will be made in one direction to cover the entire area. The second group of wipe sampling passes will be made in a direction perpendicular to the direction of the first. Prior to placing the wipe back into the storage container, synthetic grass blades, and other large debris or litter on the sides of the filter that did not contact the field will be removed to the extent possible. Finally, the wipe is folded and placed in the clean 500 mL amber glass wide mouth storage bottle with Teflon cap liner. The bottle is tightly capped and transported at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Wetted surface wipe samples for SVOC analysis will be collected at synthetic turf field sites using a wipe material (Texwipe TX312 Cleanroom Twill, 10 cm × 10 cm, cotton) that is cleaned by pre-extraction using a series of solvents including acetone and hexane prior to use. Samples will be collected 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. Samples will be collected at positions #1, #2, and#3 as shown in Figure 3, for a total of three separate samples. Samples will be collected from different areas than the areas used for metals wipe sample collection. No background sampling location wipe sample will be collected. Using clean, powderless nitrile gloves, the field sampling technician remove the wipe material from its storage container and adds 3 mL of 1:1 deionized water:isopropanol to the wipe, wetting the material evenly. Specifically, a 929 cm^2 (1-ft^2) template is placed on the surface of the field. Using one side of the wipe, the turf surface is wiped in a U-shaped pattern within the template area. After folding the wipe in half to get a fresh wipe surface, the area is wiped again in a U-shaped pattern perpendicular to the first wipe pattern. The wipe is then folded in half again and the edges near the interior portion of the template were wiped. Prior to placing the wipe in a storage tube, forceps are used to remove full size tire crumb rubber infill granules, synthetic grass blades, and other large debris or litter. Finally, the wipe is folded and placed in a pre-cleaned 60-mL amber wide-mouth glass jar. The bottle is tightly capped and transported at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

5.3.6 Field Dust Samples for Metals and SVOCs

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. At the time of research protocol preparation, no method has been selected for collection of dust samples at synthetic turf fields for metal and SVOC analysis. Method development will be performed for the dust collection method(s), conditional to gaining access to one or more synthetic turf fields for methods testing and evaluation.

5.3.7 Meteorological Information

Air temperature, field surface temperature, relative humidity, and wind speed and direction will be measured during the field measurement period. Information will also be obtained from each field's nearest NWS reporting site for sampling day temperature and wind conditions; precipitation information will be obtained for the sampling day and 6 preceding days.

5.4 Collection of Exposure Measurement Personal Samples

5.4.1 Personal Air Samples for VOCs

Personal sampling for VOCs will use a passive sampler (Radiello™ passive diffusive body containing Carbopack X sorbent, SigmaAldrich) attached to participants engaged in a sports activity on a synthetic turf field with tire crumb rubber infill. It is anticipated that sample collection durations will be approximately three hours to represent an exposure period that includes time spent at the field prior to an athletic activity, the athletic activity period ranging up to two hours, and a short time spent at the field following the athletic activity. Passive samplers will be removed from their storage containers to start sampling, and will be returned to the storage containers immediately at the end of the sampling period. The sampler will be clipped or pinned to the participant's shirt or jersey, ideally near the back neckline, to minimize interference or impacts during the sports activity. However, the field staff will discuss the placement with the participant and may chose an alternate location. All sampling start and stop times will be recorded. At the conclusion of the sampling event, filter samples will be recovered, stored in sealed transportation containers, and returned to the laboratory with ice packs at 4 °C or lower. Following receipt at the laboratory, samples will be stored at -20 °C until analysis.

5.4.2 Dermal Samples for Metals

Three dermal wipe samples will be collected for metal 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 defined areas of the forearm, and the third sample will be collected from 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.

Hand wipe samples for metal analysis will be collected using a wet (water) wipe (Environmental Express, Ghost Wipe No. 4210) conforming to American Society for Testing and Materials (ASTM) E1792 requirements (ASTM-03, 2016a). Using clean, powderless nitrile gloves, the field sampling technician removes the wet wipe from the foil packet and unfolds the wipe to its full dimensions. With moderately firm pressure, the left hand is wiped, including the back, front, and sides of the hand, fingers, and thumb. The wipe is folded with the exposed (contacted) surface on the inside and placed into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage. The tube is tightly capped and transported at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Forearm wipe samples for metals analysis will be collected using a wet (water) wipe (Environmental Express, Ghost Wipe No. 4210). Using a fresh pair of clean, powderless nitrile gloves, the field sampling technician removes the wet wipe from the foil packet and unfolds the wipe to its full dimensions. With moderately firm pressure, the top side of the left forearm is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded inside out along the 15 cm length, with the exposed (contacted) surface now on the inside. The wipe is now a 7.5 cm × 15 cm rectangle.
With the 7.5 cm × 15 cm rectangle, the bottom side of the left forearm is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded with the exposed (contacted) surface on the inside and placed into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup

No. SC475 or equivalent) for storage. The tube is tightly capped and transported at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Leg wipe samples for metals analysis will be collected using a wet (water) wipe (Environmental Express, Ghost Wipe No. 4210). Using a fresh pair of clean, powderless nitrile gloves, the field sampling technician removes the wet wipe from the foil packet and unfolds the wipe to its full dimensions. With moderately firm pressure, the outer facing side of the left calf or thigh is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded inside out along the 15 cm length, with the exposed (contacted) surface now on the inside. The wipe is now a 7.5 cm × 15 cm rectangle.
With the 7.5 cm × 15 cm rectangle, the inner facing side of the left calf or thigh is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded with the exposed (contacted) surface on the inside and placed into a pre-cleaned 50-mL polyethylene tube (Environmental Express, Disposable Digestion Cup No. SC475 or equivalent) for storage. The tube is tightly capped and transported at ambient temperature or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

5.4.3 Dermal Samples for SVOCs

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 defined areas of the forearm, and the third sample will be collected from 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.

Hand wipe samples for SVOC analysis will be collected using a wetted (1:1 water:isopropanol) cotton wipe material (M.G. Chemicals, Cleanroom Twill, 10.2 x 10.2 cm, cotton). Using clean, powderless nitrile gloves, the field sampling technician removes the wipe from its glass storage jar to its full dimensions. The wipe is evenly wetted with 3 mL of 1:1 deionized water:isopropanol using a transfer pipette. With moderately firm pressure, the left hand is wiped, including the back, front, and sides of the hand, fingers, and thumb. The wipe is folded with the exposed (contacted) surface on the inside and placed back into the glass storage jar. The jar is tightly capped and transported at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Forearm wipe samples for SVOC analysis will be collected using a wetted (1:1 water:isopropanol) cotton wipe material (M.G. Chemicals, Cleanroom Twill, 10.2 x 10.2 cm, cotton). Using clean, powderless nitrile gloves, the field sampling technician removes the wipe from its glass storage jar to its full dimensions. With moderately firm pressure, the top side of the right forearm is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded inside out along the 15 cm length, with the exposed (contacted) surface now on the inside. The wipe is now a 7.5 cm × 15 cm rectangle. With the 7.5 cm × 15 cm rectangle, the bottom side of the right forearm is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded with the exposed (contacted) surface on the inside and placed back into the glass storage jar. The jar is tightly capped and transported at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

Leg wipe samples for SVOC analysis will be collected using a wetted (1:1 water:isopropanol) cotton wipe material (M.G. Chemicals, Cleanroom Twill, 10.2 x 10.2 cm, cotton). Using clean, powderless nitrile gloves, the field sampling technician removes the wipe from its glass storage jar to its full dimensions. With moderately firm pressure, the top side of the outer facing side of the right calf or thigh is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded inside out along the 15 cm length, with the exposed (contacted) surface now on the inside. The wipe is now a 7.5 cm × 15 cm rectangle. With the 7.5 cm × 15 cm rectangle, the inner facing side of the right calf or thigh is wiped over a 48 cm^2 area using a rectangular template. The wipe is folded with the exposed (contacted) surface on the inside and placed back into the glass storage jar. The jar is tightly capped and transported at 4 °C or lower to the laboratory, where the samples are placed in a freezer at -20 °C.

5.5 Collection of Exposure Measurement Biomonitoring Samples

Urine and blood biological specimens will be collected from participants in the exposure characterization sub-study. In order to control for baseline body burden levels and allow for metabolic processes, blood and urine will be collected prior to the exposure measurement activities and post exposure measurement activities. Samples will be aliquoted at the time of collection and stored at appropriate temperatures for shipment and storage. Samples will be stored in a biorepository for the indefinite future. The purpose of indefinite storage is to determine what chemicals to look for and potentially to develop new analytical methods for analysis of these chemicals. Urine and blood sample collection, processing, shipment, and storage protocols are described in more detail in Appendix J.

5.6 Exposure Measurement Sample Analysis

5.6.1 Metals Analysis of Air, Dust, and Wipe Samples

The air, wipe, and dust samples will be extracted using EPA Method 3051A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils" (U.S. EPA, 2007a) adapted as necessary for the range of different sample media. It is anticipated that 1-g samples of dust will be used in the digestion method, but this amount may change based on experience with initial samples. For field wipe and dermal wipe samples the entire wipe will be used after removing any turf blades or pieces of the infill material that was not removed in the field. For air samples the entire filter will be used. This method is a rapid multi-element microwave assisted digestion method that does not intend to accomplish total decomposition of the sample. The method is applicable to the microwave-assisted acid extraction of 26 metals (aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdemum, nickel, potassium, selenium, silver, sodium, strontium, thallium, vanadium, zinc). The sample is placed in a fluorocarbon polymer or quartz vessel with concentrated nitric acid or a combination of concentrated nitric acid and concentrated hydrochloric acid. The vessel is sealed and heated in the microwave unit for a specified period of time. Once the extraction time is completed, the vessel is allowed to cool down and its contents are filtered, centrifuged, or allowed to settle. The extract is then diluted to volume and analyzed. The extracted samples will be analyzed by EPA Method 6020B "Inductively Couple Plasma-Mass Spectrometry" (U.S. EPA, 2014a). The method is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests. The performance acceptability for the ICP-MS method has been determined for the following analytes: aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, silver sodium, thallium, vanadium and zinc. The analyst will monitor potential sources of interferences and evaluate the performance of the method for the tire crumb rubber matrix.

5.6.2 GC/MS Analysis of Target Analyte SVOCs in Air, Dust, and Wipe Samples
SVOCs in air filter, field dust, field wipe, and dermal wipe sample media will be solvent extracted by either Soxhlet or sonication using 1:1 acetone:hexane (U.S. EPA 3540C, 2007d; U.S. EPA 3550C, 2007c) and the extract volume will be reduced as necessary. Internal standards consisting of isotopically labelled analogs of representative chemicals will be added during sample preparation to enable quantification. Interferences will be monitored and samples will be cleaned-up as necessary using an appropriate procedure as prescribed in U.S. EPA 3500C (2007b). Extracts may also be split or solvent exchanged in order to analyze using other analytical conditions or instruments.

After extraction, samples will be analyzed by gas chromatography with mass spectrometry (GC/MS) using either an Agilent model 5973 single quadrupole instrument in SIM (selected ion monitoring) mode or an Agilent model 7010 triple quadrupole instrument in MRM (multiple reaction monitoring) mode. Instruments will be standardized using autotune parameters built into the instrument software. Component-specific calibration will be performed using a least squares regression model generated from the area and nominal concentration ratios from a series of calibration standards and their associated internal standards. Component data for each sample will be calculated using the corresponding regression equation. Measured concentrations that exceed the highest calibration level by more than 30% will require the sample to be diluted to a concentration within calibration limits.

5.6.3 Non-Targeted GC/MS Screening of SVOCs in Air, Dust, and Wipe Samples
Non-targeted screening by GC/MS will be performed using the extracts from the targeted analysis if possible, otherwise a similar approach will be taken for extraction with minimal processing. Data will be acquired using an Agilent 5973 mass spectrometer with electron impact (EI) fragmentation in scan mode (50-550 m/z). Chromatographic separation will be performed using an Agilent 6890 gas chromatograph with a 60m capillary column running a thermal gradient from 40ºC to 340ºC at 5ºC per minute. Those data will be deconvoluted and compared to a screening database and the NIST.11 spectral database for tentative identification using AMDIS or Agilent Unknowns Analysis software packages.

The screening database will be populated with chemicals where standards have been prepared and analyzed using the same instrument and conditions in order to have confirmed presence/absence. Components not identified within the screening database will be ranked by abundance and match score for further analysis.

5.6.4 LC/MS Analysis of Target Analyte SVOCs in Air, Dust, and Wipe Samples
The extracts described in section 5.6.2 will be solvent exchanged from acetone:hexane into a solvent amenable for LC/MS analysis (methanol, acetonitrile). Back-calculation of the portion of the total SVOC extract will be recorded for LC/MS analysis to estimate concentrations. A personal compound database

list (PCDL) of all suspect analytes will be used to screen for presence of target analytes in SVOC extracts using an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS). The PCDL will consist of neutral monoisotopic mass, CAS# and molecular formula for suspect screening. Select compounds where standards are available and are amenable for LC/MS analysis will be quantitated. Compounds where standards are not available will be estimated in concentration based on relative response to the most similar analyte a standard is available for.

5.6.5 Non-Targeted LC/MS Screening of SVOCs in Air, Dust, and Wipe Samples

Suspect screening and nontargeted screening of tire crumb rubber will be performed by sonic extraction of the media of interest in an organic solvent amenable to HPLC-MS analysis. This will be with in acetonitrile or methanol depending on the performance on test samples. After extraction samples with be filtered for particle removal and reduced in volume for preparation for analysis. Samples with be injected onto an Agilent 6200 series Time of Flight Mass Spectrometer (TOFMS) for suspect screening and non-targeted screening analysis.

Samples with be processed according to the procedures of Rager et al., 2015. In brief, after running of samples in both positive and negative mode samples with be subjected to a molecular feature extraction (MFE) algorithm to identify peaks for further exploration. Features identified for suspect screening purposes will be compared to the US EPAs DSS-TOX database (~33,000 chemicals). Chemicals matching within 5ppm of the suspect chemical according to accurate mass and scoring >90% will be deemed as a provisional match. Features not matching will be subjected to a nontargeted screening workflow where by features will be prioritized based on occurrence and abundance into discrete data packets. Further work on these peaks may include compound discovery, verification with authentic standards and comparison to outside databases (Chemspider, Scifinder) for provisional matching. Suspect and non-targeted screening features will be summarized with descriptive statistics.

5.6.5 Biomonitoring Samples

The biological specimens, blood and urine, will be archived with the intent to analyze at a later date as more information on tire crumb rubber chemical composition and bioavailability of chemicals in the tire crumb rubber become available. However, potential analyses include urine metals, urine PAHs, urine VOCs, urine creatinine, blood metals, and serum metals. Blood samples will be collected by a trained phlebotomist from participants; the sample collection protocols indicate a blood draw of 6mL for serum metals and 5mL for blood metals (total of 11mL). The maximum blood draw per participants will not exceed 25mL.

6. Data Analysis

Measurement, questionnaire, videography data sets will be compiled into one or more analytical data sets for analysis. Data analyses will be performed to address the research objectives described in Section 2. Table 11 summarizes the data analysis plan.

Summary statistics will be prepared for each target analyte, questionnaire response, and videography metric. Categorical variables will be summarized by frequencies and response ranges, while continuous variables will be summarized by mean, standard deviation, median, and range. Tire crumb rubber characterization and exposure characterization study measured analyte concentrations will be characterized by mean and standard deviation (or geometric mean and geometric standard deviation), median, range, appropriate distribution percentile values, and percent of measurements above the detection limit.

Data quality (e.g., percent completion or detect, accuracy, precision) will be assessed to help make decisions on the statistical analyses that can be conducted with the available data. Several different types of data analyses will be applied to the different tire crumb rubber characterization and exposure characterization study data sets. These include between-group statistical tests of differences of measurement and questionnaire data where sample sizes are sufficient. For selected analytes with high detection rates, logistic modeling will be explored to assess the potential predictive power of selected facility or user characteristic variables for explaining differences in measured values. Because not all chemical analytes will be quantitatively measured, between-group statistical testing based on presence or absence will be performed for selected chemicals.

For detection limit censored data distributions, appropriate approaches for reducing bias in distributional parameter estimates will be considered (substitution, maximum-likelihood estimation, or beta-substitution, depending on the degree of censoring and sample sizes). Measurement distributions will be assessed for linearity using the Shapiro-Wilks or other appropriate normality test. Depending on the distribution, measurement values may be log-transformed to compute geometric means and geometric standard deviations. Other types of transformations and/or non-parametric analysis methods will be considered, if necessary.

Much of the data being collected under this research protocol is intended to be used in exposure screening and exposure modeling efforts. However, there will not be sufficient time to complete these analyses in time for inclusion in a 2016 report. Data and information extracted from the literature review, as well as questionnaire, videography, and measurement data will be organized for exposure modeling using Version 4.1 of the Stochastic Exposure and Dose Simulation (SHEDS) multi-media model (SHEDS, 2016). An example of an application of the SHEDS modeling approach is an assessment of children's exposure to chemicals in CCA-treated playsets and decks (Zartarian et al., 2006).