Quality Assurance Project Plan for Project 2

EPA Clean Air Research Center
Southeastern Center for Air Pollution and Epidemiology
Emory University and Georgia Institute of Technology

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Quality Assurance Project Plan for Project 1

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Quality Assurance Project Plan for
Project 2

EPA Clean Air Research Center

Southeastern Center for Air Pollution and
Epidemiology

Emory University and Georgia Institute of
Technology
1. **Background/Purpose**

The primary aim of the Southeastern Center for Air Pollution and Epidemiology (SCAPE) – Project 2, Examining In-Vehicle Pollution and Oxidative Stress in a Cohort of Daily Commuters, is to examine the effects of exposure to particulate mixtures occurring during automobile commuting and with indoor, non-commuting microenvironments (µE’s) and corresponding measures of oxidative stress-mediated response. This project integrates exposure science, environmental biochemistry, and air pollution epidemiology to trace the link between specific mixtures of particulate pollution, subclinical oxidative and inflammatory responses, and adverse clinical outcomes. The central hypotheses of Project 2 are that a) commuters are exposed to high levels of in-vehicle particulate pollutant mixtures as compared to other, indoor µE’s; and that b) these short-term exposures are associated with acute changes in oxidative stress in asthmatic and healthy adults. Results from this study will elucidate whether daily automobile commuters should be viewed as a sub-population that is vulnerable to traffic pollution-associated health effects. Further, this study will be among the first field exposure assessments to incorporate methods for measuring highly chemically-resolved particulate mixtures over short durations, focusing on specific components that may be linked to reactive oxidant species (ROS) formation including polycyclic aromatic hydrocarbons (PAHs), transition metal species, elemental carbon (EC) and ultrafine particles (UFP). Recent advancement in our ability to non-invasively measure several biomarkers of oxidative stress, lipid peroxidation and inflammation, will provide opportunities for a detailed mechanistic examination of the link between exposure and clinical response. In examining targeted biomarker levels at several time intervals following exposure, we also expect to elucidate the nature of the exposure-response function and whether these relationships differ according to baseline health status.

**Specific Project Objectives**

**Objective 1.** Characterize in-vehicle exposure to pollutant mixtures for a panel of 30 healthy and 30 asthmatic adults while commuting during peak morning traffic periods, as well as indoor exposure during non-commuting periods using ROS measurements, receptor modeling and regression based approaches.

**Objective 2.** Identify factors influencing in-vehicle pollutant concentrations, including on-road fleet compositions, traffic volumes and speeds, cabin ventilation, and ambient pollutant concentrations.

**Objective 3.** Study associations between the pollutant mixtures measured in both the commuting and non-commuting µE’s and biomarkers of acute oxidative stress, lipid peroxidation, pulmonary and systemic inflammation, and clinical response.

**Objective 4.** Compare exposure-response associations between a cohort of adults with asthma (N=30) and healthy adults (N=30).
Methods for Data Acquisition to address Project Objectives

To specifically address the project objectives, in-vehicle and indoor pollutant mixtures and acute health responses will be measured for a panel of 60 adults (30 adult asthmatics and 30 healthy adults) in the Atlanta metropolitan area. Sampling will take place over two days. Subjects will participate in a 2-hour scripted morning commute (i.e., a high traffic-pollutant exposure) and undergo repeated post-exposure health measurements at our clinical facility and at their residences on one of the days. On the other study day, subjects will spend the 2-hour morning period indoors at our clinical research facilities, rather than commuting (i.e., a low traffic-pollutant exposure) and undergo the same post-exposure health measurements. Trained study personal will conduct all health measurements. A range of size- and chemically-resolved particulate components will also be measured during the 2-hour scripted commutes and clinic visits. In addition, all of the continuous pollutant parameters will be measured throughout the entire two-day sampling period. Both health and pollutant data generated during the sampling period will be saved on the SCAPE password-protected network drive.

2. Design

In-vehicle and indoor pollutant mixtures and acute health responses will be measured for the entire panel of 60 subjects. Subjects will participate in the study over a two-day sampling period (Figure 1).

On one of the days, subjects will use their private vehicles to conduct a 2-hour scripted morning commute (i.e., a high traffic-pollutant exposure) and undergo repeated post-exposure health measurements at our clinical facility and at their residences. On the other study day, subjects will spend the 2-hour morning period indoors at our clinical research facilities, rather than commuting (i.e., a low traffic-pollutant exposure) and undergo the same post-exposure health measurements. The main difference in subject activity-patterns between the two study days will consist of whether a commute or...
Clinic exposure is scheduled. Scripted commutes may be scheduled on either the first or second study days of the study and will be chosen randomly.

The commutes will be conducted during weekday morning, peak rush hour periods and will last approximately two hours. This duration, while longer than a typical commute for most Atlantans, will allow us to collect sufficient PM mass to accurately analyze trace PM compounds. Commuting routes will be similar for all subjects, but may be adjusted based on the subjects’ residential location as well as to include areas of heavy traffic congestion. Data from the ACE and Commute Atlanta studies (see Previous Studies) will be used to identify the most congested commuting routes. Each route will begin at the subjects’ residence and finish at our clinical research facilities at Emory University.

Recruitment of study subjects. We will recruit participants for this study from several sources including an existing cohort of asthmatic research subjects in the Division of Pulmonary, Allergy and Critical Care at the Emory University School of Medicine, from a pool of Emory University students and employees living near our research clinic, and from several hundred individuals who will are participating in a transportation engineering study (the Commute Atlanta II Study) that will is being conducted by Dr. Randall Guensler of Georgia Tech.

Inclusion/Exclusion criteria. We will limit recruitment to 30 healthy adults and to 30 adults with well-controlled asthma that is stable for at least two weeks. To qualify for enrollment, the asthmatic subjects must have mild intermittent asthma defined according to criteria established in the National Asthma Education and Prevention Program’s Expert Panel Report (J Allergy Clin Immunol 2007; 120: S94-138). By definition, mild intermittent asthma is defined by symptoms less than two days per week, no interference with daily activities, normal pulmonary function, and no more than one exacerbation within the previous year. To be included, subjects must further be under the care of a physician and have no increase in symptoms (or in short-acting bronchodilator use) and no “colds” or upper/lower respiratory tract infections within two weeks prior to enrollment. Examples of asthma symptoms would include coughing, wheezing, difficulty breathing, and nocturnal asthma-related awakenings.

Exclusion criteria would include the following: 1) a hospitalization for asthma within the previous two years, 2) baseline lung function less than 80% of predicted norms, 3) a requirement for daily asthma controller medications, including inhaled or oral corticosteroids and leukotriene receptor antagonists. A subject who takes short-acting beta-agonists (e.g., albuterol sulfate) on a daily basis would be excluded because that subject would not meet criteria for mild intermittent asthma. Additionally, for most adults, baseline GSH is typically reduced with little oxidation until about age 45, when oxidation occurs at a fairly steep curve. We will, thus, recruit participants < 45 yrs old for both the healthy and asthmatic cohort. Prospective participants will also be excluded if they have been diagnosed with diabetes; have ever suffered a myocardial infarction; have been outfitted with an implantable cardioverter-defibrillator or pacemaker; are currently taking digoxin or beta blockers for the treatment of hypertension or cardiac arrhythmias; have been diagnosed with a non-asthma pulmonary disease such as COPD, emphysema, or
any type of lung cancer; smoke tobacco products or are residentially exposed to second-hand tobacco smoke.

**Criteria for Success**

For this research protocol to be successful, we intend to:

1. Recruit 60 (30 adult asthmatics and 30 healthy adult) subject to participate in this protocol;
2. Measure in-vehicle concentrations of key particulate components and pollutant mixtures associated with oxidative stress during peak morning traffic periods, as well as indoor exposures during non-commuting periods;
3. Measure acute biological responses in our subjects.
4. Measure on-road fleet compositions, traffic volumes and speeds, cabin ventilation, and ambient pollutant concentrations.
5. Generate and a comprehensive analytic database linking the measured health and pollutant measurements for each subject.
6. Conduct exposure and epidemiological analyses using appropriate statistical modeling approaches.
7. Disseminate results to the scientific community through presentations at scientific conferences and publication in peer-reviewed journals.

3. Data Gathering Methods

**Overview of pollutant sampling.** A range of size- and chemically-resolved particulate components will be measured during the 2-hour scripted commutes and clinic visits (Table 1). In addition, all of the continuous pollutant parameters will be measured throughout the entire two-day sampling period. A detailed Quality Assurance/Quality Control plan that is consistent with those prepared for our previous field studies will be developed for this study. The following section describes our pollutant sampling and analytical approach. All technician field manuals for using the commercially-available instrumentation and SOPs for all other instruments and the proposed analyses are included as appendices.

**Time-Integrated measurements.** In-vehicle coarse and fine mode PM mass will be measured gravimetrically using a 2-stage Harvard Compact Cascade Impactor (CCI) with an aerodynamic cutpoint between stages of 2.5 µm and operated at a flow rate of 30 L/min (Appendices A-B). Trace metal analysis will be performed on these filters using inductively-coupled plasma-mass spectrometry. A 47 mm quartz filter equipped with an upstream inertial impactor to remove particles larger than 2.5 µm will be operated at a flow rate of 30 L/min and analyzed using thermal-desorption gas chromatography/mass spectrometry to perform speciation of organic compounds (Appendix C). Two separate 25 mm quartz filters with upstream inertial impactors (2.5 µm cutpoints) will be operated at 10
L/min. One quartz filter will be divided in half with one half analyzed for elemental and organic carbon content using thermal-optical transmittance (Appendix D). The aqueous extract from the other half filters will be analyzed for water-soluble organic carbon (Appendix E) and ion content (Appendix F), respectively. The final half filter will be used for the analysis of particulate ROS using the fluorescent detection of oxidized dichlorofluorescin.

**Continuous measurements.** The continuous measurement of in-vehicle particulate ROS will be performed using a particle-into-liquid sampler (PILS) coupled to a fluorescence detector (Refer to the QAPP for Project 1 for a discussion of PILS standard protocol). The PM$_{2.5}$ mass concentration will be assessed continuously using a 6-channel optical particle counter calibrated against Federal reference Method measurements at Georgia Tech (Instrument Operation Manuals for all of the commercially available samplers included as Appendix G). The particle number concentration will be measured in real-time using a portable condensation particle counter (Instrument Operation Manual available upon request). An aethalometer will be used to assess the concentration of light-absorbing aerosols by continuously measuring light transmittance through an internal filter, and the concentration of particle-bound polycyclic aromatic hydrocarbons will be measured using a real-time photoionization instrument. For the proposed study, noise will be measured primarily as a means of examining its potential.

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**Table 1. Overview of pollutant measurements.**

<table>
<thead>
<tr>
<th>Pollutant Measurements</th>
<th>Temporal resolution</th>
<th>Instrument</th>
<th>Analytical Method</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse and fine mass</td>
<td>2 hr.</td>
<td>CCI</td>
<td>Gravimetric</td>
<td>Ga. Tech.</td>
</tr>
<tr>
<td>Trace elements</td>
<td>2 hr.</td>
<td>CCI</td>
<td>ICP-MS</td>
<td>U. Wis.</td>
</tr>
<tr>
<td>Organic speciation</td>
<td>2 hr.</td>
<td>47 mm quartz filter</td>
<td>TD-GC/MS</td>
<td>U. Wis.</td>
</tr>
<tr>
<td>EC/OC$^{d}$</td>
<td>2 hr.</td>
<td>25 mm quartz filter</td>
<td>TOT$^{e}$</td>
<td>Ga. Tech.</td>
</tr>
<tr>
<td>WSOC$^{f}$</td>
<td>2 hr.</td>
<td>25 mm quartz filter</td>
<td>TOC$^{g}$</td>
<td>Ga. Tech.</td>
</tr>
<tr>
<td>Ionic species</td>
<td>2 hr.</td>
<td>25 mm quartz filter</td>
<td>Ion Chromatography</td>
<td>Emory</td>
</tr>
<tr>
<td>ROS (continuous)</td>
<td>2 hr.</td>
<td>25 mm quartz filter</td>
<td>Fluorescence</td>
<td>Ga. Tech.</td>
</tr>
<tr>
<td>ROS (integrated)</td>
<td>5 min.</td>
<td>PILS-ROS$^{i}$</td>
<td>Fluorescence</td>
<td>-</td>
</tr>
<tr>
<td>PM$_{2.5}$ mass</td>
<td>1 min.</td>
<td>TSI AeroTrak</td>
<td>OPC$^{h}$</td>
<td>-</td>
</tr>
<tr>
<td>Light-absorbing aerosols</td>
<td>1 min.</td>
<td>Magee MicroAeth</td>
<td>Filter transmittance</td>
<td>-</td>
</tr>
<tr>
<td>Number Concentration</td>
<td>1 min.</td>
<td>TSI P-Trak</td>
<td>CPC$^{l}$</td>
<td>-</td>
</tr>
<tr>
<td>Particle-bound PAHs$^{j}$</td>
<td>10 sec.</td>
<td>EcoChem PAS 2000CE</td>
<td>Photoionization</td>
<td>-</td>
</tr>
<tr>
<td>Noise</td>
<td>1 sec.</td>
<td>Extech HD600</td>
<td>Noise dosimeter</td>
<td>-</td>
</tr>
</tbody>
</table>

$^{a}$ Harvard Compact Cascade Impactor; $^{b}$ Inductively Coupled Plasma-Mass Spectrometry; $^{c}$ Thermal-Desorption Gas Chromatography/Mass Spectrometry; $^{d}$ Elemental Carbon/Organic Carbon; $^{e}$ Thermal-Optical Transmittance; $^{f}$ Water-Soluble Organic Carbon; $^{g}$ Total Organic Carbon Analyzer; $^{h}$ Reactive Oxygen Species; $^{i}$ Polycyclic Aromatic Hydrocarbons; $^{j}$ Particle-Into-Liquid Sampler; $^{k}$ Optical Particle Counter; $^{l}$ Condensation Particle Counter.
confounding effect on the pollutant-outcome associations. Towards this end, in-vehicle decibel levels will be measured using a real-time noise dosimeter.

**Other measurements.** In addition to the in-vehicle and indoor microenvironmental pollutant measurements, roadside and ambient measurements will also be collected to examine the association between in-vehicle concentrations and roadside and ambient monitoring data. More details related to the inter-comparison of the sites and specific analyses are discussed in the Air Quality Core as well as in Project 1.

Traffic volumes will be available with 15-minute resolution for the major freeway corridors in the Atlanta region. In addition, a battery-operated video camera will be installed in the back seat of each vehicle to provide an alternative method for assessing traffic volume, congestion and composition in the immediate vicinity. This camera will also be used to note exceptional conditions occurring during sampling such as following a particularly dirty vehicle.

Numerous studies have shown that ventilation can influence in-vehicle pollutant concentrations. Qualitative indicators of ventilation conditions will be defined for these test commutes in order to examine the influence of ventilation on in-vehicle pollutant concentration levels. High ventilation conditions will be defined as having the front windows of the vehicles open approximately 30 cm; mid-range ventilation conditions will be defined as having all windows closed but opening the external vents and operating the interior fan, and low ventilation conditions will be defined as having all windows and external vents closed. Subjects will be asked to operate their vehicle under each class of ventilation conditions as weather permits and to record the transition times.

**The development of a portable, multipollutant sampler.** As part of the ongoing ACE study, we designed and field-tested a portable, multipollutant sampling system that contains both commercially-available and novel sampling components (Figure 2). The sampler is approximately 15” (l) x 21” (w) x 8” (h) and will be secured in the front seat of the vehicle with inlets placed near the driver’s breathing zone. A separate equipment box containing a battery and pumps required for gravimetric PM mass collection will be installed in the vehicle’s trunk. This portable sampler will be transported, as necessary, to characterize pollutant concentrations occurring in the

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Figure 2. Diagram of the portable sampling manifold and equipment.
vehicles, in the clinic, as well as in all other indoor microenvironments (i.e., subject workplace, residence) throughout the two-day sampling period.

**Overview of health measurements.** We hypothesize that exposures to particulate mixtures occurring during realistic commutes will lead to acute oxidative stress and the resulting downstream effects of pulmonary and systemic inflammation and asymptomatic clinical outcomes. To examine the progression of this biological response in our healthy and asthmatic subjects, we have selected several biomarkers of acute response along our *a priori* hypothesized pathway of response. SOPs and technician guidance documents for each of the health measurements are included as Appendix H. These biomarkers, thus, serve as indicators of specific oxidative stress-mediated processes. To follow the progression of oxidative stress related responses, we will use the non-invasive collection of exhaled breath condensate (EBC) to obtain samples at multiple time-points and use established methods for the detection of pulmonary oxidative stress and lipid peroxidation products. (All SOPs for the EBC biomarker characterization are available upon request from the Brown lab).

Given that antioxidant thiol groups readily donate electrons on contact with oxidant species, we will use a novel method of characterizing GSH/GSSG ratios in exhaled breath to observe changes in glutathione redox chemistry very quickly following exposure to oxidizing air pollution mixtures. EBC samples will be divided into aliquots immediately following collection. A 300 µL aliquot will be preserved for GSH/GSSG analysis using a preservation solution as described by Yeh et al. (2008). In addition, separate 100 µL aliquots will be prepared for the analysis of 8-isoprostane, MDA, ionic content, and pH. 8-isoprostane will be measured using commercially-available ELISA kits. GSH/GSSG and MDA will be measured using high performance liquid chromatography, and ion content will be measured using ion chromatography. EBC pH will be measured before and after deaeration with Argon to reduce dissolved CO2. Any remaining EBC will be stored at -70°C for potential future analysis. The redox potential (Eh) of the GSH/GSSG and Cys/CySS thiol pairs will be calculated with the Nernst equation, 

\[
E_h = E_0 + \frac{RT}{nF} \ln \left( \frac{[\text{disulfide}]}{[\text{thiol1}][\text{thiol2}]} \right),
\]

where \(E_0\) is the standard potential for the redox couple, \(R\) is the gas constant, \(T\) is the absolute temperature, \(n\) is 2 for the number of electrons transferred, and \(F\) is Faraday's constant and an 8-isoprostane acetylcholinesterase (AChE) conjugate (8-isoprostane tracer) for a limited number of 8-isoprostane.

In addition, we will use the minimally-invasive measurement of CRP in blood obtained from finger prick samples (Appendix I). These measurements will be conducted once per day during the pre-exposure home visit and five times per day post-exposure at our clinical facilities and at the subject’s residence. Biomarkers requiring venipuncture, such as plasma IL-6, will be measured once pre-exposure and ten hours post-exposure per day. For each procedure, 10 mL of blood will be drawn by a trained phlebotomist from an antecubital vein without venous stasis. Whole blood will undergo a complete differential cell count, including erythrocytes, platelets, lymphocytes, and polymorphonuclear leukocytes. Blood will be centrifuged to separate plasma, which will then be divided into aliquots for analysis of specific biomarkers. The plasma concentration of CRP, IL-1β, IL-4, IL-6, IL-9, IL-13, vWF,
fibrinogen and ET-1 will be assessed using commercially-available enzyme-linked immunosorbent assays (ELISA). We chose to conduct the post-exposure venipuncture approximately 10 hrs after exposure to reflect our experience investigating biochemical temporal response in oxidative-stress compounds.

To assess potential confounding of elevated psychosocial stress associated with driving, we will also measure pre- and post-exposure salivary cortisol concentrations. The salivary concentration of cortisol is an established biomarker of psychobiological stress and therefore, each of the saliva samples collected in association with EBC will be analyzed for cortisol content using a commercially-available ELISA kit.

Finally, subjects with chronic pulmonary inflammation, such as those with poorly-controlled asthma, may experience an accelerated progression of the acute response to oxidant insult. Towards this end, repeated pre- and post-commute lung function measures will also be conducted. Approximately 60 minutes before each 2-hour exposure (for both the scripted commute and the clinic visit), a trained field technician and phlebotomist will meet subjects at their residences to collect samples. Trained field staff will collect post-exposure samples as well (See Appendix H for protocol).

**Targeted biomarker measurements**

a. **Glutathione redox ratio.** GSH/GSSG will be measured in preserved EBC samples using a high performance liquid chromatography (HPLC) technique described by Yeh et al. (2008). (SOP available upon request from Brown lab).

b. **Lipid peroxidation.** The EBC concentration of malondialdehyde will be measured using an HPLC technique to assess the progression of airway lipid peroxidation reactions. (SOP available upon request from Brown lab).

c. **Cytokine upregulation.** The systemic concentration of the inflammatory cytokine IL-6 will be measured in plasma (venipuncture) using a commercially-available ELISA multiplex technique.

d. **Acute inflammation.** The concentration on the acute-phase protein CRP will be measured in blood (finger prick) using an immunoturbidometric method (QuikRead CRP).

e. **Exhaled nitric oxide.** The concentration of NO in exhaled breath will be measured using a portable analyzer (Aerocrine, NY) at multiple times on sampling days.

f. **Bronchial hyperreactivity.** BHR will be assessed by performing pulmonary function tests before and after receiving two inhalations of a bronchodilating agent.

g. **Subject stress levels.** Stress levels will be assessed using self-reported stress levels during the commute and by examining the salivary concentration of cortisol using ELISA.

h. **Symptom and time activity diary.** Subjects will be asked to complete a diary card to record the incidence and severity of asthma and other respiratory-related symptoms.
4. Data Quality

Pollutant Measurements. Data quality criteria will be determined for all in-vehicle, ambient and microenvironmental monitoring data. The proposed monitoring will consist primarily of operating and maintaining several continuous and integrated PM monitors. The criteria of the new air quality samples will include assessment of: a) accuracy; b) bias; c) precision; d) detection limits; e) completeness; and f) comparability.

a) Completeness for a parameter measured in the study is defined as the percent of valid samples from all samples scheduled using the following formula:

\[
\% \ \text{Completeness} = \left( \frac{\text{Number of valid samples}}{\text{Number of scheduled samples}} \right) \times 100\%
\]

The completeness objective of the panel studies is to achieve 90% data capture for each parameter. If data capture appears to fall below 90%, field staff and Co-Principal Investigators will work to address the data losses during field operations.

b) Precision is a measurement of correspondence between samples of the same type under the same field conditions. Precision for the relevant methods will be estimated using co-located samplers. Two samplers will be collocated at a stationary monitoring site in each location.

Percent precision of these collocated measurements will be calculated using the following technique:

1. For all collocated pairs, \( x_i = \) normal sample, \( x_i > \text{LOD} \)
   \( y_i = \) collocated sample, \( y_i > \text{LOD} \)
   \( m_i = \) the mean of sample pair \( x_i \) and \( y_i \)
   \( d_i = x_i - m_i \) (the difference between the \( i^{th} \) normal sample from the mean of the \( i^{th} \) sample pair)

2. Using the above and the sample size \( N \), calculate the Root Mean Square Difference:

\[
\text{RMSD} = \sqrt{ \frac{\sum d_i^2}{N} }
\]

3. Calculate the Overall Mean: \( M = \frac{3 m_i}{N} \)

4. Finally, calculate the \% Precision Estimate as: \( \frac{(\text{RMSD} \times 100)}{M} \)

\[
\sqrt{2}
\]

c) Accuracy is a measure of the closeness of an individual measurement or the average of a group of measurements to the true value. Accuracy for flow rates of integrated samplers is determined by flow audits of each sampling system performed in the field. Assessment of flow rates by field staff will be
evaluated by the QA manager to assess proper reading of instruments. In the field, flow measurements are taken using portable flow-calibrators. Flow rates at the start of each sampling day are adjusted to remain within 10% of normative flow rates. The flow-calibrators are calibrated by the manufacturer annually and are NIST traceable. Accuracy estimates will be based on comparison to independent or reference sampling methods, when available. Accuracy for data collected will be calculated using the following formula:

\[
\text{% Accuracy} = \left( \frac{\text{Measured value}}{\text{Known reference value}} \right) \times 100
\]

d) The field LOD or Limit of Detection for the filter based measurements will be estimated using the data from the collected blanks. It is calculated as three times the RMSE of the field blanks for each species. LOD values for the continuous instruments are typically specified according to manufacturing standards included in the operation manuals.

Field blank samples will be done at a rate of 10% for the outdoor and personal measurements.

Field Quality Control Checks

An essential quality control check for all active integrated measurements is to verify that the flows are within ±10% of the target flow rate. The HIs should be within 10% of 10 LPM.

Field activities to assess data quality for the new measurements will include scheduled sampler collocations, flow checks, routine instrument calibrations and maintenance. For all laboratory analyses, a central aspect of the acceptability criteria for the data will be an accurate reporting of the uncertainty of all reported measurements. The uncertainties of these measurements will be based on field blanks, analytical and procedural blanks, calibration standards, spiked samples and standard reference materials. Air quality data will also be obtained from several existing networks throughout the southeast including those being operated as part of the AQS/STN, ARIES, SEARCH and ASACA networks. The air quality data from these networks are secondary data sources, with their own set of compliance guidelines, information requirements, information handling procedures, and standard methods of collecting, validating, and storing air pollution data. A final determination of quality for the pollutant monitoring data will involve thorough review of data and information generated in the laboratory and in the field. For all air quality measures, the raw data will be examined using descriptive statistics. For each pollutant parameter, especially those measured continuously (i.e., reading available every second) levels will be evaluated for unusual temporal variability using frequency tables and univariate statistics.

Health Measurements. We will also apply rigorous qualitative and quantitative measures of quality assessment for the health data as has been used for our previous analyses. During and after initial data checks and cleaning procedures are completed, quantitative assessments will be conducted to validate the data. Univariate statistics and Spearman correlations will be computed and compared among the
data sources for each health parameter. The continuous time series of all continuously measured health endpoints will be evaluated for unusual patterns of temporal variability using frequency tables and univariate statistics. Any observations of within subject trends that appear inconsistent (e.g., extreme observations that appear inconsistent and implausible compared to the remainder of the timeseries) will be flagged. All invalidations will be discussed with the project co-PIs and the QA advisor and Project 2 QC reviewer and all discussions and information pertaining to each invalidation will be documented. Through an iterative procedure, the standard cleaning program will be re-run to take into account any data invalidations deemed appropriate.

5. Data Reduction

Unique IDs will be used throughout this study to track samples. Electronic copies (Excel spreadsheets) of all data will be made and verified from original field notebooks, and analytical bench sheets. Once accuracy of electronic files is ensured, sample concentrations will be calculated using SAS and Excel programs that are fully documented. The assumption of normality will be tested and if necessary, data may be log transformed. If data are not normally distributed, non-parametric tests will be employed.

Data Recording

Data will be recorded in three formats: Field logs (log sheets, notes and questionnaires), electronic data, and laboratory analysis results (electronic format). Forms and checklists have been developed to assure the accuracy and completeness of the data collection process (Appendix I). Samples with multiple detachable labels will be used to assure proper encoding and registering of each sample. (See Appendix J for technician guidance for continuous instrument programming.)

Laboratory data for mass measurements are provided to researchers in an electronic spreadsheet format. Teams of two people will enter data entry of values that are handwritten on log sheets and questionnaires. One will read log sheet values while the other enters them into a spreadsheet-based data form. Data sets are checked for entry errors after data are entered as follows: the person that entered the data will read it back to the other person, who will check it against the original handwritten data. The data will then be merged into a single data set. The data management software used will include SAS and Microsoft Excel. Any electronic data are stored on a secure Emory data server in the SCAPE drive.

Final sample values in $\mu g/m^3$ are derived by merging the processed mass, elemental, or ECOC determinations with the processed field sample volume calculations in a final validated format. Univariate statistics are determined for the final data set. Data capture is determined by finding the ratio of the total number of valid samples to the total number of samples possible and multiplying the ratio by 100.
6. Interaction of Players

The PI of the project is Jeremy Sarnat. He will direct the project and has the authority to change how the various players interact. He is supported by Co-PIs from Emory (Dana Flanders) and Georgia Tech (Mike Bergin), who will facilitate interaction between this project and the Cores. Lou Brown, Lyndsey Darrow, Anne Fitzpatrick, William Flanders, Robert Greenwald, Andrea Winquist, and Cherry Wongtrakool are co-investigators on the project; doctoral and masters level students (to be determined) will also be involved. Priya Kewada, the project coordinator will serve as the QC Reviewer once data collection activities start. All field staff and study personnel will undergo training in using the measurement methods, data downloading and processing and any relevant analytical methods. This training will be supervised by either project PI Sarnat or Dr. Roby Greenwald. The QC Reviewer will be responsible for documenting and keeping records of QA/QC procedures and any deficiencies that occur and for reporting routinely to the QA Manager. The efforts of the project will also be overseen by the Core’s QA Advisor, Guangxuan Zhu, and Mitch Klein who will work with the PI and Project Coordinator to conduct annual reviews of project methods and QA procedures. All investigators and study personnel with access to health data will maintain their CITI certification by taking refresher courses every-other-year (or as specified by Emory University). The PI will work closely with the QC Reviewer and all study personnel involved in data processing and analysis to ensure data quality procedures are followed appropriately.

This project builds on the investigators’ ongoing research efforts to examine the association between commuting exposures and acute health responses in Atlanta. We will solicit feedback from the multi-disciplinary advisory committee currently working on the ACE Study to ensure that the methods and designs used in this study optimally address the proposed hypotheses and are consist with the approaches utilized in our previous research efforts. A formal peer review of the study, prior to data publications, will include review of all programs for data quality checking, data management, and data analysis. Emory University will assign applicable in-house expertise to this activity with available project funds. This process will ensure that all data analysis summaries (e.g. descriptive statistics, effect estimates, and 95% confidence intervals, etc) from which inference and conclusions are made, as well as any resulting publications are error-free. Final evaluation will also be achieved in assessing whether the data collected successfully addressed the specific hypotheses and through the submission of manuscripts or peer reviewed scientific journals.

7. Data Management

All data handling procedures will be documented in data analysis notebooks and/or in SAS program records. Data validation is the process of filtering and accepting/rejecting data based on a set of criteria. This requires documented validity of samples and the measurement technique used. Some estimation of uncertainty will be done.
The data will be validated by project manager, who will check the data for compliance with the established data quality objectives. Checks will be done for proper sample identification, proper sample transmittal, internal consistency, and temporal and spatial consistency. Techniques for validation will include comparison to other methods, regression analysis, and plots and tests to evaluate outliers. Lab QC notes are reviewed and incorporated as needed. The project manager will document the data validation process in the form of a data validation report. The report will include all necessary supporting documents outlined throughout this QAPP. The report will include a description of the data validation process, any data anomalies identified and corrective action taken. In addition to a discussion of data validation, the report will contain the results of the preliminary data review including standard graphs and statistical parameters.

All health data recorded during the study on the Health Data Form will be entered electronically, and immediately placed on our password-protected secure network drives that are regularly backed up. All hard copies will be stored in a locked filing cabinet located in a locked office. Access will be limited to those who are on the Emory IRB for the project and have approval to work on the project as determined by the PI.

The continuous air quality data will be downloaded immediately after the commute or non-commute period by trained study personal directly onto our password-protected secure network drive (Appendix K).

Data Reporting, Documentation Control and Archiving

Each sample collected will be documented on a form by field staff at the sampling site. The project manager will keep these data sheets. These forms will include project title, parameter measured, type of sample (ambient, personal), sample identification code, date and time of collection, subject identification code, name of field staff handling the sample, and calibrator serial number. At the time of data collection a chain of custody form will be initiated and will follow the sample during all subsequent handling.

Signed consent forms and other confidential information as well as data sheets and chain of custody forms will be kept in notebooks or files by the project manager. In terms of collected data, any necessary revisions to the data set will use field data sheets for verification. Data management personnel will note any modifications to the data set. Records and data will be stored in locked file cabinets in field offices. Computer files will be stored on the network drives by each team leader and will be accessible by password only to verified users.

Sample Handling and Custody Requirements
Field data log sheets generated at the time of sample creation travel with the samples, to the field and back to the lab after exposure. In the field, operators initial each sample change on the logs, note any observations and/or problems with each sample and return the logs with the samples to the laboratory. Lab personnel record the receipt of the field samples on the sample log (date and initials) and note any observations or problems with the samples directly on the field data logs with initials when the shipments are unpacked and the samples prepared for chemical analysis. All samples should be collected, transferred, stored and analyzed by authorized study personnel. Written records will be maintained of sample handling and treatment from the time of collection through transport, laboratory analysis and disposal/final storage.

It is important to have established procedures for handling, storage, exposure and transport of samples and data to insure that they are not contaminated, lost, tampered with, or otherwise compromised. A chronological paper trail is created for each sample or set of samples. All entries are dated and initialed by the operator or lab technician or validator. Corrections are dated and initialed, taking care to preserve the original entry information as well. For logs used at field sampling sites, photocopies are made upon completion of the log and before returning them to the laboratory. These are stored on site for reference use by site operators or auditors; they also serve as backup copies of the original logs sent to laboratory.

An official listing of all secondary data sources utilized in this project will be kept by the QA Manager, and will be referred in the event of presentation or publication of results, so that appropriate identification of the data sources may be made.

Final health and pollutant datasets will be stored as “read only” on the network drive; modifications will be made to copies of these datasets as needed. The tasks of the QC Reviewer that relate to data management include:

1. Check to ensure that our Emory network drive has the most current versions of all the different air quality datasets that are on the Georgia Tech website.
2. Check to ensure that Project network folder is appropriately organized.
3. Make the original and validated health and pollutant datasets “read only” so that they will not be accidentally altered.
4. Conduct routine Data Quality Assessments and report to PI and QA Manager.

8. Technical Systems Assessment

The annual Technical Systems Assessment will be conducted by the Center’s QA Manager. The QC Reviewer will work with the QA Manager to provide documentation needed to facilitate this effort. The Assessment will ensure that progress is being made on the study, that the network drives are well organized and up-to-date, and that appropriate data cleaning procedures are being followed.
Assessment will ensure that the most current estimates following data analysis are present on the Emory network drive. The Assessment will ensure that all of the ongoing analyses have clearly documented how the data used in analyses relate to the original and validated health and pollutant datasets.

9. **Computer Hardware and Software**

Computer hardware to be utilized for this project includes Dell brand desktop computers provided by the RSPH Information Technologies. Current generation systems are Intel Core 2 Duo processors. Standard statistical software will be used to analyze the data and present results, including SAS software, R software, and Microsoft Excel software. All data will be stored on password protected servers that are regularly backed up by RSPH IT department.
Appendix A. Assembly of the Two-Stage Cascade Sampler for the EPA Commuter Study

Preparation –

Use non-powdered gloves when handling all equipment.

Do not touch the Teflon filters with your hands; use only forceps. Filters are located in the cooler and are in labeled Petri dishes.

To clean forceps using Milli-Q water or ethanol, hold them over the sink or a waste beaker and allow them to air dry.

Cover clean trays with Kimwipes.

Assembly of the Cascade sampler –

Assemble as many units as required for the monitoring. Typically 8 units will fit on a tray. See Figure 1 for all components.

Place small black o-ring into the base, place the metal screen on top. Ensure that the screen sits flat in the base. Sit the base face-up in the body (with connector barb facing down) so that it is flat. See A of Figure 2.
Place larger black o-rings into grooves on the nozzles and filter holder.

Place larger pink PUF into the larger impaction plate then place impaction plate face down in the PM10 nozzle (you should not be able to see the PUF). See B in Figure 2.

![Figure 2.](image)

Use clean forceps to place a drain disc onto the metal screen followed by a filter. Keep the Petri dish lined up with the correct unit so that it is obvious which labels correspond to which filter.

Use clean forceps to place the smaller pink PUF from the labeled Petri dish into the PM2.5 impaction plate. Place the impaction plate face down into the PM2.5 nozzle.

Stack the PM2.5 nozzle on top of the PM10 nozzle.

Attach the filter holder to the PM2.5 nozzle so that all 3 components are tightly sealed.

Place the assembled nozzle sections on top of the filter; ensure that the drain disc ring sits between the nozzles and the filter.

Carefully turnover the whole unit, hold onto the base to ensure that the base and nozzles stay together.

Once all units are loaded with filters remove the tray from the hood.
Line up the screw holes and screw together the units using the torque screwdriver.

Using the torque screwdriver tighten all the screws evenly. Place pressure on the top of the unit to ensure even tightening.

Remove both of the labels from each of the Petri dishes and attach to the side of the unit.

To ensure that all the components stay together attach the bracket and screw the body and sampling sections together. See Figure 3. Do not overtighten as the bracket will bend.

![Figure 3.](image)

Place assembled sampler into a re-sealable bag and store in the refrigerator until use.

**Disassembly of the Two-stage Cascade Particle Sampler after Exposure**

After sampling, set the sampler out on a clean tray covered with Kimwipes.

Unscrew all the screws. The sampler will then be disassembled and filters removed in the laminar flow hood (do not switch on hood though). Powder-free gloves should be worn. The following supplies are needed for this process:
Matching Petri dishes;

Wash bottle with alcohol;

Curved forceps.

To prevent mislabeling of the filters, process only one sampler at a time. Proceed as follows:

Clean the forceps with alcohol and let air dry.

Gently lift off the nozzle section and remove the drain disc ring. Using the plastic ring on the filter lift off the filter and place it into the corresponding Petri dish. Place label from sampler with TF labeling suffix into the Petri dish

Clean forceps between every sampler.

Pull apart the nozzles and remove the impaction plate. Using clean forceps remove the small PUF and place into the corresponding Petri dish. Place label from sampler with PF labeling suffix onto the Petri dish. Proceed with unloading the next sampler, until all samplers have been unloaded.

Tape Petri dishes together in ascending order and tape together in batches of 10, fill out transfer form and place a re-sealable bag in the refrigerator.

**Washing – Two-stage Cascade Particle Sampler components**

All metal components need to be washed after each use. Wash in Milli-Q water with liquid soap. Rinse twice with Milli-Q water and once with distilled water. Set out to dry on clean trays and cover in Kimwipes.

Wash o-rings and metal screens separately. Do not use alcohol to dry the O rings.
Appendix B. Gravimetric Sampling SOP
To be included.
Appendix C. Assembly of the Two-Stage Cascade Sampler for the EPA Commuter Study
Appendix D.  EC/OC Analysis SOP (Sunset labs)

Standard Operating Procedure (SOP)
For the Analysis of Organic and Elemental Carbon (OC/EC) on Quartz Fiber Filters

April 24, 2003

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Suite 4

Forest Grove, Oregon 97116
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I. Scope and Applicability

This SOP describes the analytical method to determine the levels of organic and elemental carbon in particulates collected on quartz fiber filters. The particulate samples are collected at a sampling site (outdoor, industrial, mine, etc.) by an accepted sampling method.

II. Summary of Method

This procedure is a thermal-optical transmittance (TOT) method based on NIOSH method 5040. This method uses thermal desorption in combination with optical transmission of laser light through the sample to speciate carbon collected on a quartz fiber filter. A precision tool is used to punch out a section from an aerosol sample previously collected on a quartz fiber filter. The filter punch is placed into the instrument’s quartz oven and purged with helium. The oven’s temperature is increased in ramped steps up to 900°C. Helium purges the thermally removed organic carbon and pyrolysis products into a manganese dioxide oxidizing oven where they are converted to carbon dioxide gas. The CO$_2$ gas is then mixed with H$_2$ and carried by the helium through a heated nickel catalyst which converts the CO$_2$ into methane (CH$_4$). The methane is subsequently measured using a flame ionization detector (FID). The instrument’s quartz oven is then cooled to 600°C and the carrier gas is switched to a helium/oxygen mixture and a second temperature ramp is initialized. Elemental carbon is oxidized off the sample punch and carried through the system and measured in the same manner as the organic carbon. Both external (sucrose solution) and internal (methane) carbon standards are used for FID calibration. Elemental carbon can be formed by pyrolysis (charring) of organic carbon during the initial non-oxidizing temperature ramp. An artificially low organic carbon and high elemental carbon measurement results. Because elemental carbon is a strong absorber of light, pyrolysis products formed on the quartz filter punch will cause a decrease in light transmission through it. To correct for the error produced from pyrolysis products a laser is used to determine a split point that separates the elemental carbon formed by charring from the elemental carbon that was originally in the sample. This is done by focusing a laser beam on the filter punch while it is being analyzed. If pyrolysis products form during the non-oxidizing stage of analysis, the light transmission monitored through the filter will decrease. During the second or oxidizing stage of analysis, pyrolysis products and elemental carbon originally on the punch will be burned off and light reflectance off the punch will increase to background levels for a clean quartz punch. The split point separating elemental carbon formed by pyrolysis from original elemental carbon is the point in time during the analysis at which the laser signal measured during the oxidizing stage equals the initial laser signal.
III. Working Definitions

**Organic Carbon (OC)** - Optically transparent carbon removed (through thermal desorption or pyrolysis) and char deposited when heating a filter sample to a preset maximum temperature (850 °C or 900 °C) in a non-oxidizing (helium) carrier gas.

**Elemental Carbon (EC)** - Carbon (e.g., in soot particle cores) that can only be removed from the filter under an oxidizing carrier gas (He/O\(_2\)). Optically absorbing carbon removed at high temperatures (e.g. 850 °C ) in a non-oxidizing carrier gas when internal (sample matrix) oxidants are present.

**Carbonate Carbon (CC)** - Inorganic carbon that thermally decomposes to carbon dioxide. Calcium carbonate decomposes at 825°C.

**Total Carbon (TC)** - The summation of the organic, elemental, and the carbonate carbon observed on the filter.

**OCX** - The organic carbon evolved between 550°C and the addition of oxygen. The start integration times for the fourth OC peak is optimized to begin integration at 550°C.

OCX is used to adjust the OC and EC data when comparing to data analyzed by the Interagency Monitoring of Protected Visual Environments (IMPROVE) TOR method.

**Pyrolyzed Carbon** – Light absorbing carbon (char) formed in the organic carbon portion of the analyses by incomplete combustion of OC. This is corrected by the laser transmission which defines a split point between the organic and elemental fractions.

**Split point or split time** – The transmittance of the analyzer’s laser through the filter decreases as pyrolytically formed carbon (char) is produced. The light transmittance increases as the char and original EC are removed during the oxidizing stage of analysis. The split point is the time during the analysis in which the laser transmittance matches the initial transmittance measured at the beginning of the analysis plus the transit time.

**TOT** - Thermal/Optical Transmittance

**Laboratory Duplicates** - Two filter punches taken from the same sample filter and analyzed separately with identical procedures. Analyses of laboratory duplicates indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
Field Duplicates - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

Instrument Blank - A quartz filter punch that was pre cleaned in the instrument’s oven and analyzed as a sample. This filter is not exposed to the room’s atmosphere. The instrument blank is used as a check of the instrument’s baseline.

Method Blank – A quartz filter punch that was pre cleaned, spiked with any reagents used in the method and analyzed exactly as a sample. The method blank is used to determine if method interferences are present in the laboratory environment, the reagents, or the apparatus.

ICV - Instrument calibration verification standard. An independent standard that is derived from a second source.

COC - Chain Of Custody. An unbroken trail of accountability that ensures the physical security of samples, data, and records.

I.S. - Internal Standard. A component (5% methane in helium) injected into the instrument through a fixed volume loop near the end of each analysis to provide a relative response factor for data reduction.

LCS - Laboratory Control Sample. A quality control sample that contains spiked analytes which is prepared and analyzed along with each sample batch.

MASB - Monitoring and Analytical Services Branch. The branch at NAREL is responsible for the analysis of environmental samples for radioactive and/or mixed waste contamination.

NIST - National Institute of Standards and Technology.

PM2.5 - Particulate Matter with an aerodynamic diameter less than or equal to 2.5 micrometers.

QAC - Quality Assurance Coordinator. The person with primary responsibility for overseeing the QA/QC Program.

SOP - Standard Operating Procedure. The officially approved document that describes in detail the steps of a procedure for performing a routine or repetitive task.

Transit Time - The time in seconds that it takes for the carrier gas to transfer the evolved carbon from the sample to the FID.
IV. Health and Safety Warnings

The Sunset Laboratory Thermal/Optical Carbon Analyzer uses high temperatures (up to 900\(^\circ\)C) and laser radiation to perform the required steps in this analytical procedure. Under normal operation, the analyst is protected from exposure to these energy sources. However, during repair or trouble shooting, when the instrument cover is removed, the analyst must take the following precautions:

A. Before attempting any repairs, turn off the power and wait for all heated zones to cool.

B. For most repair work, unplug power to the ovens and avoid contact with any power sources in the oven cabinet.

C. Do not look directly at the laser source as permanent eye damage can occur.

D. Use caution when handling all support gas cylinders and regulators. Always have cylinders properly secured to a safety rack.
V. Procedure

A. General Instructions

None

B. Samples

Using the “Sample Chain of Custody Form”, which will accompany the samples, document in the Laboratory Notebook, the sample name, date of receipt, and location of any observed characteristics such as color, observable moisture, tears on the filter, etc. which may influence the results.

C. Materials

See Table 1 for all required reagents and gases. Check the labels carefully to ensure that they are not expired and they are of proper purity/grade. Document the supplier, grade/purity and Lot No. as appropriate on the Analysis Data Form.

Table 1. Materials
<table>
<thead>
<tr>
<th>Material</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHP Helium (99.999 %) w/ purifiers (mol sieve and O₂ trap)</td>
<td>Carrier gas</td>
</tr>
<tr>
<td>Hydrogen (99.995 %)</td>
<td>FID combustion</td>
</tr>
<tr>
<td>Ultra Zero Air (low hydrocarbon grade)</td>
<td>FID combustion</td>
</tr>
<tr>
<td>Helium/Oxygen Mix (10 % O₂; balance UHP He)</td>
<td>EC oxidant carrier gas</td>
</tr>
<tr>
<td>Helium/Methane (5.0 % methane; balance UHP He)</td>
<td>Internal standard</td>
</tr>
<tr>
<td>Sucrose (99.9 % reagent grade)</td>
<td>Analytical standard</td>
</tr>
<tr>
<td>Calcium Carbonate (99.95 % ACS primary standard)</td>
<td>Analytical standard</td>
</tr>
<tr>
<td>Hydrochloric Acid (37 % ACS reagent grade)</td>
<td>Carbonate removal</td>
</tr>
<tr>
<td>Distilled; deionized H₂O; ultra low hydrocarbon (DDi)</td>
<td>Standard solvent</td>
</tr>
</tbody>
</table>
D. Equipment

See Table 2 for all required major pieces of equipment. Check calibration of all equipment requiring calibration to ensure it is current.

Table 2. Equipment

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forceps</td>
<td>Sample handling</td>
</tr>
<tr>
<td>Forceps (silicone rubber tipped)</td>
<td>Quartz tray manipulation</td>
</tr>
<tr>
<td>Aluminum foil</td>
<td>Work area preparation</td>
</tr>
<tr>
<td>Clean quartz filters</td>
<td>Work area preparation</td>
</tr>
<tr>
<td>Calibrated punch tool (1.5 and 1.0 cm²)</td>
<td>Removing filter portion</td>
</tr>
<tr>
<td>10 µl analytical syringe</td>
<td>Calibration</td>
</tr>
<tr>
<td>Volumetric Flasks, Class A.</td>
<td>Standard preparation</td>
</tr>
<tr>
<td>Analytical balance</td>
<td>Standard preparation</td>
</tr>
<tr>
<td>Polycarbonate Desiccator</td>
<td>Carbonate test</td>
</tr>
<tr>
<td>Glass petri dish</td>
<td>Carbonate test</td>
</tr>
<tr>
<td>Sunset Labs OC/EC Carbon Analyzer</td>
<td>Filter Analysis</td>
</tr>
</tbody>
</table>
E. Procedure

The procedure includes sample and standard preparation as well as general operation of the Sunset Laboratory OC/EC analyzer. It is assumed that the analyst has experience with routine operation of the instrument.

i. Preparation of Carbon Standard Stock Solution

a. Weigh 10.00 to 0.01 g of sucrose on the analytical balance and quantitatively transfer it into a 1 liter volumetric flask. Bring flask to volume with DDi H$_2$O and dissolve the sucrose. Record in the laboratory notebook. This will serve as the general QA/QC performance standard. Concentration = 4.21 $\mu$g C/$\mu$l solution. Date the bottle and replace the solution every 6 months. Pour about 20 mL of the standard into a clean glass vial (with Teflon cap liner) and place the rest in the refrigerator.

For Sucrose:

\[
\left( \frac{10.000 \text{g Sucrose}}{1000 \text{ml}} \right) \left( \frac{144.12 \text{g Carbon}}{342.31 \text{g Sucrose}} \right) \left( \frac{10^{-3} \text{ml}}{\mu l} \right) \left( \frac{10^6}{g} \right) = 4.21 \mu \text{g C/\mu L Solution}
\]

For KHP:

\[
\left( \frac{0.3826 \text{g KHP}}{100 \text{ml}} \right) \left( \frac{96.08 \text{g Carbon}}{204.23 \text{g Sucrose}} \right) \left( \frac{10^{-3} \text{ml}}{\mu l} \right) \left( \frac{10^6}{g} \right) = 1.80 \mu \text{g C/\mu L Solution}
\]

b. Other calibration standards can be made by diluting aliquots of the stock solution with DDi water to give you a range of concentrations to work with when performing a multipoint calibration. (Ex. Make a 1 to 10 dilution in a volumetric flask with DDi water. Concentration = 0.4207 $\mu$g C/$\mu$l of solution and can be used for the lowest concentration standard of a multi-point calibration. Date these bottles and replace the solutions every 6 months.)
ii. **Internal Standard**

The internal standard is the combination helium/methane cylinder noted in Table 1. Methane in the sample loop is automatically injected at the end of every analysis. The analytical result is normalized to the response of the methane standard to adjust for slight variations in flow rates that might occur during the day. At 760 torr and 298 K, a 5.00 % methane in helium mixture contains 24.54 μg of carbon per ml of gas. See QA/QC tests below.

iii. **Work area preparation**

a. In a designated area near the OC/EC instrument, clear an area which can be maintained free of clutter, dust and chemicals. Cover the area with 5 - 6 layers of new, clean aluminum foil. Tape the edges down so that the foiled area is secure. The foiled area does not have to be replaced daily unless damage to the surface is incurred.

b. At the beginning of each analytical session, get a new, clean section of quartz filter and roll it around the tip of the forceps forming what looks like a quartz “Q-tip”. Scrub off an area on the aluminum foil workspace about 2 inches in diameter. Observe the quartz Q-tip. It will appear gray where the hard quartz has abraded away the aluminum surface leaving a clean, uncontaminated surface for cutting filter punches. Discard the quartz filter section.

iv. **OC/EC instrument startup**

The instrument will be in the stand-by mode which it was left in at the end of the previous workday. (see shut-down procedure at the end of SOP). Take the instrument out of the stand-by mode, bring the gas flows up to the recommended settings on the computer flow table. Temporarily turn the H₂ flow up to 80 -100 ml/min and light the FID. Turn the H₂ flow down to the recommended flow rate. Check that the FID is lit by checking for condensation at the FID exhaust. Wait 20 - 30 minutes for the oven temperatures and FID to stabilize before running any samples.

a. Once the instrument is stable, choose the appropriate temperature program. (The instrument software comes with several pre-installed standard programs.) The main temperature program used will be from the EPA STN Parameters. For reference, the temperature program is shown in Table 3 below.
b. Temperature programs can be developed by setting up a parameter file (text file) with the appropriate format (see Attachment A for an example).
### Table 3. EPA STN Parameters

<table>
<thead>
<tr>
<th>Program Activity</th>
<th>Carrier Gas</th>
<th>Ramp Time (seconds)</th>
<th>Program Temperature (EC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven Purge</td>
<td>Helium</td>
<td>10</td>
<td>Ambient</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Ramp</td>
<td>Helium</td>
<td>60</td>
<td>310</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Ramp</td>
<td>Helium</td>
<td>60</td>
<td>480</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Ramp</td>
<td>Helium</td>
<td>60</td>
<td>615</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Ramp</td>
<td>Helium</td>
<td>90</td>
<td>900</td>
</tr>
<tr>
<td>Cool for EC w/fan</td>
<td>Helium</td>
<td>10</td>
<td>cool to 600</td>
</tr>
<tr>
<td>Stabilize Temp</td>
<td>Helium</td>
<td>35</td>
<td>600</td>
</tr>
<tr>
<td>He/Ox 1&lt;sup&gt;st&lt;/sup&gt; Ramp</td>
<td>Helium/Ox</td>
<td>45</td>
<td>675</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Ramp</td>
<td>Helium/Ox</td>
<td>45</td>
<td>750</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Ramp</td>
<td>Helium/Ox</td>
<td>45</td>
<td>825</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Ramp</td>
<td>Helium/Ox</td>
<td>45</td>
<td>920</td>
</tr>
<tr>
<td>Calibration</td>
<td>cal gas + He/Ox</td>
<td>30</td>
<td>cooling to ambient</td>
</tr>
<tr>
<td>Calibration</td>
<td>cal gas + He/Ox</td>
<td>50</td>
<td>cooling to ambient</td>
</tr>
<tr>
<td>Calibration</td>
<td>cal gas + helium</td>
<td>30</td>
<td>cooling to ambient</td>
</tr>
</tbody>
</table>

**Note:** Calibration gas only injected once, additional steps are to turn the blower on and facilitate purging the system.

#### v. Pre-analysis checklist

a. Check cylinders for sufficient volume and pressure
b. Re-check instrument gas flows on the computer flow table.
  c. Check the instrument pressure (typically 1 - 2 psi) prior to analysis.
d. Run Clean Oven program.

e. Ensure that the work area is clean and ready for samples

vi. External Standard preparation

The Sunset Laboratory OC/EC instrument is calibrated during the initial installation using a multipoint external standard calibration. The external standard calibration is used to determine the carbon in the internal standard gas loop. The internal standard carbon content is dependent on the volume of the loop (nominally 1 ml) and the concentration of methane in the methane/helium tank standard. Once the standard loop carbon is determined, a parameter file is set up containing: 1) the measured punch area, 2) the calibration constant, which is the number of g carbon in the loop specific to the cylinder of helium/methane mix provided at the site, and 3) the transit time, which is the time required for methane generated from a sample to reach the FID. These parameters are often stable for many months. External standards should be run according to program requirements with results monitored on a control chart. See sections on “Acceptance Criteria” and “Remedial Action” when the external standard responses fall out of accepted range.

a. Punch out three (3) new, clean 1.5 cm² sections of quartz filter with the precision filter punch.

b. Place the sections on the filter tray in the analysis oven and run the Clean Oven cycle to completely clean the filter punches.

c. Open the quartz door to the oven and pull the quartz filter boat containing the cleaned punch to the front of the oven. Use a precision syringe or the Eppendorf pippeter to deliver 10.0 – 30.0 µL of the first sucrose standard to the clean filter punch. Push the filter boat into the oven, close the quartz door of the oven and allow the filter to dry inside the oven for 8 – 10 minutes. The laser and pressure should return to approximately the same readings as they were before the punch was spiked.

d. Using the precision syringe, measure a sample volume of from 2 - 30 µL of the standard carbon (sucrose) solution and carefully apply the solution near the top of the suspended filter punch. Record the exact volume of the aliquot in the log book. A typical multi-point calibration for ambient filters would include;

1. A 10 ml sample from the “low-level” working standard yielding 2.805 g carbon/cm².
2. A 10 ml sample from the standard carbon stock solution yielding 28.05 g...
3. A 30 l sample from the standard carbon stock solution yielding 84.14 g carbon/cm$^2$.

e. Allow the filter to dry for 30 minutes before analyzing.

f. A range of 2 - 30 μl can be placed on the filter punch. If anticipating high loadings on the filters, an Analytical Standard Solution of up to 3 times the basic Standard Stock solution concentration can be made to cover the linear range of analyses measurable by the Sunset Laboratory OC/EC instrument.

vii. External Standard analysis

a. Check the OC/EC oven to determine if the temperature is cool enough to insert a sample. (Flashing green screen section on the computer monitor).

b. Open the OC/EC oven and partially slide out the quartz sample boat with the silicone tipped forceps. With the clean, steel forceps, place the standard sample on the sample boat and slide it into the oven until the tray just touches the thermocouple. The laser beam should pass through the sample.

c. Close the oven and check the oven pressure. It should read a pre-analysis pressure of 1 - 2 psi. If not, check for leaks.

d. Log in the standard and the computer “Sample Name” line in the notebook. Type in the standard description and carbon loading.

e. Start the analysis. The instrument will automatically run through the complete program and store the data in the previously noted file for subsequent analysis.

f. When the data are analyzed using the separate Sunset Laboratory OC/EC Analysis Program, the measured carbon should be within 95 % of the calculated value based on the liquid volume. See section on “Acceptance Criteria”.

viii. Internal Standard Analysis

A 5% methane in helium standard is injected automatically through a fixed volume loop at the end of each analysis. Integration of the methane peak area along with the known mass of carbon in the loop is used to calculate an instrument relative response factor for each analysis. The mass of carbon contained in the loop is calculated from the results of the external standards calibration described in Section vii as follows:

a. Analyze a series of external sucrose standards as described in Section vii.
b. Reduce the raw data using the OCECalc118 software as described in Section xii to determine the observed concentration of standard in ugC/cm².

c. Multiply the observed TC concentration (ug/cm²) of each standard by the punch area (cm²) to convert ug/cm² to total ug carbon.

d. Compute the least squares correlation coefficient and slope of the computed mass of carbon vs. the actual mass of carbon spiked using a forced origin. The slope must be \( \geq 0.95 \) and the correlation coefficient \( (r^2) \) must be 0.998. If the \( r^2 \) criteria are not met, determine the cause and correct the problem. The multipoint calibration must be repeated and a new \( r^2 \) computed. If the slope criteria are not met, a new calibration factor should be computed as follows:

1) For each standard, determine the ratio of the true concentration to calculated concentration. Ratio = True Value/Observed Value.

2) Open C:\OCECPAR\CALCPAR.TXT. (Appendix B.). Line 2 of the file contains the calibration factor which is used to correlate the methane concentration contained in the methane/helium mix to the instrument’s FID response. A new factor is calculated by multiplying the current factor by the average of the ratios determined in the previous Step. Enter the new factor in line 2 and save the file.

Note: The text file instpar.txt located in C:\ocecpar, contains information that the instrument software uses to control the operating parameters of the instrument. The latest version of instrument control software (209) requires a modification of the instpar.txt file to add new lines of text. See Appendix C. The additional data included in this file are instrument name, calibration constant and the transit time. These values will be embedded into every raw data text file associated with each analysis. By default, the calculation software (version 118) uses the information imbedded in the raw data to compute carbon concentrations. In order to over-ride the default parameters and use the parameters in the oceccalc.txt file, select the optional check box in the calculation software.

3) Verify the calibration by running an instrument calibration verification (ICV)standard. If the true value differs more than 10% from the measured value, initiate corrective action to identify and solve any problems with the instrument or standards and repeat the calibration if necessary.

ix. Routine Sample Analysis
a. Check the “Acceptance Criteria” section for the required number of instrument blanks, replicates and standards for the sample set of interest.

b. Remove the samples for analysis from cold storage and place in the desired analysis order near the sample work area. (No more than 6 samples out of cold storage at a time.)

c. On the computer, set up the base file name under which the data for the sample set will be stored. Record the base file name for the sample set in the log book for future reference.

d. Before opening the sample storage container, log the sample into the logbook and enter the sample name, number and pertinent information into the “Sample Name” screen on the instrument computer.

e. Open the sample container. With clean, stainless steel forceps, carefully pick up the filter near its edge and place it on the pre-cleaned, aluminum foil work area. Be careful not to disturb any of the filter deposit. Using the precision punch, cut out a 1.5 cm$^2$ section from the filter.

f. Check to be sure the oven is cool enough to insert a sample.

g. Open the OC/EC oven and partially slide out the quartz sample boat with the silicone tipped forceps. With the clean, steel forceps, carefully place the sample on the sample boat and slide it into the oven until the tray just touches the thermocouple. The laser beam should pass through the sample.

h. Close the oven and check the oven pressure. It should read a pre-analysis pressure of 1 - 2 psi. If not, check for leaks.

i. Begin the analysis by clicking on the “Start Analysis” button on the computer screen. The program will automatically run the desired program.

j. After the oven has cooled sufficiently (as indicated on the computer screen). Repeat the process for the remaining samples. Be sure and record each sample name as the analyses are performed to prevent mixup of results data.

k. Take precaution to insure that the required number of blanks, replicates and standards are run during the sample set.

l. The results will be stored under the “Base File Name” in order of analysis for subsequent quantification of results.

x. Non-routine Analysis

Non-routine analysis is necessary when samples contain high carbonate and/or high elemental carbon loadings. These conditions are readily recognized by the analyst. These samples must receive special attention as discussed below.
a. High carbonate levels most commonly occur in workplaces containing high levels of carbonate-containing dusts (e.g. limestone mines). A relatively narrow peak in the thermogram near the end of the non-oxidizing stage of the analysis is easily recognized when carbonates are present. If the analyst suspects high levels of carbonate based on sample appearance or history, or if the client requests carbonate information, an estimate of the carbonate carbon can be obtained as follows:

1. Verify the presence of carbonate through analysis of a second filter punch after acid treatment. In a laboratory hood, put about 5 ml of conc. HCl (37% in H₂O (w/w)) in a petri dish and put this in the bottom of the polycarbonate desiccator. Place the punch(es) on the desiccator tray (sample holder) and close the top. Allow the samples to sit in the acidified desiccator for at least one hour and then remove the entire tray. Exposure to the acid vapors will decompose the carbonate, releasing CO₂. Sit the tray out in the hood and allow the samples to “air out” for another hour to allow any trapped HCl vapors to escape. Re-analyze the samples and compare the results with the initial analysis.

2. If the carbonate is removed as a single peak (e.g., calcium carbonate), the peak can be manually integrated with the Sunset Laboratory OC/EC Analysis program (See “Calculation Section” below).

3. Analyze a carbonate standard solution in exactly the same manner as the external carbon standards (See step V.E.vi. above) to determine the carbonate peak time for use in the subsequent manual data calculations (see below).

b. High elemental carbon loadings are easily recognized because samples are black. This ordinarily occurs with samples collected in occupational settings containing high levels of elemental carbon dust (e.g. mines) or when high volume (Hi-Vol) samplers are used. When the sample is loaded into the sample oven, the laser light is essentially completely absorbed, making automatic correction for the OC/EC split point impossible. Again, such samples are readily observed because of the darkness of the sample. The analyst should:

1. If the samples are a set (i.e., contain particulate from the same source), analyze the entire set of samples. Ensure that one of the samples in the set has a loading such that the laser absorbance is acceptable for determining the OC/EC split point. This sample normally appears lighter than the others in the set. This procedure is not always valid, but it should work in some cases.

2. Analyze the raw data with the data calculation program. Look for a similar thermogram profile across the set. If the profile is essentially identical for all the samples in the set, the sample(s) having a satisfactory automatic OC/EC split
can be used as the basis for assignment of the split point for the heavily loaded samples. Note the split point time. Re-integrate the sample set manually setting the OC/EC split point at the same point throughout the set (see “Calculation Section” below). If the analyst is inexperienced, an experienced analyst should be consulted to assist with assignment of the split.

xi. Calculation of OCX Integration Start Time

a. Using a spreadsheet program, open an OC/EC raw data file with comma delimiters.
b. Select an empty cell in the first row of acquisition data.
c. Insert a formula to convert the thermocouple readings to °C. Copy the formula down the column to the last row of data.
d. Find the row number containing first temperature corresponding to 550 °C. Each row of data represents one second of analysis time, therefore the number of rows to reach 550 °C plus the transit time for that instrument is the start time for OCX which is also the beginning time for OC peak 4 integration. See Appendix D for an example.
e. Open the calcpar.txt file and enter the time for start integration of OC4.

Note: This equation is used to convert temperature from raw data produced from instruments using software previous to version 209. The thermocouple readings are in the column labeled “front_temperature” in raw data file. See Appendix D.

\[
Temperature \ (°C) = \left[ \frac{Thermocouple \ reading}{32767} \right] (1000)
\]

For instruments with software version 209 and greater:

\[
Temperature \ (OC) = 0.2063X^3 \ - \ 2.7909X^2 \ + \ 107.09X \ - \ 35955
\]

where \ \[ X = \left[ \frac{Thermocouple \ reading}{32767} \right] (1000) \]
xii. Data Analysis - Calculations

The Sunset Laboratory OC/EC Analysis Program is used to calculate the results from a sample set after the data are stored under the “Base Filename”. The OC/EC calculation program is initiated by clicking on its icon. The program then asks for the filename to perform calculations. In general, the following software parameters apply to all calculations;

a. The overall carbon response is based on a multi-point external calibration.

b. The internal standard methane for every standard cylinder is calibrated against the external multi-point calibration.

c. The internal standard is run at the end of every sample. This known amount is used to normalize the response factor for each sample. This essentially cancels out small variations in gas flows (and thus sensitivity) over the course of an analysis session. This also compensates for heavily loaded samples where the FID hydrogen flow was intentionally increased to reduce the sensitivity.

d. The software determines an initial FID baseline prior to the desorption. The area at each point along the thermogram curve minus the baseline is multiplied against the calibration response to determine the carbon. The data are summed over the range to yield the total carbon results.

e. The automated OC/EC split point is calculated from where the laser absorbance in the oxidizing (second) phase of the EC analysis matches the initial absorbance measured when the sample was first inserted in the oven. Carbon observed before the split point is considered to be organic carbon and carbon after the split is considered elemental carbon.

f. The analyst has several options in computing the results from a set of experiments. These should be exercised with caution based on the extent of the analyst’s experience.

1. The analysis program can batch process all of the sample data sets in a file without further review. It is recommended that the analyst look at each individual data set as it is processed to look for any anomalies.

2. During the processing, the analyst can calculate carbonate (if evolved as a single peak) by using the manual integration feature in the program. This must be performed by setting the left and right cursors on the beginning and ending points of the carbonate peak. When re-integrating, the program draws a
baseline between the designated points and calculates the peak area. The program will then automatically call this area carbonate and subtract it from the initial area for organic carbon (which includes carbonate, if present). This integrated value can be compared to that determined through analysis of a second portion of the same filter after exposure to HCl vapor (to remove carbonate). The difference in the carbon results for the unacidified and acidified samples gives an estimate of the carbonate carbon.

3. If necessary, the OC/EC split point can be manually set or reassigned by moving the cursor to the desired split point. The software will calculate the split based on that location. This decision is based on the analyst’s experience and information about the sample. It is normally done only when high carbonate levels are present.

xiii. Results

The analysis software automatically reduces the raw data and stores the results in a comma delimited spreadsheet format for compilation. The spreadsheet format is illustrated in “Appendix E”. The data for each sample may also be printed in graphic form with the temperature, laser absorbance, and FID profiles. The net results are tabulated and a line is provided for the analyst’s signature. An example of this results output is provided in “Attachment F”.

Concentrations of Organic, Carbonate, Elemental, and Total Carbon and OCX are reported in ugC/cm². Open the csv file using a spreadsheet program such as EXCEL. Analysis data for each sample will be displayed in the spreadsheet. OCX concentration will be listed in the column labeled as OC4.

xiv. Quartz Fiber Filter Preparation and Acceptance Testing

a. Materials
   1. 47 mm quartz fiber filters (or appropriate size for sampler)
   2. Petri dishes - sterile single use polystyrene, 50 mm.

b. Pre-cleaning storage
   1. Filters are ordered in lots of 1000. When received from the vendor, the packages are inspected for physical defects and uniformity in size.
   2. Petri dishes - clean, sterile petri dishes are inspected for physical defects or damage in shipping and stored as received.
c. Cleaning - After the filters are received, they are batch-cleaned in the following manner:
   1. Filters are removed from their shipping containers and placed in a clean crucible in batches of 100 filters.
   2. The crucible is placed in a muffle furnace with the temperature set to 900°C and a timer is set for 3 hours. The oven does not cool off at the end of the timer setting, but rather is held constant at 900°C until the samples can be processed further.

d. Acceptance testing - once the filters have been fired in the muffle furnace:
   1. The crucible is removed and placed in a clean desiccator to cool. Once cool, the crucible is removed from the desiccator and placed on the lab bench.
   2. Initially two (2) filters (per 100 cleaned) are randomly selected from the batch for acceptance testing.
   3. The two randomly selected filters are tested for total carbon immediately following their selection using the method described for filter analysis in this SOP. They must be no greater than 0.2 μg C/cm² total carbon or the lot is rejected and must be re-cleaned in the oven.

e. Packaging.
   1. A package of new, sterile petri dishes is opened and placed near the work area. The filters are individually removed from the stack using clean forceps and placed in an individual petri dish and closed.
   2. During the packing process, each filter is carefully inspected for uniformity in size, shape, thickness and appearance. Any filters which are visually flawed are discarded.
   3. The petri dishes, are wrapped in aluminum foil in groups of 10 filters.

f. Post-cleaning storage
   1. Filters will typically be shipped within 24 hours of cleaning. If for some reason this is not possible, then the cleaned, individually packaged filters will be stored under refrigeration until they can be shipped.

xv Acceptance Criteria

Acceptance criteria are summarized in Table 4.
### Table 4. Acceptance Criteria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single point external standards</td>
<td>1 per 10 samples</td>
<td>Calculated ± 5.0 %</td>
</tr>
<tr>
<td>Instrument blanks</td>
<td>2 per day</td>
<td>Value ± 0.2 :g C</td>
</tr>
<tr>
<td>Internal standard (at consistent H₂, air flow rates)</td>
<td>1 per day</td>
<td>Calculated ± 5.0 %</td>
</tr>
<tr>
<td>Multipoint external standard set</td>
<td>1 per 100 samples</td>
<td>Calculated ± 5.0 %</td>
</tr>
<tr>
<td>Sample replicates</td>
<td>1 per batch or 10% of samples - which ever is greatest</td>
<td>Value ± 10 %</td>
</tr>
<tr>
<td>Pre-fired filter blank (QC samples)</td>
<td>2 per 100</td>
<td>Value ± 0.2 :g C/cm²</td>
</tr>
</tbody>
</table>

### xvi. Remedial Action

a. External standard out of range - carefully prepare and re-run two standards. If standards are still uniformly out range then:
   1. Check gas flows
   2. Check for leaks
   3. Check system temperatures
   4. Re-make stock solution. Re-run samples
   5. Perform multipoint calibration and update calibration parameter file

b. System blanks too high
   1. Check for leaks
   2. Check system pressure
   3. Check air and hydrogen to FID

c. Internal standard out of range
   1. Check air and hydrogen flow rates
   2. Check system pressure
   3. Check for leaks
xvii. Instrument/Equipment Inspection, Testing, and Maintenance

a. Routine instrument inspection
   1. Check cylinder pressures - replace as necessary
   2. Observe oven pressure - check for leaks if out of typical range (1.0 - 1.7 psi).
   3. Observe standby temperatures of MnO₂ and methanator ovens prior to startup. If out of range, determine cause and initiate repair.
   4. Check gas flows (computer output) and verify that they are within required set points. If out of range, look for system leaks. Repair or adjust as necessary.

b. Testing
   1. Daily
      (a) Check instrument blank - if greater than 0.2 μg/cm² carbon, check for leaks, contamination of gases, and/or hardware failure.
      (b) Run methane calibration - determine if within 90% of weekly average. If out of range, look for leaks or problems with FID. If all else is operating satisfactorily and there is a trend towards lower responses over a two week period, this could be an indicator that the methanator oven needs to be replaced.
   2. Bi-weekly
      (a) Run multi-point calibration
   3. Semi-annual
      (a) Re-calibrate flow sensors using NIST traceable calibration source.
   4. Maintenance - the instrument requires virtually no routine maintenance over extended periods of time. Breakage or failure can occasionally occur with the components described below. If breakage or failure occurs, components should be replaced as necessary with replacements from Sunset Laboratory.
      (a) Replacement of quartz oven - the quartz oven/MnO₂ converter almost never fails except in the case of operator breakage or development of cracks. If this occurs, the oven will have to be replaced. Begin by cooling the oven to ambient and shutting off all gas flows. Unplug all electrical power. Begin disassembly by removing the fan, photodetector and top cover. Next, carefully remove all insulation from the oven area. Disconnect the heating coils and carefully unwrap them from the oven assembly. Unplug the thermocouples from back and underneath the instrument. Remove the desorption oven thermocouple by disconnecting the Swagelok® fitting. Remove the top oven supports and lift out the oven. Replace in
reverse order using care in installing the oven thermocouple, heating coils and insulation. Once installed, re-start gas flows to purge with helium and initiate startup sequence in the software.

(b) Replacement of methanator oven - the methanator oven may degrade with time and will be evident by declining methane calibration gas levels (assuming all other components are performing satisfactorily). If this occurs then replacement will be necessary. Begin by cooling the oven to ambient and turning off all gas flows. Unplug all electrical power to the instrument. Unhook the inlet and outlet gas lines and open up the instrument housing. Unpack the insulation and disconnect the heating coil. Unwrap the heating coil and remove the methanator tube. Replace with a Sunset Laboratory replacement part. Assemble in reverse order.

(c) Replacement of heating coils - heating coils will occasionally burn out. When this happens, they will have to be replaced. Follow the replacement steps for the appropriate component above (Maintenance, Steps 1 and 2) until the coil can be removed. Replace with Sunset Laboratory replacement part. Assemble in reverse order.

VI. Quality Assurance

A. Initial calibration. Run a complete set of calibration standards, following the procedure in V, E, vii at least once a week if performing routine analyses. Compute the linear regression of the computed concentration vs. the actual concentration of carbon using a forced origin. If the correlation coefficient (r2) is not 0.99, correct the problem and repeat the calibration.

B. No samples may be analyzed until the calibration has been verified by an independent standard (ICV) prepared from a different lot number or source of carbon (e.g. KHP). The concentration of the ICV should be at the midrange of the calibration curve. If the analyte concentration determined for the ICV differs by more than 10% from its expected value, corrective action must be taken to determine the cause and if necessary, a new initial calibration must be established.

C. Daily continuing calibration verification standard (CCV). Run a sucrose CCV at the beginning of each sample batch. The measured carbon (ug/cm^2) must be within ±5% of the true value. If the CCV does not meet the ±5% criteria, a new calibration must be performed and a new calibration factor computed.

D. Run an instrument blank, using a punch from a pre-cleaned quartz fiber filter, at the beginning of each batch. If the blank is above 0.3 ugC/cm2, determine if the problem is with the filter or the instrument, and, if necessary, initiate corrective action to identify the
problem before analyzing samples.

E. Duplicate analyses. Analyze a duplicate sample punch for each batch or 10% of the samples analyzed, which ever is greater. Acceptance criteria for duplicate measurements is dependant on filter loadings. Criteria for carbon loadings 5 ug/cm² are based on the relative percent difference (RPD) of duplicate measurements. The acceptance criterion for loadings < 5 ug/cm² is based on absolute error (±0.75 ug/cm²). See table 2 for acceptance criteria.

<table>
<thead>
<tr>
<th>Total Carbon Concentration Range</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values greater than 10 ug/cm²</td>
<td>Less than 10% RPD</td>
</tr>
<tr>
<td>5 - 10 ug/cm²</td>
<td>Less than 15% RPD</td>
</tr>
<tr>
<td>Values less than 5 ug/cm²</td>
<td>Within 0.75 ug/cm²</td>
</tr>
</tbody>
</table>

F. Internal Standard - If the internal standard area counts or FID Max for any analysis on a given day is not within ±5% of the average response for all samples run that day, then the analysis must be repeated.

G. If carbonate carbon is suspected and the manual integration method (Section V.E.xi) is used to estimate its concentration, run a CaCO₃ standard to verify the location of the carbonate peak in the thermogram. A CaCO₃ standard need only be run once for a given parameter file. 21.8 Samples with carbon concentrations above the highest calibration standard. The linearity of the instrument must re-verified by analyzing a standard higher in concentration than that of the sample. If the response is not linear, (r²< 0.99), a second, smaller punch) from the quartz filter sample must be analyzed.

H. The calibration factor must be updated when the calibration gas (CH₄) cylinder is replaced.

I. Archive raw data for each day's analytical batch to the network. Include the raw data text file, the oceccalc.txt file, the thermal profile parameter file the csv file and the instrument parameter file.

J. Check the operation of the methanator by analyzing a NIST certified 5% CO₂ in He gas. This
can be done by switching the CH4 3-way valve to the CO2 source and analyzing a sample or standard. The area counts of the CO2 should be within ±10% of the last CH4 response.

K. The transit time for each instrument must be determined in order for the software to be able to compute an accurate OC/EC split point. The transit time can be determined by analyzing a 40 ug/cm² sucrose standard using the transit.par parameter file. This parameter file omits the cooling step between the non-oxidizing to oxidizing mode of the heating profile. This permits very rapid oxidation of the char which in turn causes a rapid response of the laser signal. Open the raw data file in a spreadsheet in order to easily examine the FID and laser readings. Examine the data near the beginning of the oxygen mode. Note the row numbers at which the laser readings and the FID readings begin to increase. The difference in row numbers is the transit time in seconds. The transit time can also be determined by plotting the laser signal and FID signal. The transit time should be checked after any major maintenance is done such as replacing the quartz oven.

VII. References

VIII. Revision History

A. Revision 1 - March 1999

B. Revision 2 - February 2001
   1) Updated NIOSH method 5040 to incorporate current accepted EPA program and to remove “target temperature” column from table as the current instrument software will properly reach the assigned temperature set points.
   2) Removed references to Sodium Carbonate as a standard and replaced with Calcium Carbonate.

C. Revision 3 - February 2003
   1) Updated temperature profiles
   2) Added Quality Assurance Section

D. Revision 4 - Adapt for EPA STN Method
   1) Add calculations for OCx

VIV. Appendices

a. Available upon request
Appendix E. SOP for the characterization of particulate water-soluble organic carbon
Appendix F. SOP for the characterization of particulate ion content
Appendix G. Operation manuals for all continuous sampling instruments
Appendix H. Standard Operating Procedures and Technician Guidance Documents for Health Tests and Sample Processing

**Before the Participant Arrives:**

**Preparation of MDA and GSH28:**
Tubes are prepared in large batches, each with the relevant preserve. Label each tube on both the lid and on the side of the tube with the sample name, i.e. either MDA or GSH28. Once labeled, rinse all tubes thoroughly using milli-Q water.

**Preserve volumes to be added:**
Pipette 5 µL of BHT preserve into each of the green tubes labeled MDA. BHT preserve is prepared in our lab, and stored in the refrigerator. Pipette 28 µL of the GSH preserve into each of the yellow tubes labeled GSH28. GSH preserve is obtained from Emory Pediatrics. It is stored in the -80°C freezer. The preserve needs to be thawed before pipetting it into individual GSH28 tubes. The excess preserve is then refrozen.

Once filled with preserve, store both the MDA and GSH28 prepared tubes in the -80°C freezer.

Rinse all other study tubes thoroughly using milli-Q water. Also rinse the loose lids of the white IC tubes; remove excess water from the lids using a pipette.

Label the remaining tubes on both the top lid and on the side of the tube.

**Labeling:**

<table>
<thead>
<tr>
<th>Number of Tubes</th>
<th>Color</th>
<th>Sample Name</th>
<th>Participant ID</th>
<th>Sample Number*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Blue</td>
<td>Saliva</td>
<td>Participant ID</td>
<td>01-02 eg. HE4401 and HE4402</td>
</tr>
<tr>
<td>12</td>
<td>Pink</td>
<td>EBC</td>
<td>Participant ID</td>
<td>01-06</td>
</tr>
<tr>
<td>6</td>
<td>Pink</td>
<td>pH</td>
<td>Participant ID</td>
<td>01-06</td>
</tr>
<tr>
<td>6</td>
<td>Green</td>
<td>MDA**</td>
<td>Participant ID</td>
<td>01-06</td>
</tr>
<tr>
<td>12</td>
<td>Yellow</td>
<td>GSH28**</td>
<td>Participant ID</td>
<td>2×01-06</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>Plasma</td>
<td>Participant ID</td>
<td>4×01</td>
</tr>
<tr>
<td>1</td>
<td>Orange</td>
<td>Cells</td>
<td>Participant ID</td>
<td>01</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>Plasma</td>
<td>Participant ID</td>
<td>4×02</td>
</tr>
</tbody>
</table>
*Refers to the corresponding Sampling Round: 01 – Day before Commute; 02 – Pre Commute; 03 – Post Commute; 04 – 1 Hour post Commute; 05 – 2 Hours Post Commute; 06 – 3 Hours Post Commute.

**Both the MDA and GSH28 are prepared in advance (See above), and stored, ready for use in the -80°C freezer. The tubes are labeled with only the sample name before being stored in the freezer. They are removed as needed for each round of health tests and then labeled with the Participant ID and corresponding Sample Number.

**Downloading Data from Health Instruments:**
Refer to the ‘Continuous Sampling: Data Download’ Standard Operating Procedure.
Ensure all data from the previous commute has been downloaded and saved in the relevant folder on the secure network drive:
“T:\EohProjs\Sarnat\ACE Study\ACE Study DATA\”Assigned SubFolder“*.

**“Assigned SubFolder” – Label includes Participant ID and date of commute, e.g., HE44_01282011

**Health Instrument Programming:**
Ensure all instruments have been programmed in accordance with the ‘Continuous Sampling: Instrument Programming’ Standard Operating Procedure.

**Preparation of Health Instruments - 15 minutes before the participant arrives:**

**Exhaled Breath Condensate (EBC)**
Rinse the plastic EBC tube, including the mouth piece with milli-Q water and shake off all excess water.
The clean plastic column is stored in its original plastic bag till it is to be used by the participant.
Also rinse the metal column used to help extract the EBC sample with milli-Q water.

**Blood Pressure Cuff**
Participant’s weight, height and birth date are needed to complete programming of the device.
Place the device in the cuff and connect the grey tubing. Hold down the Start/Stop button on the device till ‘PAUSE’ mode flashes on the interface. This ensures that the pre-programmed mode set to start the device every 15 minute is not initiated; rather the device can be started as and when required by study personal by simply pressing the Start/Stop button.

**C-Reactive Protein (CRP)**
Plug the CRP machine in.
Retrieve the CRP cartridges stored in the refrigerator. They must reach room temperature before use. Make sure the gloves, alcohol swabs, capillaries, lancets, and gauze are stocked and easily accessible for use.

**Exhaled Nitric Oxide (eNO)**
Plug the instrument in and allow for a 10 minute warm up period.
Attach the mouth piece; make a small opening in the plastic bag covering the mouthpiece to attach the piece without touching it with your fingers. This ensures the mouthpiece remains clean and ready for use.

**Spirometry**
Plug the spirometry machine in.
Attach the mouth piece to the device; make a small opening in the plastic bag covering the mouthpiece to attach the piece without touching it with your fingers. This ensures the mouthpiece remains clean and ready for use.
Once the participant arrives and has consented to participation, enter the relevant weight, height, and birth date information to complete programming of the device.

**Holter Monitor**
Ensure the old AAA battery is replaced with a new one. Complete programming by adding the participant’s name and participant ID.
Clean the entire holter, including the leads, and holter clip using alcohol.
Label the adhesive electrodes corresponding to the five lead colors.
Attach the labeled electrodes to the correct leads.
Set aside a set of extra adhesive electrodes for the participant to take home with them.

**Informed Consent Process:**
The informed consent documents are emailed to the participant when they have agreed to schedule a commute.
The afternoon before the scheduled commute, study personal review all the informed consent documents together with the participant, and answer any questions they may have regarding the study. Once the participant has consented, study personal begin again to review with the participant the health tests to be completed as part of the first round of health tests.

**Health Tests:**

**First round of health tests:**
Record the participant’s age, height, weight, and mobile phone number, on the Health Test Form (See form attached in the appendix).
This information is used to complete the programming of the blood pressure cuff and spirometry machine.

The health tests are outlined below in the order that they are conducted:

**Exhaled Breath Condensate (EBC)**
Retrieve the frozen EBC sleeve from the -80°C freezer.
While at the freezer also retrieve two yellow GSH28 prepared (preserve) tubes and one green MDA prepared (preserve) tube.

The frozen metal sleeve should be placed over the plastic EBC tube.
Instruct the participant to blow out vigorously to ensure the small blue valve inside the plastic tube is not sealed shut.
(If this valve is sealed, remove the metal sleeve, and then remove the valve from the plastic tube and loosen it. Replace the valve in the tube, and place the frozen sleeve over the plastic column).
Then instruct the participant to breathe normally through the tube for approximately 10 minutes or until the desired volume of condensate is collected.
The desired volume is approximately 2500 µL, and corresponds to a blue line marker on the plastic sleeve.
While the participant is completing the EBC test measure their blood pressure and pulse.

**Blood Pressure Cuff**
This test is conducted while the participant is seated.
Ensure the device in the ‘PAUSE’ mode.
Place the blood pressure cuff on the participant’s upper left arm.
Adjust the Velcro strap so it is fitted tightly, and the device interface is easily visible.
Press the start button to inflate the cuff.
As the cuff deflates, record the reading on the Health Test Form.
(If you were unable to see the reading, record the exact time the test was conducted, the reading can then be retrieved once the study is over and the data has been downloaded from the device).
Once the reading has been recorded remove the cuff from the participant’s arm.

**C-Reactive Protein (CRP)**
While the participant continues the EBC test, measure their CRP.
Ensure the participant is still seated.
Study personal are required to wear rubber gloves.
Prepare the finger to be pricked by wiping its tip with an alcohol swab.
Remove the CRP cartridge from its foil cover and set it on the counter with the black metallic strip to the right.
Before pricking the participant’s finger ensure you have the capillary, plunger, and gauze immediately accessible on the counter.
Prepare the lancet and prick the clean finger.
Massage the lower finger to ensure enough blood pools to fill the capillary.
Exactly 50 µL is required; if there are any air bubbles within the capillary, or if the full volume is not collected, use another capillary to collect the desired volume.
Use the plunger to expel the blood into the cavity on the upper left of the CRP cartridge.
Press ‘Run’ on the CRP machine to open the cartridge tray.
Place the cartridge in the tray, press it into place without touching the metallic strip.
Once the cartridge is in place, press ‘Run’ again.
The run time is 7 minutes.
When finished running, record the reading shown on the display on the Health Test Form.
If you miss the reading, press the ‘Data’ button to show the latest recorded reading.
Once the reading has been recorded, remove the cartridge.
Press ‘Stop’ to close the cartridge tray.
Ensure that the used lancet, capillary, and used cartridge are disposed of in the sharp’s container.

Once the CRP is complete check the EBC tube to assess the volume of condensate collected.
When checking the volume, avoid sticking your finger into the top of the plastic tube.
Grip tube just above the mouth piece and slide the metal sleeve up.

**Exhaled Breath Condensate (EBC) Continued**

When the desired volume (approximately 2500 µL) has been collected, remove the metal sleeve and mouth piece from the plastic tube.
Place the tube over the metal EBC extractor and slowly pull the plastic tube down, forcing the blue valve holding the condensate up.
Attach a clean tip to the 1000 µL pipette. The same tip can be used again, simply flush it several times with milli-Q water between each scheduled health test.
The EBC tubes including 2*GSH28, 1*MDA, 2*IC, 1*pH, and 2*EBC (or as many are needed to collect all excess EBC in 250 µL aliquots) should be readily accessible on the counter.
Using the pipette, first extract 480 µL into each of the GSH28 tubes:
Then extract 245 µL into the MDA tube,
Then extract 250 µL into each of the IC tubes,
Then extract 250 µL into the pH tube,
Finally, extract 250 µL aliquots of the excess condensate into each of the EBC tubes. Use as many EBC tubes as needed to store all excess condensate.

Immediately place the pH probe into the pH tube.
Watch the meter closely and record the lowest pH value on the Health Test Form.

Turn on the Argon tank and ensure gentle bubbles are being expelled through the probe. If bubbles are too vigorous EBC condensate may bubble out of the tube and be lost. Let the Argon bubble through the labeled IC-Argon tube for 10 seconds. Attach black lid quickly.

Ensure here are no air bubbles in both IC samples. If there are, gently flick the tubes to expel them. Both samples will be placed in the IC.

Once the lowest pH reading has been recorded, place the bubbling Argon probe into the pH tube and begin the stop watch. Bubble Argon through the pH tube for 10 minutes. While the Argon is bubbling through the pH tube continue on to the eNO health test.

Ion Chromatography of EBC
Creating a new sequence
- Browser window
- File
- New
- Sequence using wizard
- Okay
- Welcome message--- Next
- Chose timebase--- Next
- Unknown samples--- Next
- Standard samples--- Next
- Methods and reporting
- Under method files:

*For cations:
- Program -- browse
- Under CATIONS folder select ‘programs’ -- ‘CS12A_EBC’ (or current program in use)
- Quantification method -- browse
- Under CATIONS folder select ‘CS12A_EBC’ (or current method in use)

*For anions:
- Program -- browse
- Under ANIONS folder select ‘programs’ -- ‘AS11HC_122810’ (or current program in use)
- Quantification method -- browse
- Under ANIONS folder select ‘122810’ (or current method in use)
Next
• Saving the sequence
• Sequence name: MMDDYY_CAT or MMDDYY_AN
• Directory: CATIONS\SEQUENCES\2011 or ANIONS\SEQUENCES\2011
• Finish -- should create a new sequence
• Copy ‘shut down’ program from top of previous sequence and paste into new one
• Add samples in sequence as follows:

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Type</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>water</td>
<td>unknown</td>
<td>99 or 100</td>
</tr>
<tr>
<td>2.</td>
<td>blank 1</td>
<td>blank</td>
<td>99 or 100</td>
</tr>
<tr>
<td>3.</td>
<td>standards</td>
<td>standard</td>
<td>copy from previous sequence</td>
</tr>
<tr>
<td>4.</td>
<td>blank 2</td>
<td>blank</td>
<td>99 or 100</td>
</tr>
<tr>
<td>5.</td>
<td>standards</td>
<td>standard</td>
<td>copy from previous sequence</td>
</tr>
<tr>
<td>6.</td>
<td>blank 3</td>
<td>blank</td>
<td>99 or 100</td>
</tr>
<tr>
<td>7.</td>
<td>participant ID</td>
<td>unknown</td>
<td>##</td>
</tr>
<tr>
<td>8.</td>
<td>water (or blank)</td>
<td>unknown</td>
<td>99 or 100</td>
</tr>
</tbody>
</table>

--- insert a blank before, in the middle, and after standards
--- copy the “name”, “type”, and “position” of standards from previous sequence
--- add water and blanks for the remaining of the sequence, alternating positions 99 and 100
--- name last sample ‘SHUT DOWN’, “position” 99 or 100, and switch “program” column to ‘shut down’
--- * when not running any participant samples, run only ANIONS sequence with water and blanks

• Menu-- batch – start (running sample should highlight in green)
• Insert participant samples (Ctrl-I) into sequence so they run as soon as they are collected

Preparing liquid reservoirs
• Control panel --‘Shut down’ anions and cations
• Anions (right side) beaker
• Fill with Milli Q water and connect to vacuum to get all air bubbles out
• Prime the pump: insert syringe, open valve, pull syringe to get air bubbles out, close valve.
Repeat at least 2 times
• Cations (left side) beaker
• Prepare eluent solution: add 48.5g stock (in refrigerator) to beaker and fill with Milli Q water to marked line
• Prime the pump: close pump from cation control panel, insert syringe, open pump, pull syringe, close pump. Repeat at least 2 times
• If not running samples:
  • Fill H2O beaker with Milli Q water
  • Control panel -- ‘Start up’

Anions-- general information
• Window -- ICS-2000 Anions
• flow rate: .5 ml/mi
• pressure: 2000-2300 psi
• cell temperature: 30°C
• auto sampler temperature: 4°C
• supp type: 2 mm
• ejection volume: 24.5 µL
• red graph: previous sample
• yellow graph: current sample
• ~ 19 standards

Cations -- general information.
• Window-- ICS 1000 Cations
• flow rate: 1.5 ml/mi (when not running: shut down, set flow rate to .25 or .5, and open eluent valve)
• pressure: 2000-2300 psi
• cell temperature: 30°C
• auto sampler temperature: 4°C
• supp type: 4 mm
• ejection volume: 24.5 µL
• ~ 6 standards

Auto sampler door
• To open: ‘door interlock bypass’- ‘yes’- ‘ENTER’
• If error message and can’t open door: disconnect auto sampler (from control panel) and select main menu options 5, 7 (on auto sampler screen).
• Don’t open door if needle is out, wait until sample begins to run and needle has moved back

Waste
• Empty waste beakers periodically (2 under IC)
Saliva Sample
Collect the saliva sample after the participant has finished breathing through the EBC tube. Saliva sample is connected twice, once during the health tests immediately preceding the commute, and then again during the health tests immediately after the commute. Participant must spit into the labeled blue tube. Approximately 100 µL of saliva is required.

Exhaled Nitric Oxide (eNO)
Ensure that the cloud interface is highlighted and the screen illuminated. Instruct the participant to hold the device up to their mouth, with their lips tightly sealed around the mouthpiece. Hold the mirror so the participant can view the instrument screen. Instruct the participant to take a deep breath in through the mouthpiece, and then blow out at a constant rate ensuring the cloud on the interface maintains a position in the middle of the blue panel in the center of the screen. Participant has to blow out at this constant rate for approximately 20 seconds and stop when the countdown on the interface reaches 0. Set the device on the counter; the final eNO value is displayed after a 2 minute countdown. Record the value on the Health Test Form.

Spirometry
Using the touch screen select ‘Patient’. Select ‘New Patient’. Enter the relevant height, weight, and age information.

Explain the steps listed below to the participant before they begin the test:

Instruct the participant to seal their lips around the green mouth piece. Participant should breathe in and out normally. Study personal should press the ‘Start’ button on the top right of the screen. When they are ready they are instructed to take a deep breath in and fill their lungs. Study personal should press the ‘Next’ button on the top right of the screen as the participant inhales. The participant is then instructed to blow out as hard and fast as you can, and to continue breathing out until the device sounds; instruct the participant to then breathe in.

The test is repeated three times to ensure the best effort is recorded. Copy the results on the Health Test Form, including the ‘Predicted’ values in the first column on the form.

When the test is complete and the results recorded:
Select ‘Patient’.
Select ‘Recall’.
Select ‘Search’ (Ensure the current participant’s last initial is selected).
Their name will appear highlighted on the lower screen.
Select ‘New Series’.
Select ‘OK’.
The spirometry machine is ready for the participant’s next round of tests.

Exhaled Breath Condensate (EBC) Continued
Once the spirometry test is complete, check the stop watch and remove the Argon probe and turn it off if it has been bubbling for 10 minutes.
Replace the Argon probe with the pH probe.
Watch the pH meter closely and record the highest pH reading on the health test form.

Blood Draws
The blood draws are completed both pre-commute and 10-hours post commute.
Once all the health tests have been completed the phlebotomist will continue with the blood draw.
For each procedure, 10 mL of blood will be drawn by a trained phlebotomist from an antecubital vein without venous stasis. Whole blood will undergo a complete differential cell count, including erythrocytes, platelets, lymphocytes, and polymorphonuclear leukocytes. The tube of blood with the blue lid is immediately placed in the centrifuge and balanced with a similar tube filled with water. Blood will be centrifuged to separate plasma, which will then be divided into aliquots for analysis of specific biomarkers.

Spin the tube of blood for 15 minutes or until all the cells and plasma are separated.
Using the pipette extract about 350 µL of plasma into each of the 4 orange labeled tubes.
When all the plasma is removed without displacing any of the cells at the bottom of the vile, extract a similar volume of blood cells into the relevant orange labeled tube.
All these samples are then stored in the -80 C freezer.

The tube of blood with the purple lid is labeled clearly with the participant’s first and last name, the date and the time of draw (either 7am or 1pm).
The blood is then stored in the refrigerator until the end of the participants study involvement that day.
Two separate Emory Medical Laboratories (EML), Grant Request Form for Clinical Trial Patients must be completed.
A copy of this form is attached in the appendix along with notes on how to complete the form.
Each sample with its corresponding form is placed in individual Ziploc bags.
The blood samples are then taken over to Emory Hospital - Medical Laboratories (Location: Floor 1. Wing D. Room 140. Telephone 404-712-5227).
Drop the samples at the window, once you have filled the sign in sheet.
Samples are processed within 48 hours.
To have the results faxed, call the laboratory with the participant’s name, and date the sample was delivered, and provide a return fax number.

**Holter Monitor**
The holter monitor is placed on the participant once the first round of health tests have been completed the afternoon before the commute day.
If a female participant is enrolled, a female member of the study personal will fit the holter monitor. If the participant is male; either a female or male member of the study personal will fit the monitor.
Direct the participant to a private exam room.
In order to place all the leads in their correct positions, it is easiest if the participant removes their shirt.
Turn the device on, and ensure that the participant’s name and ID appear on screen.
An image similar to that below will then appear on the device interface, showing with flashing points where to place the electrodes.
Remove the backing from the red adhesive electrode first and stick it on the correct position on the participant’s upper chest.
Then place the brown electrode in the correct position on the participant’s chest.
If both the red and brown electrodes have been placed on the participant’s chest in the correct positions, the flashing points on the device interface will stop flashing, indicating that they are indeed placed correctly.
The black and white electrodes are then placed in the correct positions on the chest, and lastly the green electrode is stuck to the chest.
Ensure that all the flashing points on the device interface have stopped flashing, indicated all electrodes have been placed correctly.
Scroll through the screens on the device to see the different heart rhythms.
Press the start button to begin recording.
Ensure the participant receives extra adhesive electrodes in case any fall off before the commute the following day.
When the participant returns the following day, check that all electrodes are still in place before the commute begins.

**Diagram 1: Placement of Holter Monitor Electrodes**
Placement of Electrodes for EASI Hookup

- Red (-)
- Green
- Black (+)
- Brown (+)
- White (-)
Appendix I.

PRE-RUN SETUP

- Clear trunk and load personal items on cart
- Setup batteries in parallel, connect pumps, connect tubing
- Warm-up pumps for 20 minutes
- Document Make/Model/Year/VIN#
- Window treatment and exhaust tubing
- Door Magnets
- Notebook, clock, & extinguisher in rear seat
- Setup Filters, hand tight
- Connect Pumps
- Measure Flows/Pressure
  - Initial reading on dry gas meter (PUFF)
  - QU1
  - QU2
  - GCMS
- Log Instruments
  - CPC (start early, 10 min startup)
  - PTRAK
  - AEROTRAK
  - MICRO AETH
  - PAS
  - DOSIMETER
  - GPS
  - CAMERA
  - Blood Pressure Cuff (right ear lobe)
  - Pulse Ox (left arm)
- Document subject, date, personnel, notes
POST RUN BREAKDOWN

☐ Measure Flows/Pressure
  ☐ Initial reading on dry gas meter (PUFF)
  ☐ QU1
  ☐ QU2
  ☐ GCMS

☐ Turn off Pumps

☐ Load filters in bags

☐ End Log Instruments
  ☐ CPC (start early, 10 min startup)
  ☐ PTRAK
  ☐ AEROTRAK
  ☐ MICRO AETH
  ☐ PAS
  ☐ DOSIMETER
  ☐ GPS
  ☐ CAMERA
  ☐ Blood Pressure Cuff (right ear lobe)
  ☐ Pulse Ox (left arm)

☐ Clear trunk and replace personal items

☐ Retrieve window treatment

☐ Retrieve Door Magnets

☐ Clear backseat

☐ Document subject, date, personnel.

☐ Notes/Observations
Appendix J: Continuous Sampling: Instrument Programming

Before you begin programming instruments, ensure all data from the previous commute has been downloaded and saved in the relevant folder on the secure network drive: “T:\EohProjs\Sarnat\ACE Study\ACE Study DATA\*Assigned SubFolder*”.

*”Assigned SubFolder” – Label includes Participant ID and date of commute, e.g., HE44_01282011

Refer to the Excel document saved in each participant folder, e.g., InstrumentProgramming_HE44.xls. This document serves as a checklist providing information on which instruments have been downloaded and by whom. This document is also used to save similar information regarding instrument programming. As instruments are programmed, update this document.

If data needs to be downloaded, refer to the Project 2: Standard Operating Procedures - Continuous Sampling Instrumentation: Data Download for more information.

1. Holter Monitor
   - Replace used AAA batteries with new ones
   - Plug instrument into USB attachment and connect device to the computer; USB Hardlock key needs to be inserted to ensure program-device recognition
   - Open the computer program
   - Tools
   - Recorder Utility
   - Select ‘Philips Recorder’
   - Enter patient information
   - Select ‘Write to Philips Recorder’
   - Close
   - Exit program
   - Remove device and USB Hardlock key

2. Blood Pressure Cuff
   - Replace used AAA batteries with new ones
   - Connect device to the computer and then open the computer program
   - New patient
   - Select ‘ACE’ folder
   - Complete patient information
• OK
• Configure Device
• Select Configure Device Parameters
• Period 1: 6.30am with intervals of 15 min
  Period 2: 11pm with intervals of 15 min
  Make sure that all the boxes along these two rows are checked
• Select ‘ACE’ folder on the top left
• Find the correct/current participant and select
• Send Data to ABPM device
• A prompt will ask to erase data
  o Select Yes
• OK
• Close program
• Remove device

3. Pulse OX
• Replace used AA batteries with new ones
• All programming on the device itself
• Clear Menu: Hold down the right function button; while holding it down, press the large Power button. Release power button, and then the right function button
• Press the right function button till the screen reads ‘CLR YES’, and then press the middle power button. Then press the function button again until the screen reads ‘DEL YES’, and press the middle power button again
• Date: Use the right function button to move through the time options and then the power button to select/confirm the correct year, month, day, hour and minute.
• Power instrument off once all selections have been made correctly

4. Audio/Dosi
• Replace used 9V batteries with new ones (Batteries for this device are not changed every commute, but rather every other commute)
• Hold the ‘Record’ button down, and then the ‘Power’ button. Release the ‘Power’ button and then the ‘Record’ button
• Clear memory
• Instrument needs to be off for the next two steps listed below:
• Time: Hold down the ‘Set-up’ button; press the ‘Power’ button. Release the ‘Power’ button and then the ‘Set-up’ button
• Press the ‘Level’ button to increase the date and time. To move through the different options, e.g., day, month, year, etc., use the ‘Setup’ button
  nn = minutes
  h = hours
  d = days
  H = month
  Y = year
• When you have finished setting all the date and time options, press the ‘Hold’ button

5. PAS
• Open the computer program first
• Plug instrument into computer
• Select Set Watch
• Turn the instrument on
• Dialogue box with the time will appear, set the time
• OK
• Turn instrument off
• Unplug instrument from the computer
• On the back of the instrument the first 3 white pins should be up and the last one should be down
• Turn the instrument on
• Screen will read: Clear Memory! Wait...
• When the Instrument screen reads: Clear Memory Finished
• Turn the instrument off
• Flick all the white pins down
• Plug the instrument into power supply to charge

6. BC/Aeth
• Plug instrument into computer and turn instrument on
• Open the computer program
• Erase all data
• File
• Device Settings
• Sync Time Date
• Time base: 60 seconds
• Flow: 100uL/min
• Set all parameters
- Select File Shutdown on the computer program to turn the instrument off
- Plug instrument into electrical outlet to charge

7. **Aerotrak**
   - Programming on the instrument itself
   - Set up
   - Sampling
   - Sample Timing
   - Time: 00:00:01
   - OK
   - Set up
   - Device
   - Date and Time
   - Click ‘Sync with computer’ manually
   - OK
   - To clear the memory return to the main menu
   - Set up
   - Data
   - Clear Samples
   - Yes
   - Plug instrument into electrical outlet to charge. Make sure that the device is turned on otherwise it will not recharge

8. **PTrak**
   - Replace used AA batteries with new ones
   - Open the computer program first
   - Plug instrument into computer and turn instrument on
   - Instrument set up
   - Parameters
   - Clock
   - Press the arrow keys >> Date >> Time and then press Send
   - OK
   - Instrument Set up
   - Logging Setup
   - Set log intervals to 00.01
• Send
• OK

• On the instrument itself
• Set up
• Clear memory
• Test logged memory
• Close the computer program and then turn the instrument off
• Check alcohol cartridge

Appendix K: Project 2 Continuous Sampling: Data Download

Immediately preceding a commute, ensure all data is downloaded and saved in the relevant folder on the assigned secure network drive:
T:\EohProjs\Sarnat\ACE Study\ACE Study DATA\“Assigned SubFolder”*

*“Assigned SubFolder” – Label includes Participant ID and date of commute, e.g., HE44_01282011

1. **Holter Monitor**
   • Plug instrument into USB attachment and connect device to the computer; USB Hardlock key needs to be inserted to ensure program-device recognition
   • Open the computer program
   • New
   • Select ‘Philips Recorder’
   • Next
   • Fill in patient information
   • Usually on ‘Asleep’
   • Next
   • Select ‘Standard’
   • Finish
   • Click on data (should be able to see the graphs)
   • File
   • Save As: ‘PatientID_ECG_Raw_mmddyyyy.zhr’
   • Close program and remove the device and USB Hardlock key

2. **Blood Pressure Cuff**
   • Plug instrument into computer and then open the computer program
3. Pulse OX

- Open the computer program first
- Plug instrument into computer
- File
- New Data Capture: 2500/920M Plus Series
- Hold down the right function button; while holding it down, press the large Power button. Release power button, and then the right function button: “Receiving”
- Once the download is complete, Select the data file
- Save
- Select New: Complete information about participant
- Save to desktop as ‘.mni’ file
- Open ‘.mni’ file from the desktop, and then File Export as an ‘.asc’ file
- Delete the ‘.asc’ file type and replace it with ‘.csv’ file
- Save As: ‘PatientID_PulseOx_mmddyyyy.csv’
- Close program
- Turn off the instrument and unplug from the computer

4. Audio/Dosi

- Open the computer program first
- Plug instrument into computer and turn instrument on
- Press ‘Setup’ button on the instrument
- Make sure it is showing the same reading on the computer as on the instrument screen
- If the display is not synced: Select ComPort on the top panel, and select Com5
- Select ‘Data Logger’ on the computer program
- Select the file on the left by double-clicking
- To download click on the graph on the right
- Save data
- Save As: ‘PatientID_Audio_mmddyyyy.txt’
- Turn off the instrument and then close the computer program
5. PAS
   - Open the computer program first
   - Plug instrument into computer and turn instrument on
   - Select the files found
   - Data is saved as a ‘.csv’ file on the ‘C. Drive’. Need to move it to the correct folder and convert it to ‘.dat’ file.
   - Save As: ‘PatientID_PAS_mmddyyyy.dat’
   - Turn off the instrument and then close the computer program
   - Plug the instrument into power supply to charge

6. BC/Aeth
   - Plug instrument into computer and turn instrument on
   - Open the computer program
   - Get data
   - Follow the link to where the data has been saved on the computer
   - Save
   - Move it to the correct folder
   - Save As: ‘PatientID_Aeth_mmddyyyy.dat’ file
   - Select ‘File Shutdown’ on the computer program to turn the instrument off
   - Plug instrument into electrical outlet to charge

7. Aerotrak
   - Plug instrument into computer and turn instrument on; wait till all the information has loaded
   - Open the computer program
   - Download all
   - Save As ‘PatientID_Aero_mmddyyyy.csv’ file
   - Quit
   - Turn instrument off
   - Plug instrument into electrical outlet to charge. Make sure that the device is turned on otherwise it will not recharge

8. PTrak
   - Open the computer program first
   - Plug instrument into computer and turn instrument on
   - File
• Receive
• Select the test from the larger dialogue box
• Receive
• Select the test again
• File
• Save As:
  o Save the raw file as it is ‘PatientID_Ptrak_Raw_mmddyyyy.tkp’
  o Select Test File
    - Export
    - Select the test again
    - Export and save file as ‘PatientID_Ptrak_mmddyyyy.txt’
• Close program
• Turn instrument off

9. CPC
• Run
• Receive logged data
• Select sample – Receive
• File
• Save As ‘PatientID_CPC_mmddyyyy.cox’
• File
• Export
• OK (time stamped and tab delimited)

10. GPS
• Connect to internet (not strictly necessary, but will get error messages if Google Maps is not available)
• Turn on unit (blue light will appear)
• Open GPS Photo Tagger
• Click ‘Read GPS device log’ in the upper left corner
  o Will count down from 10
• Select Track
  o Track will display in Google Maps if internet is available
  o If cursor is hovered on track, it displays lat, long, time, and velocity
• Select File, Export Track(s)
  o Select track
- Save as ‘PatientID_GPS_mmdyyyy.extension’
- Save in 3 different formats: .gpx, .kml, and .csv (for .kml select ‘yes’ when asked to simplify)
- Save as .itm
  - Select File, Clear Device Log
  - Close program, remove device, and plug into the microAeth charger
    - If the green light is on, fully charged – and can be unplugged

Notes:
Time is transmitted to the device by the GPS satellites:
- Check that the laptop is synchronized to the US Naval Observatory by clicking the bookmarked link.

Photos can be added to tracks and will be matched by time
- Requires that camera clock is synchronized to GPS

Track can also be graphed by speed to locate where traffic was stopped
- Select ‘Speed/Altitude View’ on right-hand side