PHASE I/II RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CROSS-OVER STUDY OF PREDNISONE ON AIRWAY INFLAMMATORY RESPONSE TO INHALED WOOD SMOKE

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Short Title:	Prednisone for wood smoke-induced airway inflammation
Drug Name(s):	Prednisone
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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AED	Automated external defibrillator
ADL	Activities of daily living
ATS	American Thoracic Society
BMI	Body mass index
BP	Blood pressure
BSA	Body Surface Area
CBC	Complete blood count
CEMALB	Center for Environmental Medicine, Asthma, and Lung Biology
Co57	Cobalt 57
CRP	C – reactive protein
DSMB	Data Safety Monitoring Board
EBC	Exhaled breath condensate
EKG	Electrocardiogram
FA	Filtered air
FEF_{25-75}	Maximal mid-expiratory flow rate
FEV_1	Forced vital capacity in one second
FVC	Forced vital capacity
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GSTM1	Glutathione-s-transferase mu 1
HR	Heart rate
HSF	Human Studies Facilities
IUD	Intrauterine device
LPS	Lipopolysaccharide
MCC	Mucociliary clearance
mCi	Microcurie
NCI-CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
NHANES	National Health and Nutritional Examination Survey
NSAIDs	Non-steroidal anti-inflammatory drugs
O_3	Ozone
PM	Particulate matter
PMN	Peripheral mononuclear cells (i.e. neutrophils)
RR	Respiratory rate
SAE	Serious adverse event
Tc99m-SC	Sulfur coloid
TLR	Toll-like receptor
WSP	Wood smoke particles

PROTOCOL SYNOPSIS

Study Title	Phase I/II Randomized, Double-blind, Placebo-controlled Cross-Over Study of Prednisone on Airway Inflammatory Response to Inhaled Wood Smoke
Funder	Department of Defense
Clinical Phase	Phase I/II
Study Rationale	Deployment of military personnel has been associated with increased respiratory morbidity likely due, in part, to inhalation of novel particulate matter (PM), such as from burn pits. Inflammation is a key initial response to inhaled particulates. Our center has developed a protocol using inhaled wood smoke particles (WSP) as a model agent to study PM-induced airway inflammation. Prednisone is a well-known anti-inflammatory agent used in the treatment of airway inflammatory diseases, such as asthma. Efficacy of prednisone has not been studied in the context of PM-induced airway inflammation, such as that seen in military personnel. We hypothesize that a single dose of oral prednisone will reduce neutrophilic airway inflammation following WSP exposure.
Study Objective(s)	Primary • To determine the efficacy of single dose of oral prednisone in mitigating WSP-induced neutrophilic airway inflammation, assessed via sputum %PMNs, in healthy adults Secondary • To assess the safety of WSP exposure in healthy adults by measuring vitals, symptom questionnaires, and spirometry • To determine the effect of a single dose of oral prednisone on mucus based-parameters following WSP exposure • To determine the effect of prednisone on MCC using gamma scintigraphy • To determine the effect of prednisone on airway inflammatory mediators and markers of oxidative stress in exhaled breath condensates (EBC) and induced sputum following WSP exposure • To determine the effect of prednisone on systemic inflammatory markers and serum cytokines
Study Drug/Device *(If Applicable)*	Prednisone 60 mg
Study Design	Healthy adults demonstrating a $\geq$10% increase in sputum %PMNs following WSP exposure in a separate screening protocol will be invited back to participate in this randomized, double-blind, placebo-controlled cross-over study. Those eligible for participation will undergo a baseline MCC, EBC, sputum induction, spirometry, and venipuncture. Participants will then undergo exposure to WSP after which they will be randomized to receive either a single oral dose of prednisone or placebo. Repeat MCC will be obtained starting 2 hours after WSP exposure, and EBC, sputum induction, and venipuncture are repeated 4 hours after

	WSP exposure to assess for primary and secondary endpoints. Spirometry and symptom questionnaires are also performed before and after WSP exposure to assess for safety endpoints. After at least a 1-month washout period, participants will receive the alternative treatment during the cross-over period.
Subject Population KEY criteria for Inclusion and Exclusion:	Inclusion Criteria • Subjects age 18-45 years, inclusive, of both sexes • No physician-diagnosed asthma or any symptoms consistent with asthma;; FEV_1 of at least 80% predicted and FEV_1/FVC ratio of ≥70 • Ability to provide an induced sputum sample • Demonstrate a ≥10% increase from baseline in sputum %PMNs following inhaled WSP exposure in a separate screening protocol Exclusion Criteria • Any chronic illness, which may impact safety or study results • Any infections within 4 weeks of WSP exposure • Inability to discontinue medications, which may impact study results • Pregnant/lactating women, and children <18 years of age
Number Of Subjects	14
Study Duration	Each subject's participation will last up to 4 months. The entire study is expected to last 4 years.
Study Phases **Screening** **Study Treatment** **Follow-Up**	(1) Screening: Potential participants will undergo a general screening protocol (IRB #98-0799) to identify healthy adults suitable for WSP exposure and a second screening protocol (IRB #15-1775) to identify subjects with a ≥10% increase in sputum %PMNs after WSP exposure. Following enrollment, a baseline and training visit will include: an informed consent; medical screening for eligibility; spirometry; and baseline sputum induction, MCC, and venipuncture. (2) Intervention: Participants will undergo baseline spirometry, venipuncture, and EBC followed by WSP exposure for a total of two hours after which they will be randomized to receive either a single dose of prednisone or placebo. MCC assessment will be initiated 2 hours post-exposure with measurements taken for a total of 120 minutes. EBC, venipuncture, and sputum induction will be obtained 4 hours post-WSP exposure. Pre- and post-exposure spirometry and symptom questionnaires will also be conducted. (3) Follow-up: Medical screening for safety endpoints, spirometry, sputum, and lung retention imaging will be performed the day following WSP exposure. Participants will also be contacted 3-7 days after exposure to assess for any delayed adverse events. (4) Cross-over: After a one-month wash out period, participants will be crossed over to the alternative treatment. A discontinuation visit will be performed 5 to 10 days after the last study visit.

Efficacy Evaluations	The effect of a single dose of prednisone on measures of WSP-induced airway and systemic inflammation compared to baseline measures will be assessed as follows: a) sputum induction: sputum % PMNs (primary endpoint) and mucus-based parameters b) gamma scintigraphy scan of radiolabeled Tc99 sulfur colloid: MCC c) sputum induction and EBC: airway inflammatory cytokines and markers of oxidative stress d) venipuncture: systemic inflammatory markers and cytokines All measurements will be performed at baseline and again 4 hours post-WPS exposure, except for MCC which will be performed starting 2 hours after WSP exposure with measurements taken for a total of 120 minutes. Sputum induction will also be performed 24 hours post WSP challenge. The change in these measurements (postWSP – preWSP) will be compared for each interventional arm (prednisone vs placebo).
Safety Evaluations	Vital signs, symptom questionnaire, spirometry
Statistical And Analytic Plan	Our primary endpoint is the change in sputum %PMNs from baseline to 4 hours post-WSP exposure, comparing a single dose of prednisone to placebo. This analysis will rely on a linear mixed-effects model that accounts for the treatment effect and the period effect. Analysis of secondary endpoints (change in %PMNs 24 hours post WSP challenge, total mucin concentration, mucus composition, MCC, sputum and EBC inflammatory and oxidative stress markers, and systematic inflammatory markers, comparing a single dose of oral prednisone to matching placebo) will be performed similarly. The experiment-wide rate of Type I errors will be controlled at level α = 0.05. Non-significant tests will be reported as being inconclusive.
DATA AND SAFETY MONITORING PLAN	A study coordinator will enter data into REDCap, and then a second person will verify the data. Subjects will be monitored in real time for safety by study staff. A study physician will be immediately available during inhalation and treatment procedures and will determine the severity and relatedness of any adverse events (AEs). The PI will conduct ongoing assessment of safety with strict adherence to pre-specified study-suspension rules. All serious adverse events (SAEs) will be reported to UNC IRB and funding agency within 24 hours, and all AEs will be reported to UNC IRB and funding agency annually. A formal NC TracS Data Safety Monitoring Board (DSMB) charter meeting DOD approval will be established prior to commencing study. The DSMB will address issues regarding safety concerns, efficacy concerns, termination of the trial due to pre-specified stopping criteria, and ethical concerns. The DSMB will be furnished with relevant information by the Principal Investigator to make these decisions.

1 BACKGROUND AND RATIONALE

1.1 Introduction

Military deployment is associated with exposure to novel particulate matter (PM), such as from burn pits, aeroallergens, and increased cigarette consumption (1-5). Warfighters exposed to these inhalational exposures exhibit immediate and chronic respiratory morbidity (5). For example, military service personnel surveyed in both the Republic of Korea (ROK) and Kabul, Afghanistan reported a general increase in respiratory morbidity, including asthma and chronic bronchitis, associated with their deployment (1, 6, 7). Air contaminants in the ROK were characterized by elevated levels of both $PM_{0.5-2.5}$ and $PM_{2.5-10}$. Similarly, exposures in Kabul were characterized by multiple airborne PM exposures, including those from burn pits. Burn pit PM includes metals, bioaerosols, organic by-products, and biomass combustion particles. These findings indicate that inhaled PM is a likely cause of respiratory morbidity in the field.

Inflammation is a key initial response to inhaled particulates. Wood smoke particles (WSP) serve as a model agent to study PM-induced bronchitis. WSP inhalation generates reactive oxidant (and nitrosative) species which cause local injury of airway epithelial cells and release of damage-associated molecular patterns (DAMPs) that activate toll-like receptors (TLR) and IL-1-mediated innate immune responses by resident airway macrophages. Contamination of PM with bioaerosols, which contain lipopolysaccharide (LPS), also activates innate immune responses through TLR4 activation of resident airway macrophages. These complementary processes result in recruitment of neutrophils (PMN), which mediate luminal airway inflammation with release of toxic mediators such as neutrophil elastase and myeloperoxidase that promote acute and chronic bronchitis (8).

Therefore, mitigation of PM-induced airway neutrophilic inflammation should be a key focus in order to reduce the respiratory morbidity of military personnel. We have studied a number of pro-inflammatory inhaled agents, such as nebulized LPS (9-11), ozone (O_3) (12), and WSP, as models of acute neutrophilic bronchitis against which to test a number of therapeutic agents. To this effect, we have reported that inhaled fluticasone inhibits O_3-induced (12) and LPS-induced (13) neutrophilic inflammation, and that parenteral anakinra (14) and oral γ-tocopherol (15, 16) inhibit neutrophilic responses to inhaled LPS. In this study, we will evaluate the efficacy of oral prednisone, a readily available anti-inflammatory medication commonly used in airway inflammatory diseases, in mitigating WSP-induced airway inflammation.

1.2 Name and Description of Investigational Product or Intervention

Prednisone 20 mg tablets USP (total dose of 60 mg proposed in this study) and Matching Placebo tablets

1.3 Non-Clinical and Clinical Study Findings

Systemic corticosteroids are a cost-effective and readily available therapeutic option to treat a number of inflammatory conditions. Guidelines-based care for inflammatory respiratory diseases, such as asthma and COPD, support the use of oral systemic steroids for acute exacerbations of these illnesses (17, 18). Additionally, meta-analyses have also highlighted the benefit of oral steroids in certain infectious diseases of the upper and lower airways in which neutrophilic inflammation is a key finding (19, 20). Our own group has demonstrated the use of an inhaled corticosteroid, fluticasone, in

mitigating neutrophilic airway inflammation secondary to inhaled O^3 (12) and LPS (13) challenges. As the respiratory morbidity of military personnel is driven by PM-induced neutrophilic airway inflammation as discussed previously, it is imperative to identify readily-available, cost-effective, and rapidly efficacious therapies that reduce airway inflammation for these individuals. Thus, in this study and using WSP exposure as a model of PM-induced bronchitis, we will assess the effectiveness of a single dose of 60 mg of prednisone, a standard adult dose used in the treatment of other inflammatory airway diseases, in mitigating WSP-induced airway neutrophilic inflammation in healthy volunteers. As a secondary endpoint, we will also determine whether a single dose of prednisone alters mucociliary clearance patterns after WSP-exposure.

1.4 Relevant Literature and Data

Much of the published data relevant to this proposal has been generated by investigators in the UNC Center for Environmental Medicine, Asthma and Lung Biology (CEMALB) and has been referenced in above discussions. This body of literature also highlights our extensive experience in performing the evaluation tools used in this study, including, but not limited to, WSP exposure, sputum induction and processing, MCC assessment, and spirometry. Complete references for these publications have been provided in Section 14.

2 STUDY OBJECTIVE

2.1 Primary Objective

The primary objective of this study is to examine the effectiveness of a single oral dose of prednisone 60 mg (v. placebo) on mitigating WSP-induced airway inflammation, measured by sputum %PMNs, in healthy adults 4 hours post-WSP exposure.

2.2 Secondary Objective

Secondary objectives of this study include examining the effect of oral prednisone (v. placebo) on additional features of airway and systemic inflammation such as mucus-based parameters, mucociliary clearance (MCC), inflammatory and oxidative stress markers from sputum and exhaled breath condensate (EBC), and systemic inflammatory markers. Additionally, effects of 5% HS on WSP-induced neutrophilic airway inflammation 24 hours post-WSP exposure will also be assessed.

3 INVESTIGATIONAL PLAN

3.1 Study Design

- **Type of design:** Randomized, double-blind, placebo-controlled cross-over study

- **Study phases:**
  a) Screening: Prior to enrollment in this study, participants will take part in two separate screening protocols: a general screening protocol (IRB #98-0779) and a screening WSP exposure protocol (IRB #15-1775). The general screening protocol will identify healthy volunteers suitable for WSP exposure. These healthy adults will undergo the screening WSP exposure protocol to identify those individuals with a $\geq$10% increase in sputum %PMNs following WSP exposure, who will then be invited to participate in this study.

b) Visits 0, 1, and 2 – Baseline (Period 1) and Training Day: Along with an informed consent, a medical screening, including spirometry, will be performed to confirm eligibility. Volunteers will be trained on the cycle ergometer to reach an exercise level producing a minute ventilation of 20 L/min/m^2 body surface area (BSA) while being monitored with a 12 lead electrocardiogram (EKG) per our established WSP exposure procedure. Finally, a baseline MCC scan and sputum induction will be obtained, which will provide some of the "pre-WSP" measurements for primary and secondary data analyses. A 30 minute retention scan will be performed 24 hours after baseline MCC to determine amount of Tc99m-SC retained in airways.

c) Visits 3 (Period 1) and 7 (Period 2) - WSP exposure: Participants will return within 4 weeks of Visit 0 to undergo WSP exposure. Spirometry, venipuncture, and EBC will be obtained prior to exposure. After WSP exposure, subjects will receive either a single dose of prednisone 60 mg or matching placebo. Four hours after WSP exposure, participants will then inhale radiolabeled Tc99m sulfur colloid (Tc99m-SC) to start MCC assessment. MCC measurements will be taken for a total of 120 minutes. EBC, venipuncture, and sputum induction will be obtained 4

d) hours post-WSP exposure, which will provide the "post-WSP" measurements for primary and secondary data analyses. Spirometry and symptom questionnaires will be obtained pre- and post-exposure to assess for safety endpoints.

e) Visits 4 (Period 1) and 8 (Period 2) - 24 hours post-exposure follow-up: Any symptoms post-WSP exposure will be reviewed and spirometry repeated along with EBC and sputum induction. Participants will undergo a 30-minute scan to determine amount of Tc99m-SC retained in airways.

f) Visits 5 and 6 – Cross-over and Baseline (Period 2): Participants will return after a minimum 4 week wash-out period, which is the amount of time allotted for resolution of airway inflammation post-WSP exposure. Interval medical history will be reviewed and repeat MCC, spirometry, and sputum induction obtained as pre-WSP measurements for period 2. A retention scan will be performed 24 hours after baseline MCC for period 2. Participants will then be crossed over to the alternative treatment group and undergo visits 7 and 8 as above.

g) Visit 9 – Study Completion: Five to 10 days after the second exposure, participants will return for a study completion visit in which medical history and symptom questionnaires will be reviewed. If the participant has any health symptoms or concerns, they will receive a physical examination and evaluation by a study physician.

h) Unscheduled Visits**:** We do not anticipate any unscheduled visits. However, subjects will be provided with contact information for a study physician in the event of any delayed adverse events outside of direct observation during study visits and re-evaluated as deemed necessary by the study physician. Subjects will be rescheduled if they have a change in health status that requires a delay in study

visits. If this causes a visit to be out of window, the subject can have an unscheduled visit at the discretion of the PI.

3.2 Allocation to Treatment Groups and Blinding

This is a randomized, double-blind, placebo-controlled study. Dr. Haibo Zhou, the study biostatistician, will have a colleague prepare the randomization schedule using permuted block randomization with a block size of 4 (2 prednisone, 2 placebo for the first treatment period of the protocol). The colleague will send this schedule directly to the UNC investigational drug service, who will prepare study drug or placebo for administration.

3.3 Study Duration, Enrollment and Number of Subjects

Each subject will have 10 study visits (excluding the two separate screening protocols – the general screening protocol and the screening WSP exposure protocol – as detailed above) and remain in the study for approximately three months. The expected duration of the entire study is 4 years. A total of 14 subjects will be enrolled into this study.

3.4 Study Population

Inclusion Criteria:

1. Age 18-45 years, inclusive, of both sexes
2. Negative pregnancy test for females who are not s/p hysterectomy with oopherectomy
3. No history of episodic wheezing, chest tightness, or shortness of breath consistent with asthma, or physician-diagnosed asthma.
4. FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of ≥0.70.
5. Oxygen saturation of ≥93%
6. Ability to provide an induced sputum sample.
7. Subject must demonstrate a ≥ 10% increase in sputum %PMNs 4 hours following inhaled WSP exposure, when compared to baseline sputum (to be completed in a separate protocol IRB# 15-1775).
8. Proof of being fully vaccinated to Covid-19

Exclusion Criteria:

Subjects who meet *any* of these criteria are *not* eligible for enrollment as study participants:

1. Clinical contraindications:
    a. Any chronic medical condition considered by the PI as a contraindication to the exposure study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, history of chronic infections/immunodeficiency.
    b. Viral upper respiratory tract infection within 4 weeks of challenge.
    c. Any acute infection requiring antibiotics within 4 weeks of exposure or fever of unknown origin within 4 weeks of challenge.
    d. Abnormal physical findings at the baseline visit, including but not limited to abnormalities on auscultation, temperature of 37.8° C, Systolic BP > 150mm Hg or < 85 mm Hg; or Diastolic BP > 90 mm Hg or < 50 mm Hg, or pulse oximetry saturation reading less than 93%.
    e. Physician diagnosis of asthma

f. If there is a history of allergic rhinitis, subjects must be asymptomatic of allergic rhinitis at the time of study enrollment.
g. Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
h. Medications which may impact the results of the WSP exposure, interfere with any other medications potentially used in the study (to include steroids, beta antagonists, non-steroidal anti-inflammatory agents)
i. Cigarette smoking > 1 pack per month
j. Unwillingness to use reliable contraception if sexually active (IUD, birth control pills/patch, condoms).
k. Use of immunosuppressive or anticoagulant medications including routine use of NSAIDS. Oral contraceptives are acceptable, as are antidepressants and other medications may be permitted if, in the opinion of the investigator, the medication will not interfere with the study procedures or compromise safety and if the dosage has been stable for 1 month.
l. Orthopedic injuries or impediments that would preclude bicycle or treadmill exercise.
m. Inability to avoid NSAIDS, Multivitamins, Vitamin C or E or herbal supplements.
n. Allergy/sensitivity to study drugs or their formulations
o. Positive Covid-19 test in the past 90 days

2. Pregnant/lactating women and children (< 18 years as this is age of majority in North Carolina) will also be excluded since the risks associated with WSP exposure to the fetus or child, respectively, are unknown and cannot be justified for this non-therapeutic protocol. Individuals over 45 years of age will not be included due to the increased possibility of co-morbidities and need for prohibited medications.
3. Inability or unwillingness of a participant to give written informed consent

4 STUDY PROCEDURES

4.1 Screening Visits:

General Screening: To identify healthy volunteers suitable for WSP exposure, subjects will complete a general screening protocol (UNC IRB-approved study #98-0799, *Database and Screening Protocol for Research Studies of the Center for Environmental Medicine & Lung Biology*).

Screening WSP exposure: This protocol will identify subjects who experience a ≥ 10% increase in sputum %PMNs after WSP exposure, compared to baseline sputum (UNC IRB approved study #15-1775, *To identify persons who are susceptible to WSP-induced inflammation and examine the role of GSTM1 and other factors in this susceptibility*).

4.2 Visit 0: Baseline Visit (*at least 1 month after screening WSP exposure*):

1. Informed consent
2. Medical history

3. Vital signs, including oxygen saturation
4. Urine pregnancy test
5. Physical exam
6. 12-lead electrocardigram (EKG)
7. Spirometry

4.3 Visit 1: Training Day

1. Urine pregnancy test (if not within 7 days of Visit 0)
2. Volunteers will be trained on the cycle ergometer to reach an exercise level producing a minute ventilation of 20 L/min/m^2 body surface area (BSA). Subjects will be monitored with a standard 3-lead EKG during exercise (15 minutes).
3. MCC
4. Sputum induction

4.4 Visit 2: Retention Scan (*24 hours after Visit 1*)

1. MCC retention scan (30 minutes)

4.5 Visits 3 (Period 1) and 7 (Period 2): WSP exposure *(within 6 weeks of Visit 0 for Period 1 and Visit 5 for Period 2)*

1. Vital signs, oxygen saturation
2. Symptom questionnaire
3. Physical Exam
4. Urine Pregnancy Test (if not previously performed within 7 days)
5. Venipuncture (CBC with differential, serum cytokines, CRP)
6. EBC
7. Spirometry
8. 500 µg/m^3 WSP chamber exposure for 2 hours, with intermittent exercise and rest. Subjects will alternate 15 minutes of exercise on a cycle ergometer (intensity needed to achieve 20 L/min/m^2 BSA determined at baseline visit) with 15 minutes of rest.
9. Symptom questionnaire (immediately prior to exit from the chamber)
10. Immediately following exit from chamber, subjects will receive either 60 mg of prednisone or placebo per randomization schema
11. MCC (2 hours post-exposure)
12. Symptom questionnaire (6 hours post-WSP exposure)
13. EBC (4 hours post-WSP exposure)
14. Spirometry (4 hours post-WSP exposure)
15. Sputum Induction (4 hours post-WSP exposure)
16. Venipuncture for CBC with differential, serum cytokines, CRP (4 hours post-WSP exposure)

4.6 Visits 4 (Period 1) and 8 (Period 2) *(24 hours post-WSP exposure)*

1. Review any adverse events
2. Vital signs, oxygen saturation
3. Symptom questionnaire
4. Physical exam
5. EBC
6. Retention scan
7. Spirometry
8. Sputum Induction
9. Venipuncture for CBC with differential, serum cytokines, CRP

Participants will be contacted by phone 3-7 days following Visit 4 to assess for any delayed adverse events. No phone call will be made after Visit 8 as participants will be returning for a study completion visit as below.

A 4 week washout is required between Period 1 and Period 2 for resolution of airway inflammation elicited following WSP exposure in Period 1.

4.7 Visit 5: Baseline (Period 2) (*following a minimum 4 week washout)*

1. Review medical history and adverse events
2. Urine pregnancy test
3. Vital signs, oxygen saturation
4. Physical Exam
5. MCC
6. Spirometry
7. Sputum Induction
8. Venipuncture (safety labs only – CBC w/ diff)

4.8 Visit 6: Retention Scan (Period 2) *(24 hours after visit 5)*

1. MCC retention Scan (30 minutes)

4.9 Visit 9: Study Completion *[5-10 days following WSP exposure during Period 2 (Visit 7)]*

1. Review any adverse events
2. Vital signs, oxygen saturation
3. If the participant has any health symptoms or concerns, they will receive a physical examination and evaluation by a study physician.

4.10 Unscheduled visits

Any subject who has an unexpected adverse event (AE) as a result of the study treatment will have access to and contact information for a study physician. Subjects will be invited back to the lab for vital signs collection, physical exam, and spirometry as deemed necessary by the study physician. If the subject has any AE not associated with the study, he/she will not complete the study until the AE resolves such that it does not interfere with the study or with safety. If this causes a visit to be out of window, the subject can have an unscheduled visit at the discretion of the PI.

4.11 Concomitant Medication documentation

Concomitant medications are collected at the screening visit and throughout the study visits.

4.12 Rescue medication administration

As the study population consists of healthy volunteers, we do not anticipate subjects requiring any rescue medication administration. However, in the event that subjects experience bronchospasm with symptoms of cough, chest tightness, or wheezing associated with WSP exposure, the subject will be evaluated by a study physician. If necessary, rescue medication in the form of albuterol, a short-acting bronchodilator, will be readily available and administered per study physician recommendations. There is also a code cart immediately available with epinephrine autoinjectors. Oxygen is available for supplementation if required.

4.13 Subject Withdrawal and Study Suspension Procedures

Subjects may withdraw at any point in the study and will be asked to return to the lab for a final visit if they withdraw from the study prior to completion of all study procedures to monitor safety. Subjects may be withdrawn at any time for safety concerns.

Criteria for safety prior to initiation of wood smoke inhalation challenge: (Subjects not meeting these criteria the morning of challenge will not proceed with wood smoke inhalation challenge):

1. FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of ≥ 0.70.
2. Baseline oxygen saturation of at least 93%.
3. No history of physician-diagnosis asthma or frequent cough, wheezing, or shortness of breath consistent with a history of asthma.
4. No history of viral respiratory tract symptoms within 4 weeks of challenge.
5. No current symptoms of rhinorrhea, sneezing, nasal or ocular pruritus consistent with allergic rhinoconjunctivitis.

Criteria for safety of a given individual following WSP exposure and treatment which would suspend the individual from further participation in the study will include: (Subjects will be withdrawn for the following reasons):

1. Failure of return of FEV_1 without treatment to within 90 % of baseline within 6 hours.
2. A symptom score greater than 45 (representing a ranking of “moderate” on each of 15 criteria, with a maximum score of 60, see appendix) without improvement to a score of 15 or less within 6 hours without therapy.
3. Specifically, a symptom score greater than “moderate” (3 on a scale of 0-4) for shortness of breath or cough at 24 hours post-challenge.
4. A reduction in oxygen saturation to <93% during the 4 hour observation period post-WSP exposure
5. Need for rescue albuterol therapy during the 4 hours observation period post-WSP exposure

Criteria for safety within the entire protocol (failure of which would result in suspension of further study until consultation with study sponsor, UNC IRB, and DSMB) will include the following: (Study will be suspended for the following reasons):

1. If 3 of the first 10 participants fail the individual safety criteria outlined above
2. Once 10 subjects have been enrolled, study suspension will occur if more than 25% of all participants fail the individual safety criteria outlined above
3. Occurrence of any study-related Serious Adverse Event (SAE)

4.14 Screen failure procedures

For subjects who do not meet enrollment criteria, all study related activity will stop once it is determined that the subject does not meet criteria.

5 STUDY EVALUATIONS AND MEASUREMENTS

- **List variables that will be abstracted from medical charts:** none
- **Describe baseline evaluation**

As listed above for baseline visit and training visit of period 1 (Section 4.2), vital signs, including HR, RR, temperature and BP will be collected after a thorough review of the medical history. Oxygen saturation levels will also be noted followed by a brief physical examination, including but not limited to the cervical lymph nodes, eyes, ears, nose, throat, cardiovascular and respiratory systems. Laboratory evaluations will include urine pregnancy test and venipuncture (see below for details). In addition, baseline spirometry, MCC, EBC, and sputum induction will be performed as detailed below. Subjects will also undergo training on the cycle ergometer to reach an exercise level producing a minute ventilation of 20 L/min/m^2 BSA in preparation for the WSP exposure during Visits 3 (period 1) and 7 (period 2). Baseline visit for period 2 (Section 4.5 above) will include all of the measurements obtained for baseline visit and training visit of period 1, but subjects will not require additional training on the cycle ergometer.

- **Describe how measurements will be taken.**
    a) Woodsmoke particle chamber exposure: We will employ the exposure protocol as described by Ghio and colleagues (21). We will heat red oak wood on an electric heating element (Brinkmann, Dallas, Texas, USA) in a Quadrafire 3100 woodstove (Colville, Washington, USA) at 800°F to generate wood smoke. The smoke will be extracted from the chimney and injected into the chamber air stream, and WSP concentration will be controlled using a tapered element oscillating microbalance (TEOM; Thermo Fisher Scientific, Franklin, Massachusetts, USA) to measure the chamber concentration. A Model DR-4000 DataRAM (Thermo Fisher Scientific) will also be used to measure the concentration. The particle number concentration will be measured with a Model 3775(TSI, Shoreview, Minnesota, USA) and the number and size distribution will be measured with a Model 3936L75 SMPS (TSI). The particle concentration will be verified by weighing filters obtained using a versatile air pollution sampler (VAPS; URG, Chapel Hill, North Carolina, USA). The chamber temperature and humidity will be controlled to approximately 22°C and 40%, respectively. The concentrations of WSP we will use for this study will not exceed 500 µg/m^3, with mild exercise to increase minute ventilation (20 L/min/m^2 BSA) for 15 minute periods, alternating with 15 minute rest periods across an overall 2 hour total exposure period. Subjects will be trained during Visit 0 on the cycle ergometer to reach this minute ventilation. A 12 lead-EKG will be performed prior to training for safety purposes, and subjects will be monitored with a standard 3 lead EKG during exercise.

    b) Sputum Induction: Sputum samples will be collected using a specific sputum induction protocol in which FEV_1 and FVC will be measured to determine the baseline FEV_1 and FVC values. The FEV_1 values that match a 10% and 20% fall from baseline will be calculated. An ultrasonic nebulizer will be filled with 15 mL of 3% hypertonic saline (inhalation grade for respiratory use only) to begin the test. The nebulizer will be set to the maximum output setting and turned on. The subject will be instructed to breathe normally (i.e. tidal breaths) through the nebulizer mouthpiece for 7 minutes. The saline will be nebulized through the mouthpiece in a jet stream and inhaled. The nose will not be occluded for this procedure. Prior to expectoration, subjects will blow their nose, rinse their mouth with water, and clear their throat to avoid the inclusion of non-airway fluid samples. The subject will also be encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower airways (i.e. not from the back of the throat) is ready for expectoration. The sample will

be coughed into a sterile specimen jar and capped. Following repeat measurement of FEV_1 after the first inhalation period, the concentration of saline will be increased from 3% to 4%, provided the FEV_1 falls by <10% from the baseline value. The same procedure will be followed for the final inhalation period using 5% hypertonic saline. The nebulization will be stopped at that point, or earlier, if a sputum sample of good quality is obtained (i.e. visible sputum plugs). The 4% and 5% saline solutions will be mixed for each induction by study staff just prior to induction and a volume of 15-20 mL will be used on each occasion. Participants will not be pre-treated with Albuterol as is our standard procedure with healthy volunteers. Albuterol will be available if needed. Sputum sample will be used to evaluate for mucus-based parameters, such as total mucin concentration, % solid content, and MUC5AC/MUC5B.

c) Spirometry: Spirometry will be performed according to American Thoracic Society (ATS) guidelines. Subjects will inhale as deeply as possible then exhale as rapidly and completely as possible into the spirometer. Measurements obtained from each maneuver include the FVC, the FEV_1, the maximal mid-expiratory flow rate (FEF_{25-75}) and the peak flow. The largest FVC and FEV_1, from at least 3 acceptable trials will be selected for analysis and subject must meet the appropriate inclusion/exclusion criteria based on comparison of the subject's measurements to the predicted value for an individual of his/her gender, age, race and height with NHANES III as the predicted set.

d) Exhaled breath condensate: EBC will be collected at individual study sites via established protocols (22) using the commercially available RTube (Respiratory Research, Inc., Charlottesville, VA)—a handheld, self-contained, single-use device. The device consists of a mouthpiece connected to a one-way valve that directs exhaled breath through a condenser tube cooled by a cooling sleeve. Gaseous phase liquid and aerosols in exhaled breath condense into liquid form on the inside surface of the chilled condenser tube and can be extracted using a plunger device supplied by the manufacturer. The specific collection protocol would include chilling the aluminum cooling sleeve to -20º C, then placing this cooling sleeve with insulated cover over the RTube device immediately before collection. Once the cooling sleeve is in place, the subject breathes tidally through the mouthpiece for 15 minutes. EBC samples are stored at -80º C pending analysis.

e) Mucociliary clearance: Prior to each MCC study, a transmission Cobalt57 (Co57) scan will be performed to define the lung boundaries, to assign regions of interest, and to normalize these regions for lung volume differences. A rectangular phantom containing the radioisotope Co57 (<25 microcurie (mCi)) will be placed in front (5cm) of the subject sitting with his/her back to the gamma camera for 30 seconds. Prior to the transmission scan before each MCC assessment, we will place 2 spot markers of Americium241 (0.9 mCi each, gamma 66 kilo electron volts) on the upper and lower back of each subject during scanning (both Tc99m-SC deposition/retention and Co57 transmission). With dual isotope imaging, these spot markers will allow alignment of images for more accurate determination of regional deposition/retention. These very low radiation sources have been obtained from commercially available home smoke alarms. The placement of these markers will be determined to be

outside the lung field during the transmission scan. Their location will be marked in semi-permanent ink for later placement during Tc99m deposition/retention scans. The shielded side of this source will be placed/taped onto the subject's skin.

Radiolabeled Tc99m-SD will be delivered using a modified Pari-LC Star nebulizer (MMAD 9.5 um). This is a closed delivery system that produces 80 ml/sec air flow, and therefore limits the inspiratory flow rate to this value. While seated in front of a gamma camera subjects will perform single inhalations lasting ~10 seconds each from the delivery system, and will exhale at 500 ml/sec (using feedback from a flow meter in the breathing circuit). Approximately 5 of these inhalation maneuvers will be required to deposit an adequate isotope dose to the lung. Subjects will be allowed to breathe normally (off the nebulizer) in between each inspiratory maneuver. Each volunteer will practice these maneuvers prior to the actual radioaerosol inhalation to guarantee his/her proficiency. The activity of Tc99m-SC loaded in the nebulizer will be adjusted to provide an estimated 40 mCi deposited in the lung for each MCC scan. A single crystal detector will be placed at the subject's back during inhalation to monitor dose to the lung. Total inhalation time should be less than 5 minutes in all cases. Immediately following isotope inhalation, the subject will gargle and drink water to clear activity that deposited in the mouth into the stomach. The subject will then (within a minute of final inhalation maneuver) be seated in front of a large-field-of-view gamma camera to begin acquiring particle retention images.

For each MCC measurement, the gamma image capture will capture continuously for the first 34 minutes after which 2 consecutive 2-minute images will be obtained at the start of every 10-minute period until 2 hours have passed. The subject will also return the day following each MCC measurement to sit in front of the gamma camera for 30 minutes to obtain images of the lungs to visualize retention of Tc99m-SC particles. No additional Tc99m-SC will be administered at this visit.

- **Describe rating scales, tests, psychological tools, laboratory evaluations, etc.**
  a) Urine pregnancy test (UPT): A UPT will be obtained on all females who are not s/p hysterectomy with oophorectomy at visits 0, 1, 3, 5, and 7. If positive, these women will be excluded from the study. UPTs are valid for 7 days.
  b) Venipuncture: Blood will be drawn to evaluate a CBC with differential, CRP, and inflammatory cytokine profile pre- and post-WSP exposure at Visits 3 and 7. At the time of each venipuncture, up to 50 cc will be drawn, and the total for the study will be no more than 200 cc.
  c) Symptom questionnaire**:** To access for safety throughout the study, symptom questionnaires will be performed at pre-WSP exposure (visits 3 and 7), immediately post-WSP exposure, 6 hours post-WSP exposure, and 24 hours post-WSP exposure (visit 4 and 8). The questionnaire consists of 15 symptoms, requiring a rating from 0-4 with a minimum score of 0 and maximum score of 60. See Appendix for symptom questionnaire.

5.1 Efficacy Evaluation

The effect of prednisone 60 mg (v. placebo) on WSP-induced airway and systemic inflammation will be assessed with the following measurements:

a) sputum induction: sputum % PMNs (primary endpoint) and mucus production and composition
b) gamma scintigraphy scan of radiolabeled Tc99 sulfur colloid: MCC
c) sputum induction and EBC: airway inflammatory cytokines and markers of oxidative stress
d) venipuncture: systemic inflammatory markers and cytokines

All measurements will be performed at baseline and again 4 hours post-WPS exposure, except for MCC which will be performed 2 hours post-WSP and will be completed by 4 hours post-WSP exposure. The change in these measurements (postWSP – preWSP) will be compared for each interventional arm (prednisone vs placebo). See Section 6 for detailed primary and secondary endpoints.

5.2 Pharmacokinetic Evaluation: n/a

5.3 Safety Evaluations

Safety evaluations will be performed with the following measures with strict adherence to subject withdrawal and study suspension criteria (Section 4.10):

a) Physical exam and vital signs: In addition to a limited physical exam of the eyes, ears, nose, throat, and respiratory and cardiovascular systems, we will also check vital signs (temperature, HR, BP, RR, and oxygen saturations) at each baseline visit (0 and 5), pre-WSP and 4 hours post-WSP exposure (visits 3 and 7), and 24-hours post-WSP (visits 4 and 8). Additional exams and vital signs will be performed at physician discretion if needed based on the participant's symptoms.

b) Spirometry: Spirometry will also be performed at each baseline visit (0 and 5), pre-WSP and 4 hours post-WSP exposure (visits 3 and 7), and 24-hours post-WSP (visits 4 and 8). Healthy volunteers have been shown to tolerate WSP exposure well without any changes in lung function (21). However, if participants assign a score greater than 3 ("moderate") to symptoms attributable to bronchospasm (cough, wheezing, chest tightness, shortness of breath, or pain on deep inspiration) following WSP exposure, then they will be evaluated by the study physician to determine if additional spirometry and/or rescue treatment with albuterol is necessary. Additional spirometry will be performed at physician discretion.

c) Symptom questionnaire (see Appendix)**:** This will be performed pre-WSP, immediately before exiting WSP chamber, and 4 hours post-WSP exposure (visits 3 and 7), and 24-hours post-WSP (visits 4 and 8). Symptoms may be accessed at other time points during the study per physician discretion.

d) Direct observation: Participants will remain under the direct observation of a study coordinator and study physician throughout each study visit and will be monitored for a minimum of 5 hours following each WSP exposure.

e) Post-study visit safety plan: We do not anticipate any delayed adverse events. However, all participants will have access to and contact information for a study

physician during the duration of their participation. Per study physician recommendations, they will be re-evaluated with an unscheduled visit as detailed in Sections 3.1 and 4.10. Additionally, all subjects will be contacted via phone by a study coordinator 3-7 days after visit 4 to access for any delayed adverse events as noted in Section 4.6 above.

6 STATISTICAL CONSIDERATION

6.1 Primary Endpoint

Change in sputum %PMNs [Δ (postWSP – preWSP)[prednisone] vs Δ (postWSP – preWSP)[placebo]] 4 hours post-WSP exposure and at 24 hours post -WSP exposure

6.2 Secondary Endpoints

1) Induced Sputum:
   a) Granulocyte (e.g.eosinophil) numbers/mg and percentages at 4 and 24 hours post-WSP exposure
   b) cytokine and chemokine concentrations (T_H1 and T_H2) via Mesoscale platform (pg/ml)

6.3 Exploratory Outcome measures:

1) Induced sputum:

   a) Oxidative stress markers [e.g. GSH/GSSG ratio, 8-isoprostane(pg/ml)]

   b) Mucus-based parameters including: total mucin concentration (µg/ml), % solid content, MUC5AC and MUC5B concentrations (pmol/ml) and ratio, biophysics (cohesion; macro- and micro-bead rheology)

2) EBC:
   a) Sialic acid/urea ratio
   b) purine mediators (µM)
   c) amino acids and dipeptides (µM)
   d) GSH/GSSG ratio

3) MCC measures:
   a) the central (C) vs. peripheral (P) deposition ratio (C/P), for regional airway deposition
   b) MCC, rate at which radiolabeled particles are cleared from the lung (reported as % particle retention vs time)
   c) skew, a measure of the particle deposition heterogeneity

4) Blood:
   a) Systemic inflammatory markers (granulocyte numbers/mL and percentages)
   b) Serum cytokine concentrations via Mesoscale platform (pg/mL)

6.4 Statistical Methods

Our analytic plan was developed in collaboration with Dr. Zhou, the biostatistician for the CEMALB, who will oversee all statistical analyses. For analysis of our primary endpoint, change in sputum %PMNs at 4 hours post-WSP exposure, we will employ a linear mixed-effects model used in previous studies that allows us to assess treatment effect while accounting for any period effect (14, 16). The parameter estimates obtained by fitting this model (i.e., inter- and intra-subject variance, period effect, and treatment effect) will be used to compute estimates (e.g., effect sizes, means, standard errors, confidence intervals) and to compute a p-value for the primary null hypothesis that the difference in treatment effects is exactly zero in the target population. The same linear mixed-effects regression modeling technique will be used to analyze secondary endpoints as well. Additionally, we will use tabular and graphic methods to describe/explore the basic relationship between the treatment groups, including confidence intervals for all statistical estimates. Subsequently, as part of sensitivity analyses, we will compare the Δ sputum %PMNs (post WSP – pre WSP [prednisone]) vs (post WSP – pre WSP [placebo]) using a paired-sample T-Test. Examination of the normality assumption will be performed, and a nonparametric Wilcoxon signed-rank test will be conducted in the case of evidence of extreme departure from normality. For all of the aforementioned analyses, criterion for significance will be $p \leq 0.05$, and results exceeding this threshold will be reported as inconclusive. Lastly, we will employ multiple variable regression modeling techniques to determine if specific individual risk factors (e.g.,BMI) impact either the effect of WSP exposure or the effect of treatment on the primary or secondary endpoints, similar to approaches previously described to assess genotype effects on pollutant responses (23). These analyses will only be conducted after the primary hypothesis is tested and solely for exploratory purposes.

6.5 Sample Size and Power

Our primary endpoint is the change in sputum %PMNs pre- and 4 hours post-WSP exposure, comparing prednisone to placebo. This study originally targeted enrollment of 24 subjects to yield complete 20 datasets, enrolled a total of 7 subjects and completed 6 prior to the study being put on hold for the pandemic from March 2020 till July 2021. No samples had been analyzed from this study yet. Meanwhile our ongoing WSP screen protocol was able to complete 50 screening exposures, determining that 28 met the predefined "responder" definition. These samples were collected pre-pandemic and were processed during the pandemic pause, yielding results that the responder group has a mean baseline of 24% PMN which increases to 40% at 6 hrs and 52% 24 hrs post exposure. The availability of these new data have led to concern that the Smokisone enrollment target should be amended, since the new dataset should allow a more accurate sample estimate. Using our ongoing WSP screen sputum data, estimating the prednisone effect size to be a 50% inhibition of WSP-induced sputum neutrophilia based on a previously published study of prednisone effect on sputum neutrophilia after controlled ozone exposure [2], and assuming a more conservative standard deviation for the prednisone effect size than the one reported in that study, we estimate that the N for this study to detect a significant prednisone effect should be 12 rather than 20. Anticipating 5-10% of subjects will produce inadequate samples, we propose a goal of completing 14 subjects to obtain 12 complete datasets.

6.6 Interim Analysis

We do not plan on conducting an interim analysis. Prednisone dosing is being used for its indication as an anti-inflammatory treatment. Because we do not anticipate safety concerns attributable to prednisone treatment for 1 dose, no interim analysis will be needed. Additionally, our study will be reviewed and surveyed by the Data and Safety Monitoring Board for external safety monitoring.

7 STUDY INTERVENTION

- **Description**
  Prednisone 60 mg (supplied as three Prednisone 20 mg tablets USP)

- **Receipt/Storage**
  IDS will be responsible for providing prednisone and matching placebo for each study subject and for each treatment period in a blinded fashion.

- **Packaging/Labeling**
  This is a randomized, double blind study so packaging and labeling by IDS is such that both study participants and study personnel will be blinded to the treatment (either prednisone or placebo) given.

- **Dosing**
  Study participants will receive 60 mg of prednisone (3 tablets of prednisone 20 mg) or matching placebo during period 1. They will then crossover to period 2 and receive the alternative treatment. Prednisone 60 mg is the standard clinical daily dose used to treat exacerbations of airway diseases, such as asthma and COPD.

- **Treatment compliance and Adherence**
  Subjects are observed during all dosing periods.

- **Drug Return/Destruction:** n/a

- **Drug Accountability:** n/a

8 STUDY INTERVENTION ADMINISTRATION

- **Randomization procedures**
  The randomization schedule will be generated by one of Dr. Zhou's colleagues using permuted block randomization with a block size of 4 (2 prednisone, 2 placebo) for the first treatment period of the protocol).

- **Blinding procedures**

- This is a randomized, double blind study so packaging and labeling by IDS is such that both study participants and study personnel (coordinators and physicians) will be blinded to the treatment (either prednisone or placebo) given.

- **Unblinding procedures:** Unblinding will occur at the conclusion of the study or earlier if needed for safety purposes and as dictated by the UNC IRB, study sponsor, and DSMB.

9 SAFETY MANAGEMENT

- **Definition of Adverse Event (AE) and Serious Adverse Event (SAE)**
  An AE for a given volunteer will be defined as failure of any of the safety criteria outlined above. Any symptoms that induce a volunteer to seek medical attention from any provider within 96 hours of a study visit will also be considered an AE. A SAE will be defined as any event that requires hospitalization or results in life threatening illness or injury, permanent (or likely to be permanent) illness or injury, or death if these events occur within 96 hours of a study visit (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 96 hours of a study treatment visit).

- **Grading criteria**
  - In addition to determining whether an adverse event fulfills criteria for a SAE or not, the severity of AEs experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.0. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.
  - All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade.):
    - Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
    - Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
    - Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).
    - Grade 4 = Life-threatening consequences; or urgent intervention indicated.
    - Grade 5 = Death related to AE.

- **Adverse Event/Serious Adverse Event monitoring procedures**
  Evaluations for safety throughout study visits are detailed in Section 5.3. In addition to these, review and classification of AEs will occur at each study visit with ongoing monitoring of safety throughout the entirety of the study by the PI. The UNC DSMB will be employed to review AEs throughout the study. The PI will report to the DSMA at least biannually. Any SAE;s will be immediately reported to the DSMB.

- **Adverse Event/Serious Adverse Event reporting procedures**
  All SAEs will be reported to UNC IRB, DSMB, and funding agency within 24-48 hours, and all AEs will be reported to UNC IRB and DSMB at regular predetermined intervals per protocol. Per DoD guidelines, only the following events will be reported to the HRPO: (1) All unanticipated problems involving risk to subjects or others; (2) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies; (3) Any instances of serious or continuing noncompliance with the federal regulations or IRB requirements; (4) The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research; (5) The issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any government regulatory agencies; (6) Change in subject status when a previously enrolled human subject becomes a prisoner must be promptly reported to the USAMRMC ORP HRPO with a report that includes actions taken by the institution and the IRB. The duties of the RM specifically will be to review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB and DSMB. The PI will stop a research protocol in progress, remove individual human subjects from a research protocol, and will protect the safety and well-being of human subjects until the IRB and DSMB can assess the s report. All findings from these events will be promptly reported to the HRPO.

- **Medical Emergency procedures**
  There is a study physician available for study visits, including WSP exposure. A code cart and Automated External Defibrillator (AED) are available in the event of a cardiopulmonary event. Albuterol, both inhalers and nebulizers, are available in the event of bronchospasm. If a subject has an event that is does not immediately respond to care in the research lab, he/she will be transported via EMS to UNC Healthcare for treatment.

- **Data Safety Monitoring Plan**
  Data is initially recorded on paper documents, and the documents are maintained in a binder, which is kept in a locked office within the EPA Human Studies Facility (HSF). The EPA HSF is a secure facility with a guarded entrance and requires identification for entry. This data is then entered into REDCap by an initial data entry person, and confirmed by a second user.

- **Potential Risks:**
  a) <u>WSP exposure:</u> Concentrations of wood smoke exhaust proposed for this study (500 $\mu g/m^3$) are below those reported for individuals using biomass burning for heating and food preparation and those living in the area of a forest fire or agricultural burning. No major adverse health effects were observed in a recently completed study, which exposed healthy volunteers to WSP (21). The mean PM was 485 + 84 $\mu g/m^3$ during the WSP exposure (vs. “below the detectable limit” for filtered air (FA)), and analyses of chamber concentrations of total hydrocarbon and carbon monoxide during WSP exposure were also increased compared to FA (4.8 ± 0.5 vs. 2.0 ± 0.1 ppm and 2.7 ± 0.7 vs. 0.0 ± 0.0 ppm, respectively). However, these healthy subjects had no significant

change in pulmonary function and no nasal irritation, rhinitis, inspiratory pain, shortness of breath, cough, wheezing or chest tightness after exposure to WSP or filtered air. There were complaints of fatigue, headache and eye irritation, but these were noted by volunteers as frequently before as after both the filtered air and particle exposures. Pulmonary function testing demonstrated no significant changes from pre-exposure values following exposures to either filtered air or WSP. The moderate exercise on the ergometer has a rare risk of causing leg cramps or soreness. The EKG patches cause a rare risk of skin irritation or blistering.

b) Oral prednisone: Long-term use of prednisone is associated with a number of complications such as increased risk of infections, fluid and electrolyte disturbances, gastrointestinal complications, dermatoses, ocular complications (cataracts, glaucoma), adrenal suppression, increased risk of osteoporosis, myopathy, personality changes, insomnia, among other things. However, a single dose of prednisone, as proposed in this study, is associated with minimal side effects, and may only cause temporary euphoria or irritability, hunger, or insomnia. As the half-life of prednisone is 3-4 hours, these minor side effects will last no longer than 24 hours.

c) Sputum induction: The saline solutions (3%, 4% and 5%) nebulized during sputum induction may cause throat irritation, but it is uncommon for the duration of discomfort to last more than a few minutes post procedure. The procedure also has a minimal risk of inducing bronchospasm, which is less of a concern in healthy adults.

d) Spirometry: Potential risks include possible lightheadedness or wheezing.

e) Mucociliary clearance scan: The radiation exposure from the MCC scan, including the Co57 transmission scan and the Americium 241 disks, which are used as fiducial markers, is approximately 177 mRems. This is less than the natural environmental radiation that adults receive every year, which in Chapel Hill is about 300 mRems.

f) Exhaled breath condensate: There are no additional risks associated with the EBC procedure as study subjects are simply asked to exhale into a chilled tube.

- **Protections to Minimize Risk**:

a) WSP exposure: Strict adherence to inclusion and exclusion criteria will be followed prior to subject enrollment, and medical history updated throughout study visits to affirm continued study eligibility. If participants develop any infectious processes during the washout period, period 2 will be delayed for a minimum of 4 weeks (as per exclusion criteria) following resolution of illness and/or completion of antibiotics. During WSP exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG telemetry leads attached to monitor cardiac rate and rhythm during exercise throughout the exposure. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. A physician familiar with the protocol will be available for all challenge procedures. The investigator or duty physician will end the exposure if the subject is found to be suffering from any major adverse effect. An emergency “crash cart” with standard emergency medications, IV fluids, and a defibrillator are is also readily available at the CEMALB in the unlikely event of a medical emergency during any challenge or study visit. Pulse oximetry will be performed during the exposures to wood smoke and filtered air, and the subject will be withdrawn from the chamber if the value is

<90%. The risk of discomfort associated with exercise will be limited because ability and response to exercise will be assessed at the training visit, and subjects with orthopedic issues that limit ability to pedal a bicycle will be excluded. Subjects will be encouraged to remain well hydrated to minimize risks from exercise. Should skin irritation develop from EKG patches, a topical steroid for symptomatic relief will be recommended.

b) Oral prednisone: Dosing will be limited to a single dose with minimal side effects as noted above. In addition, those with hypersensitivity to prednisone will be excluded from the study as noted in Section 3.4.

c) Sputum Induction: Throat irritation will be minimized by providing the subject with a drink (water, juice etc) and a snack after induction. An albuterol multidose inhaler will be immediately available in the event the subject experiences symptoms of bronchoconstriction; however as only healthy subjects are being enrolled in this study, no albuterol pre-treatment will be given. A physician on duty in the facility will be available during sputum inductions. If the covering physician feels that the subject's respiratory status is such that providing an induced sputum sample would place them at increased risk for significant bronchospasm, sputum sampling will be deferred. Baseline FEV_1 and FVC will be measured before the start of the induction and at the end of each level (concentration) of saline inhalation. The FEV_1 values that match a 10% and 20% reduction from baseline will be calculated and recorded. Subjects who have a 10-20% decrease in FEV_1 after the 3% or 4% level of saline will not be advanced to a higher concentration of saline and will continue at the same level. If the FEV_1 drops by > 20%, the induction will be stopped and the subject will be treated with 2 puffs of albuterol. Additional albuterol may be given if needed.

d) Spirometry: Subjects will be seated in a non-rolling chair when spirometry is performed and standard methodology conforming to the ATS guidelines for measurement of spirometry will be used. Subjects will be instructed to notify the study staff if they feel lightheaded, and albuterol will be available in the event the subject experiences any unanticipated bronchoconstriction.

e) Mucociliary Clearance***:*** Radiation history is collected. Any subject who will exceed safe annual exposure limits will not be enrolled.

f) EBC: Standard procedures are followed for EBC. No additional risk is present from an EBC.

10 DATA COLLECTION AND MANAGMENT

- **Monitoring Plan**

  We will use RedCap for data management. The data will be entered by one person (typically the coordinator or, for lab analysis, a lab staff member) and then checked by a second person. The data is not marked "complete" until the 2nd person verifies the entry. REDCap creates a data dictionary when the database is established, and this is used as the codebook.

- **Database documentation**

  All databases using equipment generated have a date and user attached to the electronic file. Data will be linked with a codebook (i.e. sample ID) using Excel. A second entry is done manually in a lab notebook, which includes date, samples assayed, assay used, any issues (like missing samples/data), reference to where data is stored, and a printout of the data (all as hardcopies in a lab notebook). Adherence

to the codebook is ensured by having a second individual to check the entries. The PI takes responsibility for data management computations.

- **Case report forms**
  Case report forms will be developed by the study team, using templates from previous studies. These forms are maintained by study coordinators, and reviewed by investigators as needed.

- **Maintaining Confidentiality**
  Subjects will be issued a subject number when they enroll into the study, and this number will only be used to label samples. All protected healthy information will be maintained by the study coordinator or the investigators and will be kept in locked areas when not in use. The identifiers that go into REDCap will only be accessible to those who need them for their job, specifically study physicians, investigators and coordinators. Subjects sometimes undergo procedures – such as sitting in front of the gamma camera – at the same time, however other than basic introductions, nothing about one subject is disclosed to the other subject.

11 RECRUITMENT STRATEGY

Informational emails at UNC will be used to recruit subjects.

12 CONSENT PROCESS

- **Describe the procedure that will be used to obtain informed consent/HIPAA authorization and assent**
  The study will be described in detail to the subject, including why the study is being conducted, the intervention being studied, the risks and benefits, and what is expected of the subject. The subject will be given adequate time to read the consent, and consent will be obtained prior to any study procedures. Consent may take place on a separate day from the baseline procedures.

- **Who will obtain consent**
  Consent will be obtained by a study coordinator or a study physician.

- **Where will consent process take place**
  The consent will take place at the UNC CEMALB, located in the EPA HSF on Mason Farm Road in Chapel Hill.

- **How will investigator assure that subjects comprehend the nature of the study, procedures, the risks and benefits**
  The subject will be encouraged to ask questions regarding the study and procedures involved. Open ended questions will be asked of the subject to solicit correct responses, to help ensure that the subject understands the study commitment, procedures and risks and benefits.

13 REFERNECES

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APPENDIX

a) Table of Study Procedures:

	V0 (Baseline)	V1 (Training)	V2 (post-MC)	Pre-WSP V3 (Prd 1) V7 (Prd 2)	Post-WSP V3 (Prd 1) V7 (Prd 2)	V4 (Prd 1) V8 (Prd 2)	V5 (Baseline-Prd 2)	V6 (post-MCC)	V9 (Completion visit)
Consent	X								
Review history/AE's	X			X	X	X	X		X
Vital Signs	X			X		X	X		X
Urine hCG (females only)	X	X		X			X		
Symptom Questionnaire				X	2X (post-WSP, hr post-WSP)	X			
Spirometry (FEV_1)	X			X	X	X	X		
EBC				X	X	X			
Physical Exam	X			X		X	X		
Venipuncture				X	X	X	X		
Sputum Induction		X			X	X	X		
2 hour WSP chamber exposure				X					
MCC		X			X		X		
MCC Retention Scan			X			X		X	
Ergometric Cycle Train		X							
Prednisone vs placebo					X				

b) Symptom Questionnaire:

Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

Symptoms	**None**	**Trace**	**Mild**	**Moderate**	**Severe**
1. Headache	0	1	2	3	4
2. Irritation of the Nose	0	1	2	3	4
3. Stuffy Nose/Sinus Congestion	0	1	2	3	4
4. Runny Nose	0	1	2	3	4
5. Dry or Sore Throat	0	1	2	3	4
6. Pain on Deep Inspiration	0	1	2	3	4
7. Unusual Fatigue	0	1	2	3	4
8. Eye Irritation	0	1	2	3	4
9. Shortness of Breath	0	1	2	3	4
10. Sneezing	0	1	2	3	4
11. Coughing	0	1	2	3	4
12. Wheezing/Whistling of Chest	0	1	2	3	4
13. Chest Tightness	0	1	2	3	4
14. Sweating	0	1	2	3	4
15. Other:	0	1	2	3	4