ADDITIONAL MATERIALS 1

Section 1.2. MOSES Manual of Operation

with Appendices A–G

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1 Summary and Introduction Materials

1.1 Study Description

The Multicenter Ozone Study in Elderly Subjects (MOSES) is a randomized clinical trial of Ozone (O_3) exposures in health older adults while undergoing a standardized exercise regimen. The study will enroll approximately 140 subjects recruited from three clinical centers/ laboratories in order to obtain data from 90 subjects who complete the entire study. Each subject will undergo O_3 exposure in a chamber while exercising. The exposures will be randomly assigned and each subject will undergo exposures to clean air, 70 parts per billion (ppb) and 120 ppb. The study is sponsored by the Health Effects Institute (HEI) and is being conducted by investigators at the University of Rochester Medical Center (URMC), Rochester, NY; the University of California (UCSF), San Francisco, CA; and the University of North Carolina (UNC) at Chapel Hill, NC. The Data and Statistical center is the New England Research Institutes (NERI, Watertown, MA). The study is registered on ClinicalTrials.gov (identifier NCT01487005).

The primary aim of MOSES is to test the hypothesis that ozone reaction products in airways surface liquid interact with lung cells (e.g., epithelial cells, macrophages, nociceptive sensory nerve endings, endothelial cells), and that these cells produce mediators that enter the pulmonary and systemic vasculature. In addition to its (local) effects on airway inflammation and lung function, ozone may have cardiovascular effects including:

(1) Altered autonomic balance (measured as changes in heart rate and heart rate variability (HRV)), cardiac arrhythmia, and repolarization;
(2) Altered systemic vascular function (measured as brachial artery flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NTG));
(3) Pro-thrombotic vascular state (measured as increase in von Willebrand factor antigen and ultra large multimers in blood); microparticle –associated Tissue Factor activity, and platelet activation.

Markers of systemic oxidative stress and inflammation will be also measured.

1.2 Study Protocol

All centers will use a Common Protocol (See Appendix A).

1.3 Informed Consent

Each center will use an Informed Consent form derived from a common template and customized to site specific rules and regulations.

2 Study Coordination and Communication

The MOSES Organizational and Management Structure and Safety Monitoring Plan in Appendix B outlines the organization of the MOSES stakeholders and the frequency and method of planned communications for the MOSES study and describes the plan for monitoring the safety of the subjects. Appendix C lists the contact information for the four centers' principal investigators and other key personnel.

2.1 Core Laboratories

Collected blood and sputum specimen, filters from the personal exposure sampler, Holter recordings, and brachial artery ultrasound images will be analyzed by core laboratories. A list of the core laboratories with shipping addresses and list of contact personnel is provided in Appendix D.

3 Subject Recruitment and Enrollment

3.1 Recruitment and Identification of Subjects

Recruitment strategies have been developed by each specific site. When a potential subject contacts a site for study information, a site-specific Subject Contact Record (SCR) is started. The SCR provides the site with a means to organize subjects from initial contact through initial phone screen. All SCRs should be filed alphabetically in a binder and stored in a locked drawer or cabinet until the subject has been deemed eligible or ineligible to participate.

3.1.1 Obtaining Informed Consent

Consent to participate is obtained at two points. Verbal consent is collected before the Initial Phone Screen Questionnaire is administered and written consent is gathered at the in-person screening assessment. When presenting the statement of informed consent, either verbally or in writing, it is important to ensure that the subject understands what is being asked of them, as well as the risks and benefits of participation, and that the subject consents freely to participate.

During the telephone screening, verbal consent is obtained by reading the standardized script part of the Initial Phone Screen Questionnaire (describing the study, the nature of the subject's involvement and possible risks and providing contact information for the principal investigator (PI), if desired. The interviewer signs Page 1 of the questionnaire to indicate that the script was read and that the subject agreed to the interview.

At the time of the in-person screening visit, a written Informed Consent (approved by the IRB) for the study is presented to the subject before any of the measures are conducted. The staff person presenting the statement of consent should allow sufficient time for the subject to read the form and ask questions. If the staff person cannot provide sufficient or satisfactory responses, the subject can be referred to the PI or a co-investigator, as determined by the site. Data collection or testing cannot proceed until written consent is obtained.

The subject shall be given a signed copy of his or her Informed Consent. The site shall retain a signed copy of the Informed Consent in a locked drawer or cabinet. Only after the subject has signed the Informed Consent will he/she be assigned a subject identification (ID) number (see section 3.4).

The subject reimbursement fee is $2,100 divided as follows: $50 for screening visit, $100 for training visit, $1,800 for all exposure visits ($150 for each pre-exposure visit, $300 for each exposure visit, $100 for each post-exposure visit, and $50 for personal monitor and Holter use), $150 bonus for completing all the visits.

3.2 Subject Eligibility and Enrollment

During the initial phone screen, once a potential subject consents to the initial phone screen, the Clinical Site Coordinator (CSC) will begin reading the questions on the Initial Phone Screen

Questionnaire. If at any point a question is answered in a bolded, grey cell, the subject is ineligible and:

- the questionnaire should be terminated;
- The thank you script at the end of the Initial Phone Screen Questionnaire should be read to the subject;
- An entry onto the Unenrolled Subject Tracking Form should be made; and
- Both the SCR and Initial Phone Screen Questionnaire should be shredded.

Once a subject completes an Initial Phone Screen Questionnaire and has been deemed eligible, and is willing to come in for a screening visit:

- The Locator Form should be filled out;
- An appointment for the screening visit should be made; and
- The screening visit should be recorded on the site-specific calendar.

The Locator Form and Initial Phone Screen Questionnaire should be stored together in a locked drawer or cabinet. Once written informed consent is obtained, the phone screen form shall be maintained with the subject's study documents.

Inclusion criteria:

1. ≥55 and ≤70 years of age; nonsmoking males and females of all ethnic backgrounds.
2. Normal spirometry (FEV1 and FVC ≥75% of predicted and FEV1/FVC ≥0.65). Predicted values will be determined using the correction factor specific for African Americans and Mexican Americans (which will include also Hispanic, Latinos, Spanish), but not for Asians, based on the NHANES III guidelines.
3. Ability to complete the training exercise regimen chosen to induce a ventilation rate of 15 to 17 L/min/m2 without exceeding 80% of predicted maximal heart rate.
4. Normal baseline 12-lead resting ECG, and absence of significant ST depression while performing the 15-minute required level of exercise targeted for the exposure period.
5. Subjects must be able to avoid the medications supplements listed in Table 1 of section #7 of the Common Protocol for 1 week before the exposure.

Exclusion criteria:

1. Non-English speaking.
2. Including, but not limited to as ascertained by the physicians: Subjects with chronic cardiovascular (such as ischemic heart disease) or respiratory (such as asthma or COPD) disease; diabetes, or other organ or system dysfunction; cerebrovascular disease; active psychiatric disorders that would interfere with the subject's ability to understand and participate in the study. Subjects who have tested positive for a disease that affects the immune system (such as HIV, lymphoma, leukemia) or current drug or alcohol abuse (defined as having more than 3 drinks per day or being unable to abstain from alcohol for 3 days).
3. Subjects with atopy or allergic rhinitis will not be excluded as long as they do not require regular treatment with antihistamines or systemic steroids.
4. Ever-smokers (smoked tobacco or marijuana during the last five years, or with history of >10 pack year for tobacco or > 1 joint year for marijuana, or living with a smoker who smokes inside the house).
5. Subject having plasma cotinine level > 3ng/mL.

6. BMI >35 and <18 (35 is the official cut off for class 1 obesity).
7. Hypertension (defined as blood pressure >140 systolic or >90 diastolic) or on anti-hypertension medications other than diuretics.
8. Pregnancy or nursing (breastfeeding).
9. On the following medications: prednisone, statins, beta-blockers, anticoagulants, current systemic estrogen therapy, and tamoxifen. Subjects will not be asked to discontinue needed prescription medications for the purpose of this study. If any of these medications becomes necessary during the course of the study, the subjects will be excluded. Use of other medications will be considered on an individual basis (see section # 7 in the common protocol).
10. Subjects taking aspirin or PDE5 inhibitors must be willing to abstain from these medications during the week preceding each exposure (see section #7 in the common protocol).
11. Occupational exposures (exposed to high levels of vapors, dust, gases, or fumes on an on-going basis)

A subject is deemed eligible and is enrolled in the study when all inclusion and no exclusion criteria have been met. This is accomplished when the In-Person Screening and Training visits have been conducted and blood analysis results have been returned and assessed. At the end of training visit the Inclusion and Exclusion Check lists should be completed and filed in the subject's file.

3.3 Randomization

There are six possible ways of ordering the exposures to three different levels of ozone. Each ordering defines an exposure plan. Participants will be randomly assigned to one of the six plans using permuted blocks with institutional balancing. This ensures that that within each center approximately the same number of subjects would receive each exposure plan. For randomization by permuted blocks, the participants are randomized in blocks of six so that within each block of six participants all exposure plans are used. The assignment of the exposure plans within a block of six participants is randomly determined.

Subjects are randomized during the first pre-exposure day, after consent has been granted, all screening and training data has been obtained, and the subject meets all inclusion and no exclusion criteria. MOSES subjects are randomized using an envelope based system. Each site has been given a package containing 36 envelopes. Each of those envelopes is identified with a label stating ORDER # n where n is between 1 and 36 inclusive. This order number is the sequence of how the envelopes are to be used – e.g. assignment order to subject.

Envelope Flow – When a subject is randomized, select the next sequential envelope from the package. Affix a subject ID label on the envelope and on the randomization tracking sheet attached to the randomization package. The envelope contains 3 white envelopes indicating which should be used at which exposure. The CSC is not to open the white envelopes as they are to be blinded to the subject's exposure (This is not feasible at UCSF).

On the pre-exposure day, the CSC will deliver the appropriate envelope (First Exposure will use Exposure 1 envelope, Second Exposure will use Exposure 2 envelope, etc.) to the exposure technician. Details of the exposure are below in Section 4.4 Exposure Characterization. Once a subject has been assigned a Randomization Envelope, no deviation shall be made in the order of ozone exposures. Should any deviation inadvertently occur, NERI should be contacted immediately and a protocol deviation form will be filled out.

The assembly of the randomization packets was documented and quality control steps were performed on each packet. The documentation and quality documentation is maintained on the NERI file share.

3.4 Subject identification (ID) and labels

Subject ID will consist of 5 digits: the first one identifies the center (1- U Rochester, 2- U North Carolina, 3- U California at San Francisco), the second, third, and fourth digits indicate the subject's order of recruitment (101, 102, etc.), the fifth is a random number generated by the program.

Labels to identify the subject will include the subject ID, a code for the visit type (SCRN-screening visit; TRAIN – training visit; PRE – pre-exposure day; EXP – exposure day; POS – post-exposure day). PRE, EXP, and POS visits will be followed by a number that identifies the order of the three sets of visits (1, 2, and 3). The combination of ID and visit type is referred to as "subject ID label".

Examples of subject ID labels are: 20035 TRAIN or 20035 PRE1.

In addition, the specimen label contains an identifier for the endpoint to be measure in that specimen.

Labels for processed blood and sputum specimen will have the visit type abbreviated as follows: PRE1=V3; EXP1=V4; POS=V5; PRE2=V6; EXP2=V7; POS2=V8; PRE3=V9; EXP3=V10; POS3=V11. Examples of labels for the various specimens are provided below.

1) **Phlebotomy at screening**. Pre-labeled tubes for the phlebotomy at screening are provided by LabCorp. However, the centers will need to collect an additional tube of blood for genotyping. This will be split into two aliquots. The labels for the two aliquots will be Subject ID V1 BL1 (or BL2).

2) **Phlebotomy PRE, EX, POS.** The label of each of the four tubes used to collect the blood (excluding the discard tube) includes the subject ID, the type of exposure (PRE1, EXP1, POS1, etc.), the tube color (e.g., blue) and the order of drawing (1, 2, 3, 4). Example of label 20035 PRE1 Blue 1, 20035 PRE1 LAV 4

   a) Labels of the tubes containing the processed blood include the subject ID followed by V3, V4, V5 (etc.), the original tube number (1, 2, 3, 4), and the endpoint. There will be a line below this information for the date to be written in.

      Tube 1 will be processed for three endpoints:
      One tube will be generated for flow cytometry (FC).
      Example of label: 20035 V3 1FC
      5 tubes will be generated for MP-TF.
      Example of label: 20035 V3 1TF1 (20035 V3 1TF2 …up to 1TF5)
      5 tubes will be generated for wWFM.
      Example of label: 20035 V3 1vWF1 (up to 1vWF5)
      Tube 2 will be processed for Assay Gate:
      One tube will be generated for soluble markers (SM).
      Example of label: 20035 V3 2SM

Tube 3 will be processed for archiving plasma (PL). 3 tubes will be generated.
Example of label: 20035 V3 3PL1 (up to 3PL3)
Tube 4 (example 20035 PRE1 LAV 4) will be sent to the lab for CBC. No additional labels are needed.

3) **Sputum sample**. The label of the collected sputum sample will include the subject ID label and the endpoint. Example 20035 POS1 SPUTUM. a) Labeling of sputum slides.
Example of label: 20035 V5 (V8, or V11) SPUT1 (up to 4). b) Labeling of tubes containing sputum supernatant (up to 5).
Example of label: 20035 V5 (V8, or V11) SPUTSN1 (up to SN5).
c) Labeling of tube for mRNA archive.
Example of label: 20035 V5 (V8, or V11) SPUTRNA

4) **BAU images** from one measurement will be copied to a CD. The CD will be identified with a subject ID label.

5) **PES filters**. The Personal Exposure Sampler will be assembled with 2 repositionable labels. These labels are affixed onto the backing of the PES and on the outside of the PES holder. The filters are sent out for analysis with the repositionable labels on the tubes.

4 Study Procedures and Measures

4.1 Scheduling Subject Visits and Subject Instructions

Subjects will participate in a total of 11 visits: Screening, Training and 3 Exposure Visits with each Exposure Visit consisting of 3 consecutive day visits. Total duration of subject participation, from the screening visit to final visit, will be approximately 1.5-3 months and could be up to 12 months depending on the clinical site's scheduling of the exposure visit(s).

The acceptable time windows between visits are the following. The period between the screening and training visit cannot be more than 8 weeks (that is, once the screening visit is completed, the training visit has to occur within 8 weeks.) If the subject does not qualify after either the screening or training visit, but returns to the clinic to repeat some procedures within the 8-week period, he/she needs the repeat only the screening/training procedures for which he/she was not eligible. After this 8- week period, if the subject did not qualify, but is recruited again, he/she should be treated as new subject, assigned a new number, and start with the Phone Screen Questionnaire. The period between screening and the last exposure (completion of the study) should not exceed one year and the period between one exposure and the next should not exceed 6 months.

The following items should be given to the subject to remind what he or she should do the week before the exposure visit; 1) a Personal Exposure Sampler (PES), which the subject needs to wear for the three days preceding the pre-exposure day (starting at noon); 2) Instructions for use of PES; 3) general subject instructions about medications and beverages not allowed; 4) a time activity diary; and 5) instructions for filling the activity diary. A subject should not get a flu show within 3

days before an exposure.

A summary of study-related visits is provided in Table 1 below.

Table 1. Schedule of Visits/Procedures

Procedure	Screening Visit	Training Visit	3-4 Days before Exp By phone	Exposure 1 Days			Exposure 2 Days			Exposure 3 Days		
				PR1	E1	PO1	PR2	E2	PO2	PR3	E3	PO3
Informed Consent	X											
Repository Consent	X											
HIPAA Consent	X											
Pregnancy Test	X											
Home & Health Questionnaire	X											
Height / Weight	X											
Heart Rate	X			X	X	X	X	X	X	X	X	X
Temperature	X			X	X	X	X	X	X	X	X	X
BP	X	X		X	X	X	X	X	X	X	X	X
Physical Exam	X			X			X			X		
12- Lead ECG	X	X										
Spirometry	X				X	X		X	X		X	X
Metabolic Profile	X											
Cotinine Levels	X											
CBC	X			X	X	X	X	X	X	X	X	X
Gene Profiling	X											
Exercise		X			X			X			X	
SpO_2 (optional)		X			X			X			X	
Minute Ventilation		X			X			X			X	
PES measurement			X									
Activity Diary			X									
Before Pre-Exposure Day Health Questionnaire			X									
Flow Cytometry				X	X	X	X	X	X	X	X	X
Soluble blood markers				X	X	X	X	X	X	X	X	X
MP-TF				X	X	X	X	X	X	X	x	X
BAU – FMD ~~and NTG~~				X	X		X	X		X	X	
Symptom Questionnaire					X	X		X	X		X	X
HRV					X	X		X	X		X	X
Sputum Induction						X			X			X

4.2 Reminder Calls

For the Screening and Training visit, the CSC telephones the subject's preferred phone number on the day before the visit, to remind the subject of their upcoming appointment.

For Exposure Visits, the CSC telephones the subject's preferred phone number 8 or 9 days before the pre-exposure visit to administer the Before Pre-Exposure Visit Health Questionnaire. The purpose is to find out whether the subject's health status has changed and remind the subject to abstain from certain medications. Another call is made 3 to 4 days prior to the exposure visit using a site-specific script to remind the subject of their upcoming visit, to start wearing the PES, and to track activities in the activity diary.

A specific timeline has been developed to allow for sites to manage all of the subjects' visits. The Timeline of Measurements, containing the overall study timeline can be found in Appendix E.

Visit-specific checklists have been defined for each interaction with the study subject. The order of activities and times indicated on the checklists (which match the Timeline of Measurements) shall be followed to the best of the site and subject's ability. All pertinent data regarding the specific interaction shall be recorded on the checklist and stored with the subject's study file. See Appendix F for a list of the checklists and questionnaires. All checklists and questionnaires are accessible on the HEI file share site (http://www.filesanywhere.com/). Tracking and other relevant forms are also listed in Appendix F.

4.3 Specimen Collection and Processing

All specimens will be collected according to the specific SOP for that specimen. See Appendix G for the list of SOPs. All SOPs are accessible on the HEI file share site (http://www.filesanywhere.com/).

4.4 Exposure characterization

The subjects will be exposed for 3 hours to filtered air, ozone at 70 ppb, and ozone at 120 ppb in a randomized order. The subjects will exercise intermittently during the exposure. Ozone will be measured continuously during each of the three exposures. The air entering the exposure chamber will be purified by passing it through several filters (Purafil, charcoal, HEPA). Ozone will be generated by a high-voltage corona-spark, silent discharge ozonator.

In addition, particle number will be measured every three month during each of the three exposures. The first measurement will be made during the exposure of the first subject. Temperature and relative humidity will be maintained at approximately 22 ^{o}C and 40%, respectively.

The CSC will give the envelope pertaining to the exposure (Envelope 1 for Exposure 1, etc.). Instruct the technician to open the envelope to find the Ozone Chamber Form contained in the envelope, which contains the concentration of ozone for that exposure. Only the exposure technician should know the type of exposure to be generated (this is not feasible at UCSF).

The ozone chamber should be set to produce the randomized amount contained on the Ozone Chamber Form. Temperature, humidity, and ozone concentration will be measured continuously during the 3-hour exposure period. The technician should be instructed to enter the average (and standard deviation) of the three sets of continuous data on the Ozone Chamber Form, sign and date it The Ozone Chamber Form should then be inserted into the envelope addressed to the

data center (NERI) and mailed. NERI staff will enter the data in ADEPT.

Follow this same procedure for each exposure.

4.5 Data Collection

Data for the MOSES study are collected in questionnaire and diary format as well as physical measures that will be reported in data forms.

The Home and Health Questionnaire is collected using a Web-based data management system (DMS), Advanced Data Entry and Protocol Tracking (ADEPT). Once the subject has entered the questionnaire data, the CSC will produce a report of the data from the ADEPT DMS and place the report in the subject's research file.

Among the question there is one about smoking history. Since the subject may have smoked different types of tobacco, the following conversion factors will be applied:

Smoking conversion table (October 1, 2012)

Type of smoking substance	Conversion factor to cigarette
Cigarette, one (factory-manufactured or hand-rolled)	1
Pipe,one[1]	2.5
Cigar, one (average of small, medium, and large)[1]	3
Hookah, average of smoking for 20 and 60 minutes[2]	56
Marihuana (joint)[3]	5

1. Based on GB National Health System Education for Scotland accessed 8/2/2012
2. Average of smoking for 20 and 60 minutes based on smoking calculator http://smokingpackyears.com/calculate, accessed 8/2/2012
3. Based on British Lung Foundation report The Impact of Cannabis on Your Lung 2012 http://www.blf.org.uk/Home

An Activity Diary will be filled out by the subject during the three days preceding the exposure day.

On each Pre-Exposure day, the CSC will collect the activity diary which the subject has filled out on paper as well as administer a paper version of the Pre-Exposure Day Health Questionnaire.

On each Exposure and Post-Exposure day, the subject will fill out a Symptom Questionnaire both before and after the exposure. See Appendix F for a list of the questionnaires. All questionnaires are accessible on the HEI file share site (http://www.filesanywhere.com/).

4.6 Subject Stipends

The subject will be compensated according to the protocol. Each site will follow their individual site's procedures for preparing and distributing the compensation.

5 Data Analyses

All data collected will be transmitted to NERI as they become available. A plan for data analysis is being developed. A brief description of the plan can be found in the Common Protocol (Appendix A).

6 Adverse Events (AEs)

6.1 Grading Adverse Events

Each AE will be graded as follows:

Grade 1 Mild: transient mild discomfort; no limitation activity; no medical intervention or therapy required.

Grade 2 Moderate: minimal noninvasive intervention or therapy (such as over-the-counter medication, application of ice, etc.) is allowed.

Grade 3 Severe: marked limitation in activity; some assistance required; medical intervention or therapy required; hospitalization possible.

Grade 4 Life-threatening: extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization probable.

6.2 Reporting Adverse Events

Any grade 3 or 4 event is to be reported within 24 hours of learning of it by entering an AE form into ADEPT. When a grade 3 or 4 AE is entered into ADEPT, an email is generated notifying the Steering Committee and HEI. Grade 1 or 2 AEs will be reported within 5 working days of learning of it, following the same data entry procedure. A report of all AEs is available within the ADEPT system.

Each site has its own individual reporting responsibility to their site specific IRB.

NERI will periodically generate a report of all adverse events and submit it to the Steering Committee, the DMB, and HEI. For more details about the plan for monitoring the safety of the subject see Appendix B.

7 Quality Assurance/Quality Control

Written records will be maintained to document all aspects of the research effort. This shall include the use of checklists, notebooks, standard forms, and computer input and output. All entries shall be made in indelible ink. The entries should be dated and signed or initialed by the individual making the entry. Notebook entries shall be made in chronological order. If a blank space is left between entries, it shall be crossed-hatched to render it unusable. Entries shall not be erased or otherwise obscured. If any entry is to be changed because it is in error or for any other reason, a single line will be drawn through the entry and a correction made in the margin. The altered entry shall carry an explanation of the reason for the change, the date of the change, and the initials or the signature of the individual making the change.

The Principal Investigator for the project shall periodically review the records to verify their completeness and accuracy. This review shall be documented by the Principal Investigator

signing and dating the reviewed record according with the center-specific QA procedures

All SOPs shall be signed by each Principal Investigators. Deviations from SOPs will be justified and documented according to center specific procedures. The degree of adherence to the SOPs may be determined during periodic independent QA audits.

7.1 Training and Certification

The Principal Investigator has the responsibility for assuring that the research is conducted with qualified personnel and in accordance with the protocol and the SOPs.

All site personnel entering data into the DMS will undergo a training and certification process. The exposure generation and characterization system will be certified by the HEI QA auditors.

7.2 Site Monitoring

HEI has contracted with Mr. David Bushes of Technical and Business Systems for Quality Assurance oversight. Mr. Bush and others designated by him will conduct periodic audits of the research centers and may also audit the core laboratory.

8 List of Abbreviations

ADEPT	Advanced Data Entry and Protocol Tracking System
AE	Adverse Event
BMI	Body Mass Index
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CSC	Clinical Site Coordinator
DMS	Data Management System
DMB	Data Monitoring Board
ECG	Electrocardiogram
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume
FMD	Flow Mediated Dilatation
FVC	Forced Vital Capacity
HIV	Human Immunodeficiency Virus
HRV	Heart Rate Variability
HEI	Health Effects Institute
IRB	Institutional Review Board
MOO	Manual of Operations
MOSES	Multicenter Ozone Study in Elderly Subjects
MP-TF	Micro Particle Tissue Factor
NERI	New England Research Institutes, Inc.
NTG	Nitro Glycerin
O_3	Ozone

PDE5	Phosphodiesterase type 5 inhibitor
PI	Principal Investigator
ppb	parts per billion
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SCR	Subject Contact Record
UCSF	University of California, San Francisco
UNC	University of North Carolina
URMC	University of Rochester Medical Center
vWf	Von Willebrand factor

9 List of Appendices

All appendices are accessible on the HEI file share site (http://www.filesanywhere.com/).

A. MOSES Common Protocol

B. MOSES Organization and Management Structure and Safety Monitoring Plan

C. Stakeholder contact information

D. Core laboratory contact information

E. Timeline of Measurements

F. List of Checklists, Questionnaires and other forms

G. List of SOPs

MOSES Manual of Operations
APPENDIX A
MOSES COMMON PROTOCOL

(see Section 1.1 of Additional Materials 1)

MOSES Manual of Operation
APPENDIX B

ORGANIZATIONAL AND MANAGEMENT STRUCTURE AND SAFETY MONITORING PLAN

1. ORGANIZATIONAL AND MANAGEMENT STRUCTURE

The study will be overseen by the HEI Research Committee, a group of scientists with diverse expertise, which is responsible for developing and managing HEI research activities. The Research Committee will be responsible for all decisions regarding the renewal of the MOSES study contracts (including review of the progress reports) and approval of the budgets and budget changes, but has delegated the scientific oversight of the study to the MOSES Oversight Panel. *Two special bodies* of MOSES investigators and members of the Oversight Panel and HEI Research Committee have *been set up to coordinate the conduct of the study, monitor the data collection, and discuss issues related to subject safety and publication of the data: a Data Monitoring Board and a Steering Committee.* The role of these three groups is summarized below.

1.1 MOSES Oversight Panel

The Panel is composed of experts in clinical medicine, statistics, and pollutant measurements appointed by the Research Committee. Two of the members of the Panel are also members of the Research Committee. The responsibilities of the Panel include: overseeing the development of the common protocol and SOPs; approving changes in the protocol (in conjunction with the Research Committee) after the study has started; working with the Data Management Center to develop analysis plans; reviewing procedures for submission of data; reviewing reports of any adverse events/protocol violations; discussing issues associated with QA/QC.

The composition of the MOSES Oversight Panel is the following:

- Mark Utell, University of Rochester (chair)
- Richard Smith, University of North Carolina, Chapel Hill (statistician, HEI Research Committee)
- Howard Rockette, University of Pittsburgh (statistician)
- Petros Koutrakis, Harvard School of Public Health (exposure)
- Jesus Araujo, University of California, Los Angeles (environmental cardiologist)
- Arshed Quyyumi, Emory University (cardiologist)
- David Christiani, Harvard School of Public Health (physician, HEI Research Committee)
- Maria Costantini & Rashid Shaikh (HEI Staff)

The members of the MOSES Oversight Panel will be consulted *ad hoc* as needed.

1.2 MOSES Data Monitoring Board (DMB)

The MOSES DMB is composed of statisticians and cardiologists (including members of the HEI Research Committee and the Oversight Panel), the Data Coordinating and Analysis Center team, and HEI staff. The DMB will establish procedures for the timely submission of data from the centers, monitor the quality of the data, monitor the implementation of the data analysis plan, and monitor subject safety through review of data on adverse events. The DMB may also perform interim analyses of the data for the purpose of revising the power calculations or *to assess whether early stopping of the study might be warranted. The DMB will report any significant findings directly to the HEI Research Committee and, as necessary, to the three clinical centers' investigators.*

The composition of the MOSES DMB is the following:

- David Christiani (HEI Research Committee) - clinician
- Richard Smith (HEI Research Committee) -biostatistician
- Howard Rockette (MOSES Oversight Committee) - biostatistician
- Anne Stoddard (NERI) - biostatistician
- Patti Nash (NERI) – Data Manager
- Maria Costantini and Rashid Shaikh (HEI)

The DMB will have a Conference call at least every four month.

1.2 MOSES Steering Committee

The Steering Committee is composed of the Principal Investigators of the four centers and members of the HEI staff and members of the HEI Research Committee and the Oversight Panel. *The Steering Committee will implement the analytical plans,* monitor subject recruitment, discuss issues that may arise during the conduct of the study, *review reports to the individual institutional IRBs of adverse events and protocol violations, report adverse events and protocol violations to the DMB in a timely fashion, prepare and decide on authorship of reports and publications, and discuss and make decisions about requests for biological samples, data, and specific analyses (from either the individual centers' investigators or other researchers).*

The Composition of the Steering Committee is the following

- David Christiani (HEI Research Committee)
- David Eaton (HEI Research Committee Chair)
- Mark Utell (HEI ACES Oversight Committee)
- John Balmes (MOSES PI, UCSF)
- Phil Bromberg (MOSES PI, UNC)
- Mark Frampton (MOSES PI, UR)
- Patti Arsenault (MOSES co-PI, NERI)
- Maria Costantini (HEI)
- Rashid Shaikh (HEI)

The MOSES Steering Committee will have a conference call every *three* months and at least one meeting per year

2. SAFETY MONITORING PLAN

The grading and process for reporting Adverse Events are provided in SOP #51.

2.1 Frequency of Safety Reviews

Safety monitoring data, consisting of reports of adverse effects, as well as of study enrollment and dropout data , provided by the Data Coordinating and Analysis Center, will be reviewed on a regular (at least monthly) basis during Steering Committee teleconferences and reported to the DMB on a semi-annual basis.

2.2 Stopping Rules (as in SOP #51)

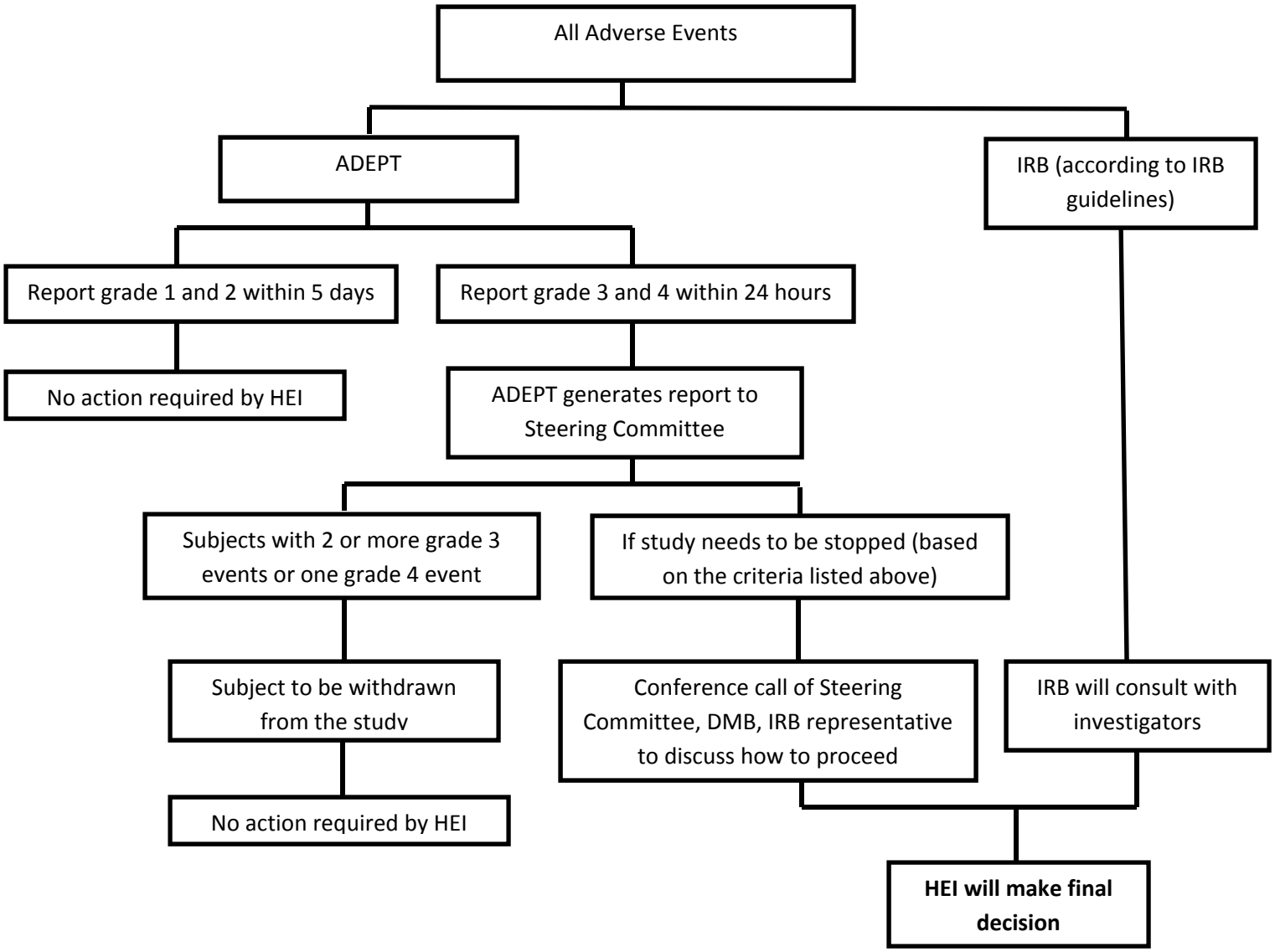
2.3 Plan for Resuming the Study if a Stopping Rule is Applied

The investigator(s) in whose institution the adverse event(s) occurred will provide an Updated Adverse Event Report that updates and expands the initial AE submitted to ADEPT. This new AE should document in more details how the event has resolved (with the exception of an event that resulted in the death of a subject) and explains when and where the event occurred, the diagnosis (including any underlying conditions and whether the adverse event could possibly be related to the study protocol, particularly exposure to ozone), and the outcome of the event. If the event followed one or more events of a lower grade, this should be specified in the Updated AE Report. The Updated AE Report should be submitted electronic to the HEI MOSES Project Manager and the HEI Director of Science. HEI staff will forward this report to the EPA and the members of the Steering Committee.

HEI staff will convene a conference call with the Steering Committee, members of the DMB, and a member of the IRB of the institution where the event(s) occurred to discuss how to proceed. The group could recommend to (1) take no action and resume the study, (2) take corrective actions that do not impact the protocol (for example replace the exercise machine) and resume the study, (3) modify the protocol before resuming the study, or (4) terminate the study at one or all the centers. After the call HEI (including the Director of Science, the HEI President, and members of the HEI Research Committee) will make a final decision on how to proceed.

- If the decision is to modify the protocol, the study will not resume until all the IRBs and the EPA have approved the protocol changes. The protocol change will be posted on the MOSES clinicaltrial.org web site.
- If the decision is to terminate the study at one center, the other two centers will resume subject recruitment (or submit any agreed upon protocol amendments to the IRB). The DMB will decide how the data collected by the center excluded from the study should be used in the data analyses. The decision will be posted on the MOSES clinicaltrial.gov web site.
- If the decision is to terminate the study in all centers the DMB will decide how to analyze the data collected up to that point. The decision will be posted on the MOSES clinicaltrial.org web site.

FLOW CHART FOR PROCESS TO REPORT ADVERSE EVENTS AND STOP AND RESTART THE STUDY



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MOSES Manual of Operations
APPENDIX E
Timeline of Measurement

(see Section 1.3 in Additional Materials 1)

MOSES Manual of Operations
APPENDIX F
List of Checklists, Questionnaires, and Other Forms

Checklists

Inclusion Exclusion Checklist
Screening Visit Checklist
Training Visit Checklist
Pre-Exposure Day Checklist
Exposure Day Checklist
Post-Exposure Day Checklist

Questionnaires and Diary

Before Pre-Exposure Visit Phone Questionnaire
Health and Home Screening Questionnaire
Initial Phone Screen Questionnaire
Medication List for Health and Home Screening Questionnaire
Medication List for Phone Screening
Pre-exposure Health Questionnaire
Symptoms Questionnaire
MOSES Activity Diary

Data Forms

Adverse Event Report Form
Vital Signs and Blood Pressure Screening Training Data Form
Vital Signs and Blood Pressure — PRE and POST Data Form
Vital signs and Blood Pressure Log Exposure Day
Training Exercise Data Form
Exposure Day Exercise Data Form
Spirometry Data Form
Randomization Packet Assignment

Instructions and Other Forms

Subject Instructions for Use of PES
Subject Instructions Before Exposure Visit
Subject Instruction for Filling Out the Activity Diary
Adverse Event Report Form
Protocol Deviation form
Locartor Information
Subject Withdrawal Form
BMI Table Metrics System
Spirometry NHANES III FVE1 Reference Values Accessed 12 15 11

Tracking and Shipping Forms

Unenrolled Subject Tracking Form
Brachial Artery Ultrasound (BAU) Tracking Form
Brachial Artery Ultrasound (BAU) Case Report Form
Sputum Supernatant and Slide Tracking Form
Holter Card Tracking Form
Pre- Exp- Post- Blood Tracking Form
Filters and PES Data Log Sheet
PES Return and Process
Ultrasound Maintenance Tracking Form
LabCorp Test Requisition Form
AssayGate Service Order Form
PES Filters Chain of Custody (shipping)

MOSES Manual of Operations
APPENDIX G
List of MOSES SOPs

Clinical Center Common SOPs
#1 Height and Weight (H&W)
2 Vital Signs(VS)/BP SCREEN AND TRAIN
3 Vital Signs (VS)/BP PRE, EXP, POS
#4 Exer MINVEN ECG BP (MINVEN) Training (TRAIN)
#5 Exer MINVEN ECG BP (MINVEN) Exposure Visits (EXP)
#6 Spirometry (SPIR) UR #6 Spirometry (SPIR) UNC #6 Spirometry (SPIR) UCSF
#7 Sputum Induction Processing Shipping and Analysis (SPUTUM)
#11 Phlebotomy (PHLEB) SCREEN
#12 Phlebotomy (PHLEB) EXP
#13 Flow Cytometry (FLOW)
#21 12-Lead ECG (ECG) Screening
#22 Holter Recording (HOLTER)
#31 Brachial Artery Reactivity and Doppler (BAU Analysis)
#32 Brachial Artery Ultrasound (BAU)
#41 PES Preparation, Storage, and Use (PES)
#42 Tech Instructions for TAD (OZ DIARY)
#51 Adverse Event Reporting Process

Clinical Center-Specific SOPs
SOP C18 MOSES Exposure Chamber and Ozone Analyzer Calibration (UofR)
SOP #71- Exposure Chamber and Ozone Analyzer Calibration (UNC)
SOP Human Exposure Laboratory Chamber WebCTRL (UCSF) SOP Human Exposure Laboratory Chamber LabVIEW (UCSF) SOP Human Exposure Laboratory Ozone Generation (UCSF)