# Post Approval Submissions

## Modification Information

*To modify an approved study, edit the individual answers that make up the application. The questions below are intended solely for the IRB to have a summary statement of your requested action. The modifications cannot be processed until the actual changes have been made throughout the application.*

1.Provide a brief non-technical summary of any changes you will be making to the study. The text you enter here will be reproduced in the IRB approval document, and should contain the details that you and/or your sponsor find relevant (e.g., master protocol/amendment version number and date). Typical summaries are 50-100 words. Include a list of any documents that have been modified or added. PLEASE NOTE: THIS SECTION MAY BE EDITED BY THE IRB FOR CLARITY OR LENGTH.

We are currently approaching our upper limit for study enrollees and would like to increase the number of volunteers that we can enroll in the study. Of our original maximum enrollment (40 participants) we have had six people complete the study, 13 that are currently enrolled, and 16 that have either failed to qualify for the study after enrolling or have been removed from the study. We anticipate needing to enroll approximately 20 additional volunteers to achieve the number of study completions (n=26) that our power calculation indicates.

We have changed the potential number of enrolled participants from 40 to 60 as follows:

Adult Consent Form (page #3, "How many people will take part in this study"

Protocol:

General Information, Question #2

Section A.2.1, A.2.2, and A.2.3

2.Is this modification being submitted in response to an unanticipated problem/adverse event or new findings?

No

3.Do any of the proposed changes increase risk?

No

4.Does this modification involve new information that requires reconsent of CURRENT subjects?

No

5.Is this study permanently closed to enrollment of subjects, all interventions and follow-up complete, and open for DATA ANALYSIS ONLY?

No

## Continuing with Modifications

*Click the "save and continue" button to access your existing application.*
*You may make any changes to the application that you are requesting at this time.*

# General Information

## 1. General Information

1.Project Title

Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.

2.**Brief Summary**. Provide a brief non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content. PLEASE NOTE: THIS SECTION MAY BE EDITED BY THE IRB FOR CLARITY OR LENGTH.

Purpose: The purpose of this study is to determine whether exposure to ozone ($O_3$) or nitrogen dioxide ($NO_2$) has a "priming" effect on the healthy adult human body in such a way that the cardiopulmonary effects of sequential exposure to these two air pollutants would be greater than exposure to either pollutant alone.

Participants: Sixty (60) healthy men and women between the ages of 18 – 45 years.

Procedures (methods): Each study participant will be exposed in environmentally controlled exposure chambers to four exposure regimens. Each regimen will involve a two hour exposure with intermittent, moderate exercise on two consecutive days with a third follow-up day. The first day (Day #1) of the series will consist of an exposure to either clean air (CA), 300ppb $O_3$, or 500ppb $NO_2$. Participants will return the following day (Day #2) for a subsequent exposure to either 300ppb $O_3$ or 500ppb $NO_2$. Finally, the participants will return the next day (Day #3) for a follow-up visit. Each regimen will be separated by at least 13 days. Techniques measuring cardiac physiology and lung function will be performed pre- and post-exposure on both exposure days as well as 20 hours after the second exposure day. Primary endpoints will include Holter monitoring of cardiac electrophysiology, spirometry, and pulse wave analysis to measure arterial stiffness. Secondary endpoints will include analysis of blood clotting/coagulation factors and other soluble factors present in plasma.

## 2. Project Personnel

1.Will this project be led by a STUDENT (undergraduate, graduate) or TRAINEE (resident, fellow, postdoc), working in fulfillment of requirements for a University course, program or fellowship?

Yes

This study will require the identification of a single faculty advisor, who should be added in Project Personnel on this page. This should be the faculty member who will mentor this research, who may or may not be your academic faculty advisor.

The faculty advisor will be required to co-certify with the student/trainee PI. You should also make sure this person has a chance to review and edit the submission before you submit.

Choose the status of the student/trainee:

postdoc

2.List all project personnel beginning with principal investigator, followed by faculty advisor, co-investigators, study coordinators, and anyone else who has contact with subjects or identifiable data from subjects.

- List ONLY those personnel for whom this IRB will be responsible; do NOT include collaborators who will remain under the oversight of another IRB **for this study**.
- If this is Community Based Participatory Research (CBPR) or you are otherwise working with community partners (who are not functioning as researchers), you may not be required to list them here as project personnel; consult with your IRB.
- If your extended research team includes multiple individuals with limited roles, you may not be required to list them here as project personnel; consult with your IRB.

The table below will access campus directory information; if you do not find your name, your directory listing may need to be updated.

| Last Name | First Name | Department Name | Role | Detail |
|---|---|---|---|---|
| McCullough | Shaun | Environmental Protection Agency (EPA) | Principal Investigator | view |

| | | | | |
|---|---|---|---|---|
| Devlin | Robert | Environmental Protection Agency (EPA) | Faculty Advisor | view |
| Diaz-Sanchez | David | Environmental Protection Agency (EPA) | Co-investigator | view |
| Schmitt | Michael | Environmental Protection Agency (EPA) | Project Manager or Study Coordinator | view |
| Rappold | Ana | Environmental Protection Agency (EPA) | Co-investigator | view |
| Bassett | Maryann | Environmental Protection Agency (EPA) | Co-investigator | view |
| Montilla | Tracey | Environmental Protection Agency (EPA) | Co-investigator | view |
| Ghio | Andrew | Environmental Protection Agency (EPA) | Co-investigator | view |
| Case | Martin | Environmental Protection Agency (EPA) | Project Manager or Study Coordinator | view |
| Cascio | Wayne | Environmental Protection Agency (EPA) | Co-investigator | view |
| Wood | Julie | Environmental Protection Agency (EPA) | Co-investigator | view |
| Mirowsky | Jaime | Center for Environmental Medicine Asthma and Lung Biology (CEMALB) | Co-investigator | view |
| Kahle | Juliette | Environmental Protection Agency (EPA) | Co-investigator | view |
| Bowers | Emma | Toxicology Curriculum | Co-investigator | view |
| Best | Carissa | Epa | Research Assistant | view |
| Lavrich | Katelyn | Medicine Administration | Co-investigator | view |
| Morgan | David | Environmental Protection Agency (EPA) | Research Assistant | view |
| | | | | view |

*NOTE: The IRB database will link automatically to UNC Human Research Ethics Training database and the UNC Conflict of Interest (COI) database. Once the study is certified by the PI, all personnel listed (for whom we have email addresses) will receive separate instructions about COI disclosures. The IRB will communicate with the personnel listed above or the PI if further documentation is required.*

3.If this research is based in a center, institute, or department (Administering Department) other than the one listed above for the PI, select here. Be aware that if you do not enter anything here, the PI's home department will be AUTOMATICALLY inserted when you save this page.

| | |
|---|---|
| Department | Environmental Protection Agency (EPA) |

## 3. Funding Sources

1.Is this project funded (or proposed to be funded) by a contract or grant from an organization EXTERNAL to UNC-Chapel Hill?

Yes

Funding Source(s) and/or Sponsor(s)

| Sponsor Name | UNC Ramses Number | Sponsor Type | Prime Sponsor Name | Prime Sponsor Type | Sponsor/Grant Number | Detail |
|---|---|---|---|---|---|---|
| Environmental Protection Agency (epa) | Currently Not Available | Federal | | | Intramural EPA Funds | view |

2.Is this study funded by UNC-CH (e.g., department funds, internal pilot grants, trust accounts)?

No

3.Is this research classified (e.g. requires governmental security clearance)?

No

4.Is there a master protocol, grant application, or other proposal supporting this submission (check all that apply)?

- ✖ Grant Application
- ✖ Industry Sponsor Master Protocol
- ✖ Student Dissertation or Thesis Proposal
- ✖ Investigator Initiated Master Protocol
- ✖ Other Study Protocol

## 4. Screening Questions

*The following questions will help you determine if your project will require IRB review and approval.*

*The first question is whether this is RESEARCH*

1.Does your project involve a systematic investigation, including research development, testing and evaluation, which is designed to develop or contribute to generalizable knowledge? PLEASE NOTE: You should only answer yes if your activity meets all the above.

Yes

*The next questions will determine if there are HUMAN SUBJECTS*

2.Will you be obtaining information about a living individual through direct intervention or interaction with that individual? This would include any contact with people using questionnaires/surveys, interviews, focus groups, observations, treatment interventions, etc. PLEASE NOTE: Merely obtaining information FROM an individual does not mean you should answer 'Yes,' unless the information is also ABOUT them.

Yes

3.Will you be obtaining identifiable private information about a living individual collected through means other than direct interaction? This would include data, records or biological specimens that are currently existing or will be collected in the future for purposes other than this proposed research (e.g., medical records, ongoing collection of specimens for a tissue repository).

Yes

*The following questions will help build the remainder of your application.*

4.Will subjects be studied in the Clinical and Translational Research Center (CTRC, previously known as the GCRC) or is the CTRC involved in any other way with the study? (If yes, this application will be reviewed by the CTRC and additional data will be collected.)

No

5.Does this study directly recruit participants through the UNC Health Care clinical settings for cancer patients **or** does this study have a focus on cancer or a focus on a risk factor for cancer (e.g. increased physical activity to reduce colon cancer incidence) **or** does this study receive funding from a cancer agency, foundation, or other cancer related group? (If yes, this application may require additional review by the Oncology Protocol Review Committee.)

No

6.Are any personnel, organizations, entities, facilities or locations in addition to UNC-Chapel Hill involved in this research (e.g., is this a multi-site study or does it otherwise involve locations outside UNC-CH, including foreign locations)? You should also click "Yes" if you are requesting reliance on an external IRB, or that UNC's IRB cover another site or individual. See guidance.

No

# Exemptions

## Request Exemption

*Some research involving human subjects may be eligible for an exemption which would result in fewer application and review requirements. This would not apply in a study that involves drugs or devices, involves greater than minimal risk, or involves medical procedures or deception or minors, except in limited circumstances.*

*Additional guidance is available at the OHRE website. Exemptions can be confusing; if you have not completed this page before, please review this table with definitions and examples before you begin.*

1.Would you like your application evaluated for a possible exemption?

No

# Part A. Questions Common to All Studies

## A.1. Background and Rationale

1.Provide a summary of the background and rationale for this study (i.e., why is the study needed?). If a complete background and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive background and literature review, including references.

Despite improvements in air quality over the past several decades, over 100 million people in the U.S. still live in counties that do not meet the National Ambient Air Quality Standards (NAAQS) for one or more pollutants. During the course of daily living individuals are exposed to multiple pollutants from various sources of both natural and anthropogenic origin. It has become increasingly clear that air pollutant exposure is a risk factor for exacerbation and perhaps even progression of pulmonary and cardiovascular disease. The majority of controlled human exposure studies have examined individual pollutants; however, real-world exposures occur in the context of a complex mixture of pollutants. Different pollutants reach peak levels at different times during the day, which raises the concern that exposure to one pollutant may sensitize an individual so that their response to a subsequent exposure may be enhanced. Thus the sequence of exposure to these agents may affect their relative health effects and result in certain exposure scenarios being more deleterious than others.

To define multipollutant exposures that are relevant to real world scenarios we consulted experts in the EPA Office of Air and Radiation (OAR), who advised us to study the effects of sequential exposure to $NO_2$ and $O_3$, two ubiquitous NAAQS criteria pollutants. Ambient diurnal profiles of these two pollutants indicate that levels of $NO_2$ often peak in the evening and morning hours, which are followed by peak ambient $O_3$ concentrations during mid-day. Using this information we designed the study described here to determine whether sequential exposure to $NO_2$ and $O_3$,

or $O_3$ and $NO_2$, will result in greater pulmonary and cardiovascular effects than exposure to either pollutant alone. Ozone is a major component of photochemical smog and is one of the most thoroughly studied gaseous pollutants. Controlled human exposure studies have been critical in demonstrating that it can cause airway inflammation [1-3], including increases in neutrophil infiltration into the lung and the production of pro-inflammatory mediators [4,5], and ultimately decrements in lung function [6-12]. More recent studies have shown that ozone can also increase vascular inflammation, as well as alter autonomic nervous system control of heart rate [13]. Nitrogen dioxide is an oxidant that is produced by natural and anthropogenic processes. The majority of man-made $NO_2$ results from large-scale combustion-related processes, such as automobile emissions and the generation of electricity. Although traffic-related exposures account for the majority of $NO_2$ emissions [14,15]. Emissions from natural gas cooking appliances and kerosene-fueled space heaters with inadequate ventilation can serve as a significant source of human exposure to $NO_2$ indoors. Previous studies have shown that $NO_2$ concentrations can reach 600ppb in the area surrounding an operating gas stove [16], and peak levels may exceed 2000ppb [17]. Controlled human exposure studies have indicated that exposure to $NO_2$ alone (ranging from 110-2000ppb) results in little to no observable decrement in lung function; however, $NO_2$ exposure has been associated with increases in airway hyper-responsiveness, susceptibility to pulmonary infection, and increased pulmonary inflammation [18]. More recently, exposure to 500ppb $NO_2$ has been associated with changes in cardiac electrophysiology [19]. Recent epidemiological data indicate that exposure to $NO_2$ from vehicle emissions were associated with both respiratory and cardiovascular-related mortality [20]. Previous studies have shown that sequential exposure to $NO_2$ and $O_3$ (at concentrations similar to those proposed in this study) results in greater lung function decrements and increased non-specific airway responsiveness compared to $O_3$ exposure preceded by clean air exposure in young women [21]. Additional studies have demonstrated that sequential exposure to ozone, separated by 24 hours, resulted in greater lung function decrements, assessed as forced expiratory volume in the first second of exhalation ($FEV_1$), following the second exposure than was observed after the first [22]. Ozone exposure has also been shown to have a priming effect for subsequent exposure to sulfur dioxide ($SO_2$) in adolescent asthmatics [23] and allergen-induced responses of perennially allergic asthmatics [24]. Additionally, ongoing research at the EPA Human Studies Facility has demonstrated that sequential exposure of humans to diesel exhaust and ozone can result in greater lung function decrement than exposure to either pollutant alone. Given the complex nature of pollutant exposure, we are interested in determining if exposure to one pollutant can sensitize a person so that subsequent exposure to a second pollutant would cause a more pronounced response than would be expected based on exposure to just the second pollutant alone. Thus, in this study we will examine two exposure scenarios involving sequential exposures of $NO_2$ and $O_3$. The first involves determining whether an initial exposure to $NO_2$ will "prime" an individual to a subsequent $O_3$ exposure. The second involves determining whether an initial exposure to $O_3$, at a concentration that results in small cardiopulmonary changes that resolve within 24 hours, will augment a subsequent exposure to $NO_2$. Generally speaking, exposure to $NO_2$ alone is not associated with robust changes in metrics of cardiopulmonary function; however, we believe that it can modify, and be modified by, ozone exposure. Specifically, this study will test two general hypotheses. First, we hypothesize that pre-exposure to a relatively low concentration of $NO_2$ will "sensitize" individuals to a subsequent $O_3$ exposure and lead to greater changes in cardiopulmonary function compared to $O_3$ exposure preceded by CA exposure. Second, we hypothesize that pre-exposure to $O_3$, at a concentration that has been previously associated with small changes in cardiopulmonary function, will prime individuals to have a greater response to $NO_2$ compared to pre-exposure to clean air. The information obtained during the course of this study will enable the EPA to better evaluate the risks associated with sequential multipollutant exposure and potentially provide advice on activities to mitigate the effects.

1. Devlin, R.B., et al., *Time-Dependent Changes of Inflammatory Mediators in the Lungs of Humans Exposed to 0.4 ppm Ozone for 2 hr: A Comparison of Mediators Found in Bronchoalveolar Lavage Fluid 1 and 18 hr after Exposure.* Toxicology and Applied Pharmacology, 1996. **138**(1): p.

176-185.

2. Devlin, R.B.M., W.F.; Mann, R.; Becker, S.; House, D.E.; Schreinemachers, D.; Koren, H.S., *Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung.* Am. J. Respir. Cell Mol. Biol., 1991. **4**(1): p. 72-81.

3. Schelegle, E.S.S., A.D.; McDonald, R.J., *Time course of ozone-induced neutrophilia in normal humans.* Am. Rev. Respir. Dis., 1991. **143**: p. 1353-1358.

4. Bascom, R., *Environmental factors and respiratory hypersensitivity: the Americas.* Toxicol. Lett. , 1996. **86**: p. 115-130.

5. Peden, D.B.S., Jr., R.W.; Devlin, R.B., *Ozone exposure has both a priming effect of allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics.* Am. J. Respir. Crit. Care Med. , 1995. **151**: p. 1336-1345.

6. Adams, W.C., *Relation of Pulmonary Responses Induced by 6.6-h Exposures to 0.08 ppm Ozone and 2-h Exposures to 0.30 ppm Ozone via Chamber and Face-Mask Inhalation.* Inhalation Toxicology, 2003. **15**(8): p. 745-759.

7. Adams, W.C., *Comparison of Chamber 6.6-h Exposures to 0.04–0.08 PPM Ozone via Square-wave and Triangular Profiles on Pulmonary Responses.* Inhalation Toxicology, 2006. **18**(2): p. 127-136.

8. Folinsbee, L.J.M., W.F.; Horstman, D.H., *Pulmonary function and symptom responses after 6.6-hour exposure to 0.12ppm ozone with moderate exercise.* JAPCA, 1988. **38**(1): p. 28-35.

9. Hazucha, M.J., *Relationship between ozone exposure and pulmonary function changes.* Journal of Applied Physiology, 1987. **62**(4): p. 1671-1680.

10. Horstman, D.H.F., L.J.; Ives, P.J.; Abdul-Salaam, S.; McDonnell, W.F., *Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12ppm.* Am. Rev. Respir. Dis., 1990. **142**(5): p. 1158-1163.

11. McDonnell, W.F.K., H.R.; Abdul-Salaam, S.; Ives, P.J.; Folinsbee, L.J.; Devlin, R.B.; O'Neil, J.J.; Horstman, D.H., *Respiratory response of humans exposed to low levels of ozone for 6.6 hours.* Arch. Environ. Health, 1991. **46**(3): p. 145-150.

12. Silverman, F., et al., *Pulmonary function changes in ozone-interaction of concentration and ventilation.* Journal of Applied Physiology, 1976. **41**(6): p. 859-864.

13. Devlin, R.B., et al., *Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects* . Circulation, 2012. **126:** 104-111.

14. Lee, K., W. Yang, and N.D. Bofinger, *Impact of Microenvironmental Nitrogen Dioxide Concentrations on Personal Exposures in Australia.* Journal of the Air & Waste Management Association, 2000. **50**(10): p. 1739-1744.

15. Son, B., et al., *Estimation of occupational and nonoccupational nitrogen dioxide exposure for Korean taxi drivers using a microenvironmental model.* Environmental Research, 2004. **94**(3): p. 291-296.

16. Goldstein, I.F.L., K.; Andrews, L.R.; Foutrakis, G.; Kazembe, F.; Huange, P.; Hayes, C., *Acute respiratory effects of short-term nitrogen dioxide exposure.* Arch. Environ. Health, 1988. **43**: p. 138-142.

17. Leaderer, B.P.S., J.A.; Zagranski, R.T.; Quing-Shaung, M.A., *Field study of indoor air contaminant levels associated with unvented combustion sources (abstract).* 77th Annual Meeting of the Air Pollution Control Association, 1984. **33**: 3.

18. Hesterberg, T.W.B., W.B.; McClellan, R.O.; Hamade, A.K.; Long, C.M.; Valberg, P.A., *Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: Evidence for NO2 no-effect levels.* Crit. Rev. Toxicol. , 2009. **39**(9): p. 743-781.

19. Huang, Y.-C., et al., *Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans.* Inhalation Toxicology, 2012. **In Press**.

20. Brunekreef, B., et al. *Effects of long-term exposure to traffic-related air pollution on respiratory and cardiovascular mortality in the Netherlands: the NLCS-AIR study*. Res. Rep. Health Eff. Inst., 2009. **139:** 5-71.

21. Hazucha, M., et al. *Lung function response of healthy women after sequential exposures to $NO_2$ and $O_3$*. Am. J. Respir. Crit. Care Med. **150:** 642-647.

22. Devlin, R.B., et al., *Inflammation and cell damage induced by repeated exposure of humans to ozone.* Inhalation Toxicology, 1997. **9**(3): 211-235.

23. Koenig, J.Q.C., et al., *Prior exposure to ozone potnetiates subsequent response to sulfur dioxide in adolescent asthmatic subjects*, 1994. Am. Rev. Respir. Dis., 1990. **141**: 377-380.

24. Peden, D.B., et al., *Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics.* Am J of Resp and Crit Care Med, 1995. **151**: 1336-45.

2.State the research question(s) (i.e., specific study aims and/or hypotheses).

The goal of this study is to determine whether sequential pollutant exposure has a priming effect on cardiovascular and pulmonary endpoints in healthy 18-45 year-olds compared to single pollutant exposure. Specifically, we will examine the effect of sequential exposure scenarios using $NO_2$ and $O_3$.

## A.2. Subjects

1.Total number of subjects proposed across all sites by all investigators (provide exact number; if unlimited, enter 9999):

60

2.Total number of subjects to be studied by the UNC-CH investigator(s) (provide exact number; if unlimited, enter 9999):

60

3.If the above numbers include multiple groups, cohorts, or ranges or are dependent on unknown factors, or need any explanation, describe here:

A group of 26 healthy adults (men and women) between the ages of 18 and 45 years will participate and complete the study. However, because of potential dropouts and early terminations we will recruit 60 participants to have 26 individuals complete all four exposure regimens. Enrollment in this study will not be restricted by race or ethnicity. All study participants must be non-smokers for at least five years (with a lifetime smoking history of less than five pack-years) and have no active cardiac or respiratory disease. Women who are pregnant, trying to become pregnant, or breast feeding will not be accepted. All potential participants will undergo medical screening (previously approved IRB protocol #95-0518), including a detailed medical history, complete physical examination, baseline 12-lead EKG, and routine chemical and hematologic screens. All participants are required to be moderately active so that they can sustain intermittent periods of moderate exercise. During their study training day all volunteers will be monitored on our telemetry system

while exercising at levels similar to those that will be asked of them during the pollutant exposures. All participants will be recruited without regard to their genetic profiles; however, with their expressed consent they will be genotyped and classified for presence or absence of polymorphisms of select genes that are know to modify the response to air pollutants.

4.Do you have specific plans to enroll subjects from these vulnerable or select populations:
Do not check if status in that group is purely coincidental and has no bearing on the research. For example, do not check 'UNC-CH Employees' for a cancer treatment study or survey of the general public that is not aimed at employees.

| |
|---|
| ✖ Children (under the age of majority for their location) |
| Note that you will be asked to provide age ranges for children in the Consent Process section. |
| ✖ Non-English-speaking |
| ✖ Prisoners, others involuntarily detained or incarcerated (this includes parolees held in treatment centers as a condition of their parole) |
| ✖ Decisionally impaired |
| ✖ Pregnant women |
| ✖ HIV positive individuals |
| ✖ UNC-CH Students |
| Some research involving students may be eligible for waiver of parental permission (e.g., using departmental participant pools). See SOP 32.9.1 |
| ✖ UNC-CH Employees |
| ✖ People, including children, who are likely to be involved in abusive relationships, either as perpetrator or victim.<br>This would include studies that might uncover or expose child, elder or domestic abuse/neglect. ( See SOP Appendix H) |

5.If any of the above populations are checked, describe how you plan to confirm status in one or more of those groups (e.g., pregnancy, psychological or HIV testing)

No Answer Provided

6.If any of the above populations are checked, please describe your plans to provide additional protections for these subjects

No Answer Provided

7.Age range of subjects:

| | |
|---|---|
| Minimum age of subject enrolled | 18 |
| | years |
| Maximum age of subject enrolled | 45 |
| » If no maximum age limit, indicate 99 | |
| | years |

## A.3. Inclusion/exclusion criteria

1.List required characteristics of potential subjects (i.e., inclusion and exclusion criteria). If not covered, list also characteristics that would preclude their involvement.

We are interested in studying healthy volunteers between 18-45 years of age because they can serve as a low-risk study model. Further, we can compare the results of this study with those of previous and ongoing studies at the EPA Human Studies Facility with similar demographics.

Inclusion Criteria:

Healthy men and women between 18 and 45 years of age.

1. Physical conditioning allowing intermittent, moderate exercise for two hours.
2. Ability to complete the exposure exercise regimen without reaching 80% of predicted maximal heart rate.
    - Predicted maximal heart rate will be calculated using the equation (described by Tanaka *et al.* [2001] *J. Am. Coll. Cardiol.*): [208bpm-((0.7) x (age in years))]
3. Normal baseline 12-lead resting EKG, or if the automated reading is not normal the EKG must be approved by a study cardiologist.
4. Normal lung function
    - Forced vital capacity (FVC) > 75% of that predicted for gender, ethnicity, age and height (according to NHANESIII guidelines).
    - Forced expiratory volume in one second ($FEV_1$) > 75% of that predicted for gender, ethnicity, age and height.
    - $FEV_1$/FVC ratio > 75% of predicted values.
5. Oxygen saturation >96% on room air.

Exclusion Criteria:

1. Individuals with a history of acute or chronic cardiovascular disease, chronic respiratory disease, diabetes, rheumatologic diseases, or immunodeficiency state.
2. Individuals with a Framingham risk score (Hard Coronary Heart Disease; HCHD; 10-year risk) ≥10.
3. Individuals with asthma or a history of asthma.
4. Individuals who are allergic to chemical vapors or gases.
5. Females who are pregnant, attempting to become pregnant, or breastfeeding.
6. Individuals that are unwilling or unable to stop taking vitamin C or E, or medications that may impact the results of ozone challenge such at least two weeks prior to the study and for the duration of the study. Medications not specifically mentioned here may be reviewed by the investigators prior to an individual's inclusion in the study.
7. Individuals who have smoked tobacco during the last five years or those with a history of >5 pack years.
8. Individuals living with a smoker who smokes inside the house.
9. Individuals with a body mass index (BMI) >35 or <18. Body mass index is calculated by dividing the weight in kilograms by the square of the height in meters.
10. Individuals with occupational exposures to high levels of vapors, dust, gases, or fumes on an on-going basis.
11. Individuals with uncontrolled hypertension (≥150 systolic or ≥90 diastolic).
12. Individuals that do not understand or speak English.
13. Individuals that are unable to perform the exercise required for the study.
14. Individuals that are taking beta blocker medications.
15. Individuals with a history of skin allergies to adhesives used in securing EKG electrodes.
16. Individuals with unspecified diseases, conditions, or medications that might influence the responses to the exposures, as judged by the medical staff.
17. Individuals that are unwilling or unable to stop taking over-the-counter pain medications such as aspirin, ibuprofen (Advil, Motrin), naproxen (Aleve), or other non-steroidal anti-inflammatory ("NSAID") medications for 48 hours prior to the exposures and post-exposure visits.
18. Individuals that are taking systemic steroids or beta-blocker medications.

19. Individuals with a hemoglobin A1c (HbA1c) level > 6.4%.

Temporary Exclusion Criteria

1. Individuals with active seasonal allergies during the time of participation in the study.
2. Individuals suffering from acute respiratory illness within four weeks prior to any of the study exposure series.
3. Individuals that have been exposed to smoke and fumes within 24 hours of any study visit.
4. Individuals that have consumed alcohol within 24 hours of any study visit.
5. Individuals that have engaged in strenuous exercise within 24 hours of any study visit.
6. Individuals that have been exposed to ozone-based home air purifiers within 24 hours of any study visit.
7. Individuals that have been exposed to unvented household combustion sources (gas stoves, lit fireplaces, oil/kerosene heaters) within 48 hours of any study visit.

2.Justify any exclusion based on race, gender or ethnicity

Individuals will not be excluded from this study on the basis of race, gender, or ethnicity. Further, we will make every effort to recruit women and minorities into this study.

3.Will pregnant women or women who become pregnant be excluded?

Yes

If yes, provide justification and describe the type and timing of pregnancy testing to be used:

We will exclude women that are pregnant, or actively trying to become pregnant, from this study due to the unknown nature of the effects of air pollutant exposure on the fetus.

We will test for pregnancy using a chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine.

## A.4. Study design, methods and procedures

*Your response to the next question will help determine what further questions you will be asked in the following sections.*

1.Will you be using any **methods or procedures commonly used in biomedical or clinical research** (this would include but not be limited to drawing blood, performing lab tests or biological monitoring, conducting physical exams, administering drugs, or conducting a clinical trial)?

Yes

2.Describe the study design. List and describe study procedures, including a sequential description of what subjects will be asked to do, when relevant.

This is a randomized crossover single blind study with four arms separated by at least 13 days. We will measure cardiopulmonary responses following exposure to $NO_2$ (mean concentration of 500ppb, +/- 50ppb, over the two hour exposure period) and $O_3$ (mean concentration of 300ppb, +/- 30ppb, over the two hour exposure period) exposure, both singularly and sequentially, and clean air control in healthy adults while undergoing moderate intermittent exercise. Each study participant will be exposed randomly to all four exposure regimes (Fig. 1, below) with each one separated by at least 13 days. For all regimes, participants will be exposed to the pollutant or clean air for two hours while undergoing moderate intermittent exercise during the exposures. Moderate exercise will be carried out on an ergometer (stationary bicycle or treadmill) in the chamber for 15-minute intervals beginning after the first 15 minutes of exposure and resting for 15 minutes after each time for a total of one hour of exercise per chamber exposure. Ventilation rate measurements will be taken during each exercise session to monitor inspired ventilation rates. The target inspired ventilation rate is 20

L/min/ $m^2$ BSA, which in most individuals may be approximately 50 L/min.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Participants will be monitored continuously by EPA personnel for signs of distress, such as shortness of breath or chest pain, and an on-call physician will be available in the building. Study participants will be able to end their exposure and exit the chamber at any time. Total exposure times will be two hours. The exposure atmosphere will be at approximately 40% ± 10% relative humidity (RH) and approximately 22 ± 2 $^{o}$C. Clean air will be passed through an air purification system to ensure minimal presence of pollutant gases ($O_3$ and $NO_2$), organic vapors, or particles. Nitrogen dioxide and $O_3$ concentrations will be monitored continuously in real time. During exposures, gaseous pollutant delivery will be immediately halted if the target concentration is exceeded by >10%. There may be a need to enroll two volunteers for the same exposure series. If this is the case, the volunteers can opt out of this request on the consent form.

***Physical Examination Day***

Prior to recruitment into the study, volunteers will undergo a physical exam under a separate protocol (IRB protocol #95-0518). During this visit, the volunteer's medical history will be obtained, he or she will undergo a physical examination with a resting 12-lead EKG, and his or her vital signs, height, and weight will be assessed in order to determine eligibility. Up to 25mL of blood may be collected for a complete blood count with differential, basic metabolic panel, and complete lipid panel.

***Facilities***

A detailed description of the physical facilities in the Human Studies Facility (HSF) of the U.S. EPA Health Effects Research Laboratory located on the UNC-CH campus have been published as U.S. Government Publication EPA-600/1-78-064, and is on file in the office of the UNC Committee on the Protection of the Rights of Human Subjects. The medical station of the Human Studies Facility includes a fully-equipped medical examination room, a cardiac/respiratory emergency cart, a participant recovery room, a nurse's station, a waiting room and conference rooms. The facility is staffed by qualified Registered Nurses, and on-site physicians are available to respond to a medical emergency in a timely manner.

***Training Day***

1. Eligible study participants will report to the EPA Human Studies Facility for a training session that will last approximately three hours.
2. Informed consent will be obtained. Additionally, the volunteers will be made aware that more than one person may be enrolled for the same exposure series. The volunteers will be given the ability to opt out of this request.
3. Vital signs will be taken and recorded (temperature, pulse, respiratory rate, and blood pressure, oxygen saturation).
4. Pregnancy tests will be administered to any women who may have child-bearing potential that have not previously undergone a hysterectomy.
5. Participants will perform spirometry.
6. Participants will be trained on the ergometer (stationary bicycle or treadmill) and the workload to elicit a minute ventilation (inspiration) normalized for body surface of approximately 20 L/min/$m^2$ BSA will be determined. In most participants this may be 40 L/minute (i.e. $VO_2$ of approximately 2.0 L/m).
    - The participant's ability to complete the level of exercise required for the study will be assessed. Additionally, the participant must be able to perform the required level of exercise while maintaining a blood oxygen saturation of ≥92%.
    - If at any time during the training visit the participant indicates that he or she is having chest pain, shortness of breath, or other signs of distress, then the training activity will be terminated immediately and the on-duty physician will be called to assess the physical state of the volunteer.

**Exposure Day (**Same protocol for both Day #1 and Day #2)

Study participants will be randomly exposed to four individual regimens, which are shown below in Table #1.

| | Day #1 | Day #2 | Day #3 |
|---|---|---|---|
| **Exposure Series #1** | Clean Air | $O_3$ | Follow-up |
| **Exposure Series #2** | $NO_2$ | $O_3$ | Follow-up |
| **Exposure Series #3** | Clean Air | $NO_2$ | Follow-up |
| **Exposure Series #4** | $O_3$ | $NO_2$ | Follow-up |

**Table #1. Description of individual exposure series.**

***Pre-exposure (Days #1 and #2):*** On the day of the exposure, study participants will report to the medical station in the HSF at which time their general health will be evaluated and the appropriate pre-exposure measurements (vital signs, cardiac electrophysiology, pulmonary function by spirometry, pulse wave analysis, and blood sampling) will be completed.

- ***Ambulatory EKG measurements*** will be collected with a Holter monitor (Mortara, Inc.; Boston MA). EKG electrodes will be placed on clean skin, which will be shaved if necessary, to ensure proper attachment and function. Following placement of the EKG leads participants will relax for 20 minutes in a reclined position, after which 10 minutes of resting cardiac physiology measurements will be obtained.
- ***Blood pressure and heart rate measurement*** will be measured pre-exposure at the medical station by a blood pressure monitor.
- ***Pulmonary function*** will be measured by spirometry.
- ***Venous blood sampling*** **:** approximately 25 ml of blood will be collected.
- ***Pulse wave analysis*****:** baseline measurements will be obtained to serve as a comparison to post-exposure measurements.

***Exposure (Days #1 and #2) :*** The participants will be exposed to one of the exposure series shown in Table #1. All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. The participants will engage in intermittent moderate exercise on an ergometer (stationary bicycle or treadmill) for 15-minute intervals throughout the exposure period. During the exposure, participants will be monitored continuously by visual camera monitor, continuous pulse oximetry, and telemetry. An on-call physician will be available. The participants will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be two hours per exposure day (Days #1 and #2).

***Immediate Post-exposure (Days #1 and #2) :*** Lung function will be assessed by spirometry. Approximately 25 ml of venous blood will be collected. Ambulatory cardiac electrophysiology measurements will be taken with the Holter monitor. Once the Holter measurements have been collected the Holter monitor will be removed. Radial arterial pulse waves will be recorded for analysis.

***Follow-up Day (Day #3):*** Study participants will return to the HSF the morning after completing the Day #2 exposure of each exposure series to have their lung function assessed by spirometry and undergo electrocardiography for 30 minutes at rest using a Holter monitor. At this time they will also have approximately 25mL of venous blood collected and undergo pulse wave analysis. Following the completion of Holter monitor data collection, the Holter monitor will be removed before the participant is discharged.

**Outcomes:**

Our primary endpoints will be cardiac electrophysiology measurements, pulmonary function, and pulse wave analysis. We will also monitor levels of soluble markers of inflammation and clotting/coagulation in venous blood as secondary endpoints. It is possible that not all procedures will be performed on every participant.

***Ambulatory EKG monitoring:*** Cardiac electrophysiology data will be collected on each study participant before and after the exposures on Days #1 and #2, as well as during the follow-up appointment on Day #3. Participants will be asked to relax for 20 minutes in a reclined position in a quiet room after which 10 minutes of resting Holter monitor data will be obtained. Specific five-minute epochs will be analyzed for frequency domain and repolarization variables. Study participants will wear a Holter monitor during the exercise regimens and immediate post-exposure follow-up measurements on Days #1 and #2 of each exposure series. Prior to EKG electrode placement, the skin will be prepared appropriately, which may include shaving of the electrode placement area in men. Electrodes are attached and connected to the beeper-sized Holter monitor, which participants wear around their waist.

***Pulmonary Function***: Study participants will perform spirometry before and after the exposures on Days #1 and #2, and during the follow-up visit on Day #3. Spirometry will be performed using a Sensor Medic Vmax pulmonary function system (dry rolling seal spirometer) according to the standard procedure published by the American Thoracic Society. This test measures the volume of air inspired and expired as a function of time. Participants will inhale as deeply as possible, then exhale as rapidly and completely as possible into the spirometer. Measurements obtained from each maneuver will include forced vital capacity (FVC), forced expiratory volume in the first second of exhalation ($FEV_1$), and maximal mid-expiratory flow rate ($FEF_{25-75\%}$). The largest FVC and $FEV_1$ from at least three acceptable trials will be selected for analysis. The flow rates will be selected from the trials with the largest $FEV_1$+FVC.

***Peripheral Venous Blood S ample:*** Peripheral venous blood will be collected from each study participant before and after the exposures on Days #1 and #2, as well as during the follow-up appointment on Day #3. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet will be applied and blood will be drawn from an antecubital or other appropriate vein. Endpoint measurements may include, but not be limited to, the following: biomarkers for inflammation, coagulation factors, and vasoactive factors. With the participant's permission, a portion of the peripheral blood sample will be used for genetic testing, to identify potential polymorphisms that may make the participant more susceptible to air pollutants. In addition, samples may be stored for as yet undesignated research. Consent for genotyping will be included with the general consent form. Consent for storage is a separate form.

***Pulse Wave Analysis:*** Pulse wave analysis will be performed on each study participant before and after the exposures on Days #1 and #2, as well as during the follow-up appointment on Day #3. The study participant will sit in a recliner with the arm supported in a relaxed and comfortable position. The operator will palpate the point of maximal impulse of the radial artery and mark it with a pen. The participant’s forearm will be placed in a positioning splint and immobilized with Velcro straps (one over the fingers with the hand facing up and the second strap over the forearm at the opposite end of the splint). Neither strap will be too tight as to cause discomfort or occlude blood flow. Next the pulse wave sensor (tonometer) will be positioned over the marked area and placed approximately at the level of the heart. A blood pressure cuff will be placed on the opposite arm. The participant will rest for at least 10 minutes. After the rest period the operator will begin the pulse wave analysis. The first step is to take the blood pressure followed by adjusting the tonometer until an acceptable waveform is obtained. Once an acceptable waveform has been reached, the operator will start the pulse wave reading. The participant’s blood pressure will be assessed a second time by the pulse wave machine followed by approximately 30 seconds of pulse wave reading/recording by the tonometer. Once this has been completed the data will be printed on a standard form. The blood pressure cuff, tonometer, and the splint will all be removed.

***Exercise measurements:*** Study participants will breathe for up to three minutes through a pneumotachograph while performing exercise during the course of each exercise period of each exposure to allow for the acquisition of ventilation parameters, including minute ventilation, tidal volume, and breathing frequency.

3.If subjects are assigned or randomized to study "arms" or groups, describe how they are assigned.

The study participant's exposure condition will be determined by TRC Pollutant Control System operators utilizing a computer program that simulates a random drawing from a pool of identical objects.

4.Describe any follow up procedures.

We will follow-up with study participants the day after they complete the second exposure in each series. During the follow-up visit the study participants will be seen by medical professionals, have blood drawn for analysis, and have their lung function and cardiac electrophyisiology assessed.

5.What are your proposed start and stop dates for this study?

We anticipate that this study will take 18-24 months for completion.

6.Will this study use any of the following methods?

| | |
|---|---|
| ✘ | Audiotaping |
| ✘ | Videotaping or filming |
| ✘ | Behavioral observation - (e.g., Participant, naturalistic, experimental, and other observational methods typically used in social science research) |
| ✘ | Pencil and paper questionnaires or surveys |
| ✘ | Electronic questionnaires or surveys |
| ✘ | Telephone questionnaires or surveys |
| ✘ | Interview questionnaires or surveys |
| ✘ | Other questionnaires or surveys |
| ✘ | Focus groups |
| ✘ | Diaries or journals |
| ✘ | Photovoice |
| ✘ | Still photography |

7.If there are procedures or methods that require specialized training, describe who (role/qualifications) will be involved and how they will be trained.

Spirometry will be performed by a qualified professional with experience in both clinical and research duties.

Venipuncture will be performed by a qualified professional registered nurse (RN).

Placement of the ambulatory EKG electrodes will be done by a registered nurse with previous experience.

Pulse wave analysis will be performed by a qualified professional with previous experience.

Controlled air pollutant exposure conditions will be monitored by an engineer with several years of previous experience in contolled human exposure studies at the EPA Human Studies Facility.

8.Are there cultural issues, concerns or implications for the methods to be used with this study population?

No

## A.4.A. Biomedical methods and procedures

1.Is this an interventional study that involves treatment, evaluation or diagnosis of a medical disease or condition?

No

2.Is this a Clinical Study?
Check YES if this study involves research using human volunteers that is intended to add to medical knowledge. There are two main types of clinical studies: clinical trials and observational studies. Do NOT check yes merely because you are conducting research in a clinical setting or using clinical data.
Click here for additional definition of "Clinical Study"

Yes

Will this clinical trial be listed in ClinicalTrials.gov, either by you or the sponsor?

Yes

Choose the appropriate Phase designation for this clinical trial.

- ✗ Pilot Study
- ✗ Phase I
- ✗ Phase I/II
- ✗ Phase II
- ✗ Phase III
- ✗ Phase IV
- ✓ Other

If other, please explain
Controlled human exposure to environmental pollutants

3.If the study involves the use of placebo control, provide justification

Not applicable.

4.Will this study involve drugs, biologics or other substances (such as a botanical or dietary supplement)?
For guidance on dietary supplements, see Section VI, C FDA guidance document UCM229175.pdf

No

5.Is there an Investigational New Drug application (IND) for this study?

No

Please check below:

This study does not involve drugs, biologics or other substances.

6.Will this study involve investigational devices, instruments, machines or software?

No

7.Does your study involve any of the following? (check all that apply)

- ✗ Embryonic stem cells
- ✗ Fetal tissue
- ✓ Genetic testing (see GINA and GWAS)
- ✓ Clinical laboratory tests

If McLendon Labs will do the testing, you must complete the appropriate form found at UNC Health Care and submit to them for review.

- ✗ Testing for communicable diseases that have mandated reporting requirements (link to state guidance)
- ✓ Point of Care Testing (POCT), which is CLIA-approved testing done at the "bedside" or site of care by hospital or clinic personnel (not by subject). Examples include urine pregnancy testing, glucose monitoring, etc.

If McLendon Labs will do the testing, you must complete the POCT form found at UNC Health Care and submit to them for review.

- ✗ Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise if not participating in this research study. Do not check if all radiation is administered as standard of care. ( Human Use of Radiation in Research)
- ✗ Gadolinium administered as a contrast agent
- ✗ Recombinant DNA or gene transfer to human subjects

8.Will your study involve storage of specimens for future unspecified research?

Yes

Please explain:

No Answer Provided

Will any personal identifiers or codes be retained with the specimens that would allow anyone to link the specimen back to an individual subject?

Yes

## A.5. Benefits to subjects and/or society

1.Describe how this study will contribute to generalizable knowledge that will benefit society.

The results of this study may contribute to the overall assessment of air pollution effects in the U.S. and thereby influence health policy. The study participants will not benefit personally from being in this research study other than by undergoing a free physical examination and screening.

2.Does this study have the potential for direct benefit to individual subjects in this study?

No

*Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the*

*individual subject, say so here and in the consent form, if there is a consent form. Do not cite monetary payment or other compensation as a benefit.*

Explain

Not applicable

3.Are there plans to communicate the results of the research back to the subjects?

No

## A.6. Risks and measures to minimize risks

*For each of the following categories of risk you will be asked to describe any items checked and what will be done to minimize the risks.*

1.Psychological

- ✘ Emotional distress
- ✘ Embarrassment
- ✘ Consequences of breach of confidentiality (Check and describe only once on this page)
- ✘ Other

2.Describe any items checked above and what will be done to minimize these risks

No Answer Provided

3.Social

- ✘ Loss of reputation or standing within the community
- ✘ Harms to a larger group or community beyond the subjects of the study (e.g., stigmatization)
- ✔ Consequences of breach of confidentiality (Check and describe only once on this page)
- ✘ Other

4.Describe any items checked above and what will be done to minimize these risks

Risk of breach of confidentiality is minimal. All study participants will be assigned a study number which will be used for data recording – not the participant's name. The study number is all that will be entered into computer databases. All paper files that may contain the participant's name or screening number are secured in a locked cabinet in a locked room at the US EPA facility with controlled building access and a security guard positioned at the building entrance 24 hours a day 365 days a year. Any abnormal medical findings (CBC, EKG, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her personal physician. Samples will be stored in a secured room at the U.S. EPA HSF. A numeric coding system will be used to ensure that study participants cannot be directly identified from the samples alone.

5.Economic

- ✘ Loss of income
- ✘ Loss of employment or insurability

- ✗ Loss of professional standing or reputation
- ✗ Loss of standing within the community
- ✗ Consequences of breach of confidentiality (Check and describe only once on this page)
- ✗ Other

6.Describe any items checked above and what will be done to minimize these risks.

No Answer Provided

7.Legal

- ✗ Disclosure of illegal activity
- ✗ Disclosure of negligence
- ✗ Consequences of breach of confidentiality (Check and describe only once on this page)
- ✗ Other

8.Describe any items checked above and what will be done to minimize these risks

No Answer Provided

9.Physical

- ✗ Medication side effects
- ✗ Pain
- ✓ Discomfort
- ✓ Injury
- ✗ To a nursing child or a fetus (either through mother or father)

10.Describe any items checked above, including the category of likelihood and what will be done to minimize these risks. Where possible, describe the likelihood of the risks occurring, using the following terms:

- Very Common (approximate incidence > 50%)
- Common (approximate incidence > 25%)
- Likely (approximate incidence of 10-25%)
- Infrequent (approximate incidence of 1-10%)
- Rare (approximate incidence < 1%)

This study might involve the following risks and/or discomforts:

***Ozone Exposure:*** Potential risks may include decrements in lung function, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. These symptoms typically resolve within 2-4 hours after exposure, but may last longer for particularly sensitive people. Ozone exposure also induces an inflammatory reaction that may last for 24 hours after the exposure and may increase the chance of you catching a cold. Very recently some studies have suggested that elderly people, particularly those with underlying cardiovascular disease, are at increased risk for getting sick and even dying during episodes of high ozone pollution. In addition, there may be uncommon or previously unknown risks that might occur. While we cannot exclude the possibility that study participants may have an adverse reaction to breathing ozone other human exposure studies have utilized 300ppb ozone, or more, for many years. Additionally, the total amount of ozone that study participants will be exposed to during the two-hour period is equivalent to what they would be exposed to in a city at the current eight-hour national standard. The current National Ambient Air Quality Standard for ozone is 75ppb for an eight-hour period, which results in a

cumulative exposure to 600ppb ozone during the eight-hour period. This is equivalent to a two-hour exposure to 300ppb (cumulative exposure = 600ppb), as will be done in this study. Participants will be monitored by direct observation, via closed-circuit television, cardiac telemetry, and pulse oximetry during exposure periods.

***Nitrogen Dioxide Exposure:*** Potential risks include decrements in lung function, irritation to the nose, eyes, throat and airways, pain on deep inspiration and cough. Previous controlled human exposure studies have utilized $NO_2$ concentrations equal to or higher (up to 2000ppb) than those that will be used in this study. Additionally, the $NO_2$ levels that will be used in this study are lower than those that have been measured around an operating gas stoves [16,17]. Participants will be monitored by direct observation, via closed-circuit television, cardiac telemetry, and pulse oximetry during exposure periods.

***Effects of sequential $NO_2$ and $O_3$ exposure:*** Because this exposure scenario has not been conducted at our facilities or elsewhere before, we do not yet know whether or not this exposure will include the same risks as $NO_2$ and $O_3$ exposures alone. It is possible that exposure to $NO_2$ prior to ozone exposures will yield greater effects compared to exposure to $O_3$ alone. The purpose of this study is to determine the effects of the sequential exposure scenario.

***Blood Sampling by Venipuncture:*** Insertion of the needle may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the participant may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Participants are closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility, if they are stable.

***Breathing tests (Spirometry):*** Pulmonary function tests are standard clinical tests that are commonly performed in hospitals and entail little or no risk to the participants. However, cough or dizziness may occur during these tests. If these symptoms occur, they are usually temporary. Participants will remain seated in a chair until symptoms disappear. There is an extremely small chance that the subject could have a bronchospasm or faint upon performing a forced breath expiratory maneuver for spirometry.

***Exercise:*** Moderate exercise on a stationary bicycle or treadmill entails the potential, although minimal, risk of occasional muscle soreness, cramps or general fatigue. These discomforts are temporary and not harmful. Heart rate and rhythm will be monitored using a cardiac telemetry device. Heart rate will be kept below 80% of the age related maximum (i.e., [(208bpm – [(0.7) x (age in years)]]; according to Tanaka *et al.* (2001)) during the exercise periods. As is common with studies involving this population, we use 80% of calculated maximum heart rate as a safety cut-off. If a study participant reaches 80% of their calculated maximum heart rate then we will reduce the exercise level to reduce his or her heart rate. Exercise will be terminated at any time upon the request of the participant or if the investigator/medical staff observes signs of distress. During exercise, participants will be monitored by direct observation, via closed-circuit television, cardiac telemetry, and pulse oximetry during exposure periods.

***Heart Rate and EKG Monitoring:*** EKG and heart rate variability are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the participant. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, temporary skin discoloration in some sensitive individuals, itching, or soreness in some participants.

***Pulse Wave Analysis:*** There are no known risks associated with imaging of the radial artery. There may be mild pressure caused by the tonometer pressing down on the radial artery but blood flow will not be occluded. There will be a red, minor indentation in the skin over the radial artery, but this usually fades within a few hours.

***Unknown/Unforeseen Risk:*** In addition to the potential risks outlined above, there is always the

possibility for uncommon or previously unknown/unforeseen risks to occur. The investigators and medical staff take precautions during the course of the study to generally reduce risk to study participants. During all exposures and testing, participants will be monitored by EPA personnel. The investigators or on-call physician will end the exposure if the subject is found to be suffering from any signs of distress, such as significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Heart rate will be kept below 80% of the age related maximum (i.e., [(208bpm – [(0.7) x (age in years)]]; according to Tanaka *et al.* (2001)) during the exercise periods. As is common with studies involving this population, we use 80% of calculated maximum heart rate as a safety cut-off. If a study participant reaches 80% of their calculated maximum heart rate then we will reduce the exercise level to reduce his or her heart rate. Exercise will be terminated at any time upon the request of the participant or if the investigator/medical staff observes signs of distress. During exercise, participants will be monitored by direct observation, via closed-circuit television, cardiac telemetry, and pulse oximetry during exposure periods. For safety reasons, if a study participant exhibits a significant heart rhythm abnormality or if their $O_2$ saturation value is ≤92% their exercise will be terminated, they will be removed from the chamber, and they will be evaluated by a physician. An on-call physician will be available in the building to respond to an emergency during all study visits. A group of physicians employed by EPA and UNC Center for Environmental Medicine, Asthma & Lung Biology have primary responsibility for medical coverage of studies conducted in the EPA Human Exposure Facility. This facility is also equipped with an emergency "crash cart" with standard emergency medications, IV fluids, and a defibrillator in the unlikely event of a medical emergency during any exposure or exposure-related procedure. The EPA Human Studies Facility is in close proximity to the University of North Carolina (UNC) Hospitals Emergency Room, where UNC Hospital System physicians are also available to assist in treatment of an emergency.

11. Unless already addressed above, describe procedures for referring subjects who are found, during the course of this study, to be in need of medical follow-up or psychological counseling

Study participants will be given any new information gained during the course of the study that might affect their willingness to continue to participate in this study, and regarding our decision to not allow volunteers to participate in the study (e.g. physical findings, abnormal blood tests, etc). Volunteers will be provided with a copy of the report (e.g. CBC with differential, spirometry) to take to their physician. They will also be informed that neither EPA nor UNC is financially responsible should they decide to follow-up with their physician regarding any abnormal findings identified during the study.

12. Are there plans to withdraw or follow subjects (or partners of subjects) who become pregnant while enrolled in this study?

No

## A.7. Data and safety monitoring

1. When appropriate, describe the plan for monitoring the data to ensure the safety of participants. These plans could range from the investigator monitoring subject data for any safety concerns to a sponsor-based data and safety monitoring board or committee (DSMB, DSMC, DMC), depending on the study. For studies that do not raise obvious safety concerns, you may still describe your plans for monitoring the study as it progresses.

The safety of participants will be monitored throughout the course of the study by the investigators and EPA medical staff. There will be monitoring of participants by either direct observation or by closed circuit television during each exposure. During each exposure, every study participant will have their heart rate and rhythm monitored by telemetry. Additionally, the participant's pulse oximetry will be monitored during the two hour exposure/exercise period. Registered Nurses will monitor the participant from the Medical Station during the exposure. Additionally, a study physician will be immediately available by pager during all study visits. A fully

equipped medical-cart will be accessible at all times with resuscitation medications and equipment. In the event of an emergency during which it becomes necessary to call 911, the UNC Chapel Hill Hospital Emergency Department is in close proximity to our facility. Participants will be medically assessed before, during and at the completion of each exposure. Participants will be asked to refrain from strenuous physical activity for 24 hours before and after each exposure.

Hematologic markers (e.g. hemoglobin, electrolytes) will be measured following each visit and analyzed for abnormalities. Additionally, Holter monitor data will be downloaded within 24 hours following the study visit and will be assessed by a Registered Nurse prior to the beginning of the next exposure series. If the assessment indicates that any significant arrhythmias occurred the Holter monitoring results will be reviewed by a licensed physician. Individuals will be removed from the study if they experience arrhythmias that pose a safety risk, such as symptomatic bradycardia, sustained superventricular tachycardia, and either sustained (≥30 sec or symptomatic for any duration) or unsustained (≥3 consecutive ventricular beats) ventricular tachycardia. Any detected abnormality will be brought to the attention of the investigators and the medical staff who will review the information and determine if there is adequate concern to remove the participant from the study. ny abnormality detected will be conveyed to the subject with appropriate advice for follow-up with their primary care physician or sub-specialty physician, when warranted. Any Unanticipated Problem(s) (UP) or Adverse Event(s) (AE), as defined by the UNC IRB, will be reported to the IRB as directed by the published Standard Operating Procedures (19.0).

Subjects will be aware of their right to terminate their participation in the study at any time without prejudice or loss of monetary compensation.

2.If not already addressed above, describe the plans for aggregate review of unanticipated problems (including but not limited to adverse events) across all sites, in order to monitor subject safety.

Not applicable

3.What are the criteria that will be used to withdraw an INDIVIDUAL SUBJECT from this study or halt the research intervention (e.g., abnormal lab tests, allergic reactions, failure or inability to comply with study procedures, etc.)?

Individual participants will be withdrawn from the study if they exhibit abnormal lab test values that, in the opinion of the investigators and study physician(s), put them at a reasonable risk for an adverse reaction to any aspect of the study procedure. Individual participants will also be withdrawn from the study if they exhibit signs of physical distress (ex. chest pain or shortness of breath) during the study.

Additionally, if an individual study participant exhibits an adverse reaction to either $O_3$ or $NO_2$ exposure, or is unable to comply with the study procedures, he or she will be removed from the study.

4.Are there criteria that will be used to stop the ENTIRE STUDY prematurely (e.g., safety, efficacy, unexpected adverse events, inability to recruit sufficient number of subjects, etc.)?

Yes

Please explain

The entire study will be stopped if we are unable to recruit a sufficient number of study participants. Additionally, after every five participants complete each exposure series we will assess the incidence of significant arrhythmia, such as symptomatic bradycardia, sustained superventricular tachycardia, and either sustained (≥30 sec or symptomatic for any duration) or unsustained (≥3 consecutive ventricular beats) ventricular tachycardia and the potential risk that they would pose to the study population. If greater than 50% (cumulative for all participants that had completed that

specific exposure series at the time of assessment) of study participants exhibit significant arrhythmia or lung function decrements ($FEV_1$) greater than 40% (comparison of absolute values between pre- and post-exposure measurements) then the study will be suspended.

5.Will this study involve a data and safety monitoring board or committee?

No

## A.8. Data analysis

1.Summarize the statistical analysis strategy for each specific aim.

This study will be a four arm crossover design study during which participants receive a sequential combination of ozone (O3), nitrogen dioxide (NO2), or clean air (CA) in the following combinations 1.) CA-NO2, 2.) CA-O3, 3.) O3-NO2, 4.) NO2-O3. The order in which each subject will receive these four exposure sequences will be randomized. Each subject will receive an equal dose of clean air, 500 ppb NO2, or 300 ppb O3 during a two-hour exposure with exercise. The main hypothesis of the study is that exposure to a pollutant will lead to greater effects following a prior pollutant exposure then following a prior clean air exposure:

Primary Hypothesis 1:

- H0: Response following O3-NO2 is equal to the response following CA-NO2
- HA: Response following O3-NO2 is not equal to the response following CA-NO2

Primary Hypothesis 2:

- H0: Response following NO2-O3 is equal to the response following CA-O3
- HA: Response following NO2-O3 is not equal to the response following CA-O3

A secondary hypothesis will be aimed at confirming the results of single pollutant studies.

Secondary Hypothesis 1:

- H0: Response following NO2 exposure is equal to the response following CA
- HA: Response following NO2 exposure is not equal to the response following CA

Secondary Hypothesis 2

- H0: Response following O3 exposure is equal to the response following CA
- HA: Response following O3 exposure is not equal to the response following CA

The study is focused on cardiovascular effects of pollutant exposure with the high frequency component of heart rate variability (HRV) defined as primary endpoints. These include “High Frequency” (HF) and “Normalized High Frequency" (HFn) parameters. Endpoints are measured at five time-points for each of the four study Arms.

Three time points are used to address primary hypothesis and they are referred to as “pre” exposure measured on day 1 of the study arm, “post” exposure measured on day 2 of the study arm and “follow up” (FU) measured on day 3 of the study arm. Based on previous studies conducted by investigators at Human Studies Facility we have accepted a convention of normalizing ”post” and ”follow up” measurements by the corresponding “pre” exposure measurement prior to analysis by dividing “post”/”pre” and “FU”/”pre”. The normalization helps to control for day to day variability of the baseline level in the individuals and allows us to express all endpoints as “percent of the baseline”. All endpoints are measured on the continuous scale.

In order to maximize statistical power in the crossover design we pair normalized “post” responses and normalized “FU” responses between Arm 3 and Arm 1 to test Hypothesis 1 and separately between Arm 4 and Arm 2 to test Hypothesis 2. Responses at “post” time points of Arms 3 and 4 are

compared to the paired "post" responses at Arms 1 and 2. Similarly follow up time point measurements are compared only to follow up. Post and follow up time points cannot be compared due to diurnal variation. To compare these changes we use paired t-test with type I error rate of 5%. Results will be displayed with a point estimate and corresponding 95% confidence intervals of the difference between two exposure arms in terms of "percent point differences" relative to baseline. This study will examine secondary endpoints in a similar manner.

*Missing data*

This study follows a crossover design where each subject's response is paired across different exposure treatments. By design therefore we only use data for subjects that have complete data needed to test the hypothesis and do not impute missing values. From the past experiences of similar studies subjects miss an exposure arm for one out of three reasons; 1) Subjects do not return for all study arms for personal reasons, 2) Subjects may be disqualified for medical reasons, 3) Subject may be temporarily put on medical hold for reasons such as cold, flu, or allergies. All subjects undergo physical examination and screening which contributes to only small portion of subjects missing for the first two reasons. In case of medical hold subjects are rescheduled for a later date according to the protocol. Occasionally only a portion of endpoints are not available for unpredictable reasons such as equipment failure, failed ELISA etc. In this case, only a particular set of end-points are excluded from the analysis.

*Other Considerations:*

To minimize carry over effect in a crossover design exposures are randomized and separated by a minimum of two weeks.

To minimize the effect of unmeasured confounders a number of restrictions are imposed on study design;

- All subjects are selected or excluded according to the basis of inclusion-exclusion criteria
- All exposures are done at approximately the same time of the day following exact protocol
- All measurements are collected at approximately the same time
- All endpoints are normalized and paired within subjects for statistical analyses.

Multiple testing, and as a consequence the potential for false-positive results, can be a problem; however, we will look at response patterns rather than statistical significance and health outcomes/markers of similar pathways (e.g. inflammatory pathway) as well as within each marker to get a consistent picture of the influence of air pollution on our health outcomes.

2.What are the practical objectives of the study?

This study will be a four arm crossover design study during which participants receive a sequential combination of ozone ($O_3$), nitrogen dioxide ($NO_2$), or clean air (CA) in the following combinations 1.) CA-$NO_2$, 2.) CA-$O_3$, 3.) $O_3$-$NO_2$, 4.) $NO_2$-$O_3$. The order in which each subject will receive these four exposure sequences will be randomized. Each subject will receive an equal dose of clean air, 500 ppb $NO_2$, or 300 ppb $O_3$ during a two-hour exposure with exercise. The main hypothesis of the study is that exposure to a pollutant will lead to greater effects following a prior pollutant exposure then following a prior clean air exposure. This study is focused on cardiovascular effects of pollutant exposure with a primary endpoint defined as the high frequency component of heart rate variability (HRV): "High Frequency" (HF) and "Normalized High Frequency" (HFn) parameters.

3.If this is a pilot study, please describe the future study and say how its study design, aims, sample size, and methods differ from the pilot study you are proposing.

Not applicable.

4.Provide a compelling justification for the proposed sample size in terms of the likelihood of achieving each aim.

To estimate the required sample size for the power of 80% with a type I error rate of 5% for a two-sided alternative hypothesis we used data from two previous studies conducted by the co-investigators. One such study examined synergistic effects of gas and particle exposure in comparison to clean air. One of the arms of the study included an exposure to 500ppb of $NO_2$ in young healthy individuals. The results of the published study indicated that there was a 12% decrease in the HFn parameter following $NO_2$ exposure relative to CA. This study was a randomized single-blinded study however only a small portion of subjects received both $NO_2$ and CA exposure. We made an assumption of testing responses in two independent samples with standard deviation of 16% and 15% for CA and NO2 exposure, respectively (in standardized units), and estimated that 26 subjects will be needed for the proposed exposure study. The assumption is conservative as it should serve as an upper estimate of sample size by not accounting for the correlation between the two independent samples.

The second sample size estimation is based on the published result of 51% decrease in HF following $O_3$ exposure relative to clean air in a cross over design. Using the standard deviation of 59% from this study we estimated that 25 subjects are needed for power of 80% and type I error of 5%.

Based on these two studies we conclude that the minimum sample size for testing the above hypothesis is 26 subjects.

5.Summarize the plans for data management.

The Principal Investigator, Shaun McCullough, Ph.D., will be responsible for ensuring data management quality. Statistical computations will be performed by Ana Rappold, Ph.D. The study investigators will be assisted by their data management staff in monitoring adherence to protocol, which will be overseen by Mike Ray, the QA Officer for the Environmental Public Health Division. The majority of data inputs are automated from instrumentation into databases, reducing the chances of human error in data input. A 5% random sample of data inputs, stratified by data type, will be compared against the database. Remarkable data findings will be subjected to increased scrutiny.

## A.9. Identifiers

1.Check which of the following identifiers you already have or will be receiving, or select "None of the above."

| | |
|---|---|
| ✔ | Names (this would include names/signatures on consent forms) |
| ✔ | Telephone numbers |
| ✔ | Any elements of dates (other than year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older |
| ✔ | Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes (e.g. GPS coordinates), except for the initial three digits of a zip code |
| ✘ | Fax numbers |
| ✔ | Electronic mail addresses |
| ✘ | Social Security numbers |
| ✘ | Medical record numbers |

- ✗ Health plan beneficiary numbers
- ✗ Account numbers
- ✗ Certificate/license numbers
- ✗ Vehicle identifiers and serial numbers (VIN), including license plate numbers
- ✗ Device identifiers and serial numbers (e.g., implanted medical device)
- ✗ Web universal resource locators (URLs)
- ✗ Internet protocol (IP) address numbers
- ✗ Biometric identifiers, including finger and voice prints
- ✗ Full face photographic images and any comparable images
- ✗ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher
- ✗ None of the above

2.For any identifiers checked, how will these identifiers be stored in relationship to the research data?

- ✗ with the research data (i.e., in the same data set and/or physical location)
- ✓ separate from the research data (i.e., coded with a linkage file stored in a different physical location)

Describe:
No personal identifying information will be labeled on the samples, only an alphanumeric code. Similarly, only the alphanumeric code assigned to the subject will be stored in relation to the acquired data. No participants will be identified in any report or publication about this study. Study samples will be stored in a secured room with restricted access. With signed consent from the participant, the samples will be stored indefinitely for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in a locked cabinet in a locked room within the EPA Human Studies Facility located on the UNC campus (104 Mason Farm Road, Chapel Hill, NC). All participant data and questionnaires are encoded and held in strict confidentiality in a locked filing cabinet in a secured building and /or on a secure computer.

3.Are you collecting Social Security Numbers to be used as a unique identifier for study tracking purposes for national registry or database? (Do not check yes if collecting SSN *only* for payment purposes; this will be addressed later.)

No

## A.10. Confidentiality of the data

1.Describe procedures for maintaining confidentiality of the data you will collect or will receive (e.g., coding, anonymous responses, use of pseudonyms, etc.).

No personal identifying information will be labeled on the samples. No study participants will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. With signed consent from the participant, the samples will be stored indefinitely for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded

samples will be sent. All medical records generated during this study will be kept in a locked cabinet in a locked room within the EPA Human Studies Facility located on the UNC campus (104 Mason Farm Road, Chapel Hill, NC). The EPA Human Studies Facility has limited access to authorized individuals only, 24 hours a day, seven days a week. All participant data and questionnaires are encoded and held in strict confidentiality in a locked filing cabinet in a secured building and /or on a secure computer.

2.Describe how data will be transmitted among research team (i.e., personnel listed on this application).

In general, information will be transmitted between the study investigators with paper, verbal, and encrypted electronic transmissions. The information will include pertinent medical history, physical examination, lab studies, and information relating to study endpoints (*e.g.* spirometry, and cardiac physiology data). Within the EPA building, there will be communication between the FEFA Recruitment Office and the study investigators with regards to participant qualification.

3.Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc?

No

4.Do you plan to obtain a federal Certificate of Confidentiality for this study?

No

5.If relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

The potential for deductive disclosure is not likely in this study since the variables collected could not reasonably be used in aggregate to identify a single individual in this population.

6.Will any of the groupings or subgroupings used in analysis be small enough to allow individuals to be identified?

No

## A.11. Data sharing and transmission

1.Check all of the following who will receive **identifiable data** (contains any of the 18 identifiers listed above) outside the immediate research team (i.e., not listed as personnel on this application)? *

- ✔ No one
- ✘ Coordinating Center
- ✘ Statisticians
- ✘ Consultants
- ✘ Other researchers
- ✘ Registries
- ✘ Sponsors
- ✘ External labs for additional testing
- ✘ Journals
- ✘ Publicly available dataset
- ✘ Other

2.For any recipients checked above, explain the confidentiality measures to be taken

No Answer Provided

## A.12. Post-study disposition of identifiable data or human biological materials

1.Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. If you plan to destroy linkage codes or identifiers, describe how and when this will be done.

The study data will be archived with identifiers by storage in a locked room in the secured USEPA HSF building. If offsite storage space is ever required for the data, the data will be transferred to offsite storage according to the USEPA's record keeping guidelines. All specimens remaining after the completion of the study will be stored with only an alphanumeric specimen number identifier in a secured freezer in accordance with the EPA Human Studies Facility protocol, titled "Repository for storage of human specimens" (IRB approved protocol #07-1768). Identifying information in records that could be used to link participants to specimen numbers will be protected by an "honest broker" system in which only EPA medical station personnel will be able to link specimen numbers to the associated personal identifying information. The honest broker system is described in Appendix O of the UNC-CH Human Research Protection Program's standard operating procedures.

Only the Principal Investigator, Co-investigator and laboratory technical staff identified on the protocol will have access to the repository. The Co-investigator will assume contingency responsibility for security of participant files, specimens, and future studies in the absence of the Principal Investigator. Specimens and data deriving from this study may be released to other investigators for more comprehensive studies with appropriate US EPA approval. In the event of their release to other investigators the specimens and/or data will carry only the alphanumeric code originally assigned and will be identifiable only to the Principal and Co-investigator. Specimens from subjects who opt not to allow for storage will be destroyed at the end of the study. Subjects at any time may request, in writing, that their samples no longer be stored in the repository. Any analysis in progress at the time of the request or already performed prior to the request being received by the researcher will continue to be used as part of the research study. Once the researcher has obtained written notification, the requesting subject's specimens will be destroyed.

# Part B. Direct Interaction

## B.1. Methods of recruiting

1.Check all the following means/methods of subject recruitment to be used:*

- ✔ In person
- ✘ Participant pools
- ✘ Presentation to classes or other groups
- ✔ Letters
- ✔ Flyers
- ✘ Radio, TV recruitment ads
- ✔ Newspaper recruitment ads
- ✔ Website recruitment ads
- ✔ Telephone script
- ✔ Email or listserv announcements

✔ Follow up to initial contact (e.g., email, script, letter)

✖ Other

2.Describe how subjects will be identified

Volunteers will be recruited for this study by the FEFA Corporation. The manner in which this will be done is similar to past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for EPA Studies (IRB #95-0518).

Potential volunteers will self identify in response to the advertising described in section B.1.1. The exception will be for those identified from a pool of previous volunteers who are selected based on study eligibility criteria. Any previous volunteers that meet the study criteria will be contacted via an IRB approved email or phone script. After they are provided information about the study, they will elect whether or not to respond.

3.Describe how and where subjects will be recruited and address the likelihood that you will have access to the projected number of subjects identified in A.2.

Volunteers will be asked to call the recruitment office. During the telephone interview, the volunteers will receive information regarding the study and their eligibility for the study will be assessed. Volunteers who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per IRB protocol #95-0518.

Based on past experiences with similar studies, we will be able to recruit the projected number of participants required for this study.

4.Describe how you will protect the privacy of potential subjects during recruitment

Emails will be sent from password-protected computers with email stored on secure servers. Email subject lines will state either “Study at the US EPA” or “Your appointment at the EPA Human Studies Facility”. All phone calls and phone screening interviews will be conducted from private offices in the EPA Human Studies Facility. On site screening will also be conducted in private offices so that no personal information is shared with other research volunteers. More than one volunteer may be present in the waiting room at one time, but personal information will not be discussed in that area.

5.Describe how subjects will be contacted, if not addressed above

Potential volunteers will contact FEFA by phone, or by email from the recruitment web site (www.epastudies.org). FEFA will respond either by phone or by email.

6.Describe who will do the recruiting

FEFA, Inc. will provide recruitment services to support this study. FEFA is a Contract Research Organization under contract with the US EPA to provide support services for human research at the Human Studies Facility in Chapel Hill, NC. FEFA staff members are CITI and COI trained and certified.

7.Describe efforts to ensure equal access to participation among women and minorities

Every effort will be made to include women and minorities in this research. Advertising will be placed in a variety of locations to allow widespread access to recruitment information.

## B.2. Protected Health Information (PHI)

*Protected Health Information (PHI) is any identifiable information about the subject's health that relates to their participation in this research and is obtained from sources other than the subject, such as medical records, health care providers, insurance plans, etc.* *more*

1.Are you requesting a limited waiver of HIPAA authorization?
If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a limited waiver of HIPAA authorization (see SOP 29.3). This does not apply to situations where you will never contact subjects directly (e.g., retrospective chart review), in which case you should request a full waiver under section D.

No

2.Will you need ongoing access to PHI (e.g., medical records) to conduct the study, beyond the identification of potential subjects as addressed above? In this case you will need to obtain a signed HIPAA Authorization from each subject.

No

## B.3. Subject Contact, Duration and Privacy

1.Number of contacts per subject

Approximately 13-15

2.Duration of each contact. If multiple contacts, provide the range or average time for each contact.

Individual visits will vary between two and seven hours.

3.Total duration of individual subject's participation, including follow up evaluation, if applicable

8-10 weeks.

4.List the locations where subjects will be studied, both on and off the UNC-CH campus.

Participants will be studied at the United States Environmental Protection Agency Human Studies Facility located at 104 Mason Farm Road, Chapel Hill, NC 27514.

5.Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope)

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard’s desk without an escort. Additionally, participants will need to initial the consent form indicating whether or not they would be willing to take part in the study with another participant present.

## B.4. Incentives for participation

1.Are there incentives (monetary or non-monetary) for subjects to participate?

Yes

A. Please describe

Study participants will receive monetary compensation for their time and some procedures in the study (See below). In addition, participants traveling from areas beyond Chapel Hill will be reimbursed for travel expenses commensurate with the US Government mileage rate ($0.555/mile as of April 2012) in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the participant requests otherwise.

A participant who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Participants who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be compensated for his/her participation through the day that they are dismissed in addition to 50% of the amount indicated in Section B.4.B for any remaining day(s) in that particular scheduled three-day exposure series.

In the event a scheduled study visit must be cancelled by the investigators with less than 72 hours prior notice, the participant will be paid 50% of the amount indicated in Section B.4.B for the remaining day(s) of the exposure series. If an exposure series has been started, but must be cancelled by the investigators prior to its completion the participant will be compensated at the full rate for the day in progress and for 50% of the amount indicated in Section B.4.B for the remaining day(s) in that particular exposure series. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the participant will be rescheduled.

Money received by participants in research studies is normally treated as ordinary income by taxing authorities and payments made to participants will be reported to the Internal Revenue Service as required by law. WESTAT will collect participants' social security numbers for income reporting purposes as indicated in IRB #95-0518. The money is not intended to coerce completion or participation, but to emphasize the importance of all the visits, and to signify the importance of participants' time and commitment to the research study.

B. Specify the schedule for incentives and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

**Training Day = $36**

- Approximate duration of three hours

**Each Exposure Series:**

Day 1: $235

- Approximate duration of seven hours
- Blood draws (2X)
- Holter monitoring
- Spirometry (2X)
- Pulse wave analysis (2X)
- Lunch

Day 2: $235

- Approximate duration of seven hours
- Blood draws (2X)
- Holter monitoring
- Spirometry (2X)
- Pulse wave analysis (2X)
- Lunch

Day 3: $50

- Approximate duration of two hours
- Blood draw (1X)
- Holter monitoring
- Spirometry (1X)
- Pulse wave analysis (1X)

**Total payment First Exposure Series** = $520.00

**Second Exposure Series** = $520.00

**Third Exposure Series** = $520.00

**Fourth Exposure** Series = $520.00

Completion Bonus (for completing all four exposure series) = $100

**APPROXIMATE TOTAL (including training day and all four exposures)** = $2,216.00

C. For compensation in foreign currency, provide a US dollar equivalent.

Not applicable

D. Discuss the potential for coercion, given factors like the amount of the incentive, the age of the subjects, the purchasing power in foreign countries, the time involved and complexity of procedures, etc.

Based on hourly rates of compensation that were approved in other studies with similar protocols and/or levels of risk, the above-detailed pay schedule was felt to be commensurate with other existing studies' pay structure.

E. If the subjects are children who will receive the compensation, i.e., the child, the parents or both?

Not applicable

2.Are you collecting Social Security numbers for payment and/or tax-related purposes?

No

## B.5. Costs to be borne by subjects

1.Will there be any costs that subjects will incur related to participation in the study? Do not include costs for standard care for which patients would be billed if they were not in this study. Also do not include the time spent participating in the study.

No

# Part C. Existing Data, Records, Specimens

## C.1. Data Sources

1.What existing records, data or human biological specimens will you be using? (Indicate all that apply or select 'None of the above'): *

✖ Data already collected from another research study

Were the investigators for the current application involved in the original collection? --

✖ Patient specimens (tissues, blood, serum, surgical discards, etc.)

Has the clinical purpose for which they were collected been met before removal of any excess? --

✖ Data already collected for administrative purposes

✖ Student records (You will need to satisfy FERPA requirements: see SOP 24.6.2 for guidance)

✖ UNC Health Care System Medical records in any format.

If you access the records of fewer than 50 patients under a full or limited waiver of HIPAA, submit a copy of your IRB approval letter and a completed Research Disclosure Form to Health Information Management (HIM). Do not submit this information to the IRB. For additional information about this process, you should contact HIM directly at 595-5691 or 966-1255.

✖ UNC Dental Records

✖ Data coming directly from a health plan, health care clearinghouse, or health care provider?

✖ Publicly available data

✖ Other

✖ None of the above

For EACH data source checked above, provide a description of the data, proposed use, how data were collected (including consent procedures), and where data currently reside.
--

2.Describe your plans for obtaining permission from the custodians of the data, records or specimens (e.g., pathology dept, tissue bank, original researcher):

Not applicable

3.Do the custodians of the data, records or specimens require a data use agreement?

No

## C.2. Coding and Data Use Agreements

1.When you receive these data, records or human biological specimens will they be coded? Coded means identifying information that would enable the research team to readily ascertain the individual's identity has been replaced with a number, letter, symbol, or combination thereof (i.e., a code). If you will not be using existing materials, check "No."

No

# Part D. The Consent Process

## D.1. Obtaining informed consent from subjects

*The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances. If you will be requesting a waiver answer "not applicable" for any of the following questions that will not pertain to this study. You will be asked to provide relevant information in the section below on waivers.*

1.Will children under the age of majority in their locale (18 years in NC) be enrolled?

No

2.Will adult subjects be enrolled in your study?

Yes

Explain the process for obtaining consent from the subject or the subject's legally authorized representative, if relevant

The participants will be required to read and sign a consent form asserting that they have read and understood the following:

1. Participation is strictly voluntary
2. The purpose of the study
3. The nature and extent of participation
4. Rights to withdraw from the study at any time
5. Right to privacy and that if they agree, there may be another participant present during the exposure series
6. The risks associated with participation
7. The method and schedule of compensation
8. The limits of liability with respect to the EPA and the PI.

The PI or Co-Investigator will then review the contents of the consent form and go through a checklist highlighting important points from the consent form before the volunteer signs it. Subjects will have the opportunity to ask questions at any time during the study by contacting the PI or Co-Investigator.

3.Will decisionally-impaired subjects be enrolled in your study? (includes unconscious patients, some psychiatric disorders, others who lack the capacity to give consent)

No

4.Are you planning to obtain consent from any Non-English speaking subjects?

No

5.Describe who (by role) will be obtaining consent or parental permission.

One of the individuals listed on the front page of the application in the role of study Principal Investigator, Co-investigator, or Study Coordinator will be responsible for obtaining informed consent from the study volunteers.

6.Discuss the potential for influencing the subject's decision to participate. Describe steps that will be taken to minimize undue influence during the consent process. These might include a waiting period between the initial consent discussion and obtaining consent, or obtaining consent by someone other than a person with perceived authority (e.g., professor, employer, treating physician).

Informed consent will not be obtained during the initial phone/letter contact. This will occur at the training visit prior to enrollment into the study. Informed consent will be obtained by the Principal Investigator, a study Co-investigator, or Study Coordinator.

7.Has the sponsor of this study provided a model consent form?

No

## D.2. Waiver of written documentation of informed consent

*The default is for subjects to sign a written document that contains all the elements of informed consent. Under limited circumstances, the requirement for a signed consent form may be waived by the IRB. For example, this might occur for phone or internet surveys, when a signed consent form is either impractical or unnecessary, or in circumstances where a signed consent form creates a risk for the subject.*

1.Are you requesting a waiver of any aspect of written (signed) documentation?

No

## D.3. Full or partial waiver of consent

*The default is for subjects to give informed consent. A waiver might be requested for research involving only existing data or human biological specimens. More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.*

1.Are you requesting any of the following:

- ✖ a waiver of informed consent in its entirety
- ✖ a waiver or alteration of some of the elements of informed consent
- ✖ a waiver of HIPAA authorization (If you are accessing patient records for this research, you must also request a waiver of HIPAA authorization)

2.If your request for a waiver applies to some but not all of your subject groups and/or consent forms, please describe and justify

No Answer Provided

3.Does this request for waiver support a study design that involves deception or withholding of information?

No

## Consent Forms

### This submission requires the following consent forms

| Template Type |
|---|
| Adult Consent Form |
| Stored Specimens with Identifiers |

### This submission includes the following consent forms

| File Name | Document Type |
|---|---|
| ENDZONE - Adult Consent Form - 101514.docx | Adult Consent Form |
| ENDZONE - Consent Form Checklist - 063014.docx | Other Consent Materials |
| ENDZONE - Re-enrollment Consent Form - 063014.docx | Other Consent Materials |
| ENDZONE - Consent For Stored Specimens with Identifiers - 063014.docx | Stored Specimens with Identifiers |

view consent forms

## Attachments

### This submission requires the following attachments

| Document Type |
|---|
| Letter for Recruitment |
| Flyer for Recruitment |
| Newspaper Ad for Recruitment |
| Website for Recruitment |
| Telephone Script for Recruitment |
| Email or Listserv Recruitment |
| Recruitment Follow Up |

### This submission includes the following attachments

| File Name | Document Type |
|---|---|
| ENDZONE - Email - 063014.docx | Email or Listserv Recruitment |
| ENDZONE - AdFullPage - 012714.docx | Flyer for Recruitment |
| ENDZONE - Recruitment Letter - 012614.docx | Letter for Recruitment |
| ENDZONE_DisplayAd_09052013.docx | Newspaper Ad for Recruitment |
| ENDZONE - Appointment Confirmation - STUDY VISITS - 063014.docx | Other Materials for Recruitment |
| ENDZONE - CraigsList Ad - 012714.docx | Other Materials for Recruitment |
| ENDZONE - Appointment Confirmation - TRAINING VISITS - 063014.docx | Recruitment Follow Up |
| ENDZONE - Automated Calls - 012614.docx | Telephone Script for Recruitment |
| ENDZONE - Phone Questionnaire - 012414.docx | Telephone Script for Recruitment |
| ENDZONE - Recruitment Script - 063014.docx | Telephone Script for Recruitment |
| ENDZONE_Web_Site_09052013.docx | Website for Recruitment |
| 030614 - TRC Memo Regarding NO2 Exposure.pdf | Other |
| 033114 - TRC Letter Certifying Completion of Training Plan.pdf | Other |
| CITI_training_TraceyMontilla_9-26-13.pdf | Other |
| Devlin - Inflammatory response in humans exposed to 2.0ppm nitrogen dioxide.pdf | Other |
| ENDZONE - Payment Voucher - 012414.docx | Other |
| SSN Exemption_Dan Nelson_11-8-07.pdf | Other |

view attachments

## Addenda

Data Security Requirements

view addenda

**By certifying below, the Principal Investigator affirms the following:**

I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

This study proposes research that has been determined to include Security Level 2 data security requirements. I agree to accept responsibility for managing these risks appropriately in consultation with departmental and/or campus security personnel. The Data Security Requirements addendum can be reviewed here.

**If PI is a Student or Trainee Investigator, the Faculty Advisor also certifies the following:**

I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

## Certifying Signatures:

**Signature:** Electronic Signature Received **Date:** 10/15/2014 07:07:55 PM
Shaun McCullough

**Signature:** Electronic Signature Received **Date:** 10/16/2014 08:31:54 AM
Robert Devlin

**University of North Carolina at Chapel Hill**
**Consent to Participate in a Research Study**
**Adult Participants**

**Consent Form Version Date:** 15 October 2014
**IRB Study #** 13-0459
**Title of Study**: Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.
**Principal Investigator**: Shaun McCullough, Ph.D.
**Principal Investigator Department**: Environmental Protection Agency (EPA)
**Principal Investigator Phone number**: 919-843-8031
**Principal Investigator Email Address**: mccullough.shaun@epa.gov
**Co-Investigators:** Robert Devlin, Ph.D.; David Diaz-Sanchez, Ph.D.; Michael Schmitt, MSPH; Ana Rappold, Ph.D.; Andrew Ghio, M.D.; Martin Case, BS; Maryann Bassett, R.N.; Tracey Montilla, R.N.; Julie Wood, R.N.; Jaime Mirowsky, Ph.D.; Juliette Kahle, Ph.D.; Wayne Cascio, M.D., Emma Bowers, M.S., Carissa Best, B.S., David Morgan, B.S., Katelyn Lavrich, B.S.

**Faculty Advisor**: Robert Devlin, Ph.D.
**Faculty Advisor Contact Information**: devlin.robert@epa.gov

**Funding Source and/or Sponsor:** United States Environmental Protection Agency (EPA)

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**What are some general things you should know about research studies?**
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

**What is the purpose of this study?**
The purpose of this research study is to understand whether sequential exposure to the common air pollutants nitrogen dioxide, a product of combustion such as vehicle exhaust, and ozone results in greater health effects than exposure to either pollutant alone.

To accomplish this we will:

- Expose you to nitrogen dioxide (at a concentration of 500 parts per billion [ppb]), ozone (at a concentration of 300ppb), and clean air under several exposure scenarios. During these exposures you will undergo moderate exercise on a stationary bicycle or treadmill in 15-minute intervals for a total of one hour of exercise over a two-hour exposure period. Ozone at or above 300ppb has been used in human exposure studies for many years. Nitrogen dioxide levels at 500ppb are below levels that have been previously shown in other studies near an operating gas stove.
- Look at changes in your lung function (you will inhale and exhale through a machine, called a spirometer, which measures your breathing) after each exposure.
- Look at changes in components of your blood involved in inflammation and clotting.
- Look at whether air pollutant exposure changes the ability of your nervous system to correctly regulate your heart rate and rhythm.
- See if you have genes, which have been reported in scientific literature that makes individuals more likely to have a response to air pollutant exposure. We will compare the presence or absence of these genes with your response to air pollutants in this study.
- With additional permission from you, we will store your blood samples before and after pollutant exposures (as described in the accompanying consent form "*Consent for Storing Biological Specimens with Identifying Information*") for potential future examination if new markers of exposure are identified.

You are being asked to be in the study because you are a healthy adult between 18 and 45 years of age.

**Are there any reasons you should not be in this study?**

You should not be in this study if:

- You have a history of acute and/or chronic cardiovascular disease, chronic respiratory disease, diabetes, rheumatologic diseases, or immunodeficiency state. You are an asthmatic or have a history of asthma. You are allergic to chemical vapors or gases.
- You are pregnant, attempting to become pregnant, or are breastfeeding. You are unwilling or unable to stop taking vitamin C or E (commonly found in over-the-counter multivitamins), or medications that may impact the results of ozone exposure at least two weeks prior to the study and for the duration of the study.
- You currently smoke or have smoked tobacco in the last five years or have a lifetime history of >5 pack-years.
- You are currently living with a smoker that smokes inside the house.
- You have a body mass index (BMI) >35 or <18. Body mass index is calculated by dividing your weight in kilograms by the square of your height in meters.
- You have exposures to high levels of vapors, dust, gases or fumes on an on-going basis where you work.
- You have uncontrolled hypertension (≥150 systolic, ≥90 diastolic).
- You do not speak or understand English.
- You are unable to perform the exercise required for the study.

- You have unspecified diseases, conditions, or medications (to be determined by the study physician) that might influence your ability to safely complete the activities associated with this screening visit.
- You are on systemic steroids or beta-blocker medications.
- You have a history of skin allergies to adhesives used in securing heart rate monitor (ECG/EKG) electrodes.
- You have a hemoglobin A1c (HbA1c) above 6.4%.

You should not participate in this study if you are unwilling or unable to comply with the following requirements:

- Avoid smoke and chemical/exhaust fumes for 24 hours prior to all study visits.
- Avoid drinking alcohol for 24 hours prior to all study visits.
- Avoid strenuous exercise for 24 hours prior to all study visits.
- Avoid the use of ozone-based home air purifiers for 24 hours prior to all study visits.
- Minimize exposure to unvented household combustion sources (gas cooking appliances, lit gas/propane/wood fireplaces, oil/kerosene heaters) for 48 hours prior to all study visits.

**How many people will take part in this study?**

If you decide to participate, you will be one of approximately 60 participants in this research study.

**How long will your part in this study last?**

If you are eligible and decide to take part in this study your participation will involve approximately 13 and 15 visits to the research facility over the course of approximately 8-10 weeks. The duration of each individual visit will vary between 2-7 hours. There will be no follow-up after your completion of study-related activities. With your permission, blood samples taken from you during the course of the study may be stored as described in a separate consent form ("*Consent for Storing Biological Specimens with Identifying Information*"). You will complete the training day once before beginning any of the study-related exposures. After that you will complete four different exposure series, each of which will have an Exposure Day #1, Exposure Day #2, and a Follow-up Day. The approximate length of time that you will be at the EPA Human Studies Facility for each of these days is shown in the table below.

| | **Training Day** | **Exposure Day #1** | **Exposure Day #2** | **Follow-up Day** |
|---|---|---|---|---|
| **Approximate Amount of Time** | 3 hours | 7 hours | 7 hours | 2 hours |

**What will happen if you take part in the study?**

**General Study Design**

This study contains four "arms," which means that there are four different sets of exposure regimens, each with two days of pollutant or clean air exposure and one follow-up day. If you complete the entire study you will be exposed to all four regimens. The order in which you are exposed to the different regimens will be random and the order of exposure will vary between different study participants. This study is "single blind," which means that you will not be told the order in which you will be exposed to the four regimens; however, the investigators will be aware of the order of your exposure regimens. To help complete this study, there may be the possibility that two subjects will be performing the study at the same time in the same exposure room. We will only do this with your consent and will ask you to initial a special section in this consent agreeing to this study request.

Before you agree to participate in this study, you must read the consent form completely. The research and medical staff will then answer all of your questions and explain all of the risks involved in this study to your satisfaction. You should have already undergone a general screening visit and physical examination to certify that you are a suitable candidate. The screening visit and physical exam has a separate informed consent form.

You will perform the following procedures during the course of this research study:

**Training Day**

- You will report to the EPA medical station at the requested time.
- We will review the inclusion and exclusion criteria and any medical conditions that you have or medications that you are currently taking. We will go over the study in detail so that you will know what we expect from you as a participant and what you should expect from us as investigators. If you agree to participate in the study you will sign two copies of this consent form. We will give one copy to you. We will check your vital signs (heart rate, blood pressure, oxygen saturation level) and do a pregnancy test if applicable. For the pregnancy test, you will be asked to provide a urine sample. The purpose of this test is to provide evidence that you are not pregnant at the time of the study. The nurse will also ask you when your most recent menstrual cycle began. The results of the pregnancy test will be held in strict confidence. A positive pregnancy test will result in your removal from the study since we do not know how air pollutant exposure affects the developing fetus.
- You will undergo a breathing test, which is known as spirometry. You will inhale and exhale through a filter into a machine that will measure your breathing, such as how much air you can inhale and how much you can exhale over a certain amount of time. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times. You will be trained on the exercise bicycle or treadmill (you will be able to choose which one you would prefer to use for study-related exercise) and we will determine the work load required to obtain a breathing rate, normalized to your body surface area, of approximately 20 L/min/$m^2$ BSA (inspiration).
- We will determine whether you will be able to perform the physical activity required during the study. We will determine your ability to complete the required physical activity

in two ways. First, we will determine if you are able to exercise without exceeding 80% of your predicted maximal heart rate, which will be determined relative to your age. Second, using a non-invasive method known as pulse oximetry we will determine whether you are able to exercise at the required level while maintaining a blood oxygen saturation level ≥92% (oxygen saturation is a measure of how much oxygen the blood is carrying as a percentage of the maximum that it could carry). We will monitor your heartbeat with a safety system we have in our building called telemetry.

**Exposure (Days #1 and #2):** You will be asked to eat a normal breakfast and arrive at the EPA medical station at 8:00 AM on Day #1 and 8:00 AM on Day #2. You will need to wear comfortable clothes and shoes that are suitable for exercise and bring a lunch. Due to the timing of the study procedures you may be asked to have a "late lunch" in the early afternoon.

**Prior to exposure, you will:**

- Have your vital signs checked (heart rate, blood pressure, oxygen saturation level) and you will have a pregnancy test if applicable. If your medication history is not within previously established limits, respiratory tract illness has occurred within the previous four weeks, or if respiratory symptoms are present then testing will be postponed.
- Have a small portable heart monitor ("Holter monitor") placed on you so that we can collect measurements of your heart rate and rhythm. We will take 30-minute resting heart rate and rhythm measurements before and after the exposure. These measurements will consist of a 20-minute rest period and a 10-minute data collection. These monitors will allow us to determine if air pollutants cause small changes in the ability of your nervous system to regulate how your heart beats. This monitor will be removed at the end of the day.
- Have up to 25 mL blood drawn (less than two tablespoons). We will test this blood to see if ozone or nitrogen dioxide exposure affects the ability of your blood to clot correctly, and to look at changes in proteins and/or blood cells.
- Have a breathing test (spirometry). You will breathe through a filter into the machine as described above in the "Training Day" section. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have the stiffness of your arteries tested using a technique called pulse wave analysis. During this procedure you will sit in a reclined position and have a blood pressure cuff placed on one arm and a pulse wave tonometer (a sensor that takes measurements from your radial artery) placed on your other forearm. The procedure will take approximately 10 minutes and includes two blood pressure readings and approximately 30 seconds of data collection from the tonometer.

**During the exposure, you will:**

- Be exposed to either clean air, ozone (at a concentration of 300ppb), or nitrogen dioxide (at a concentration of 500ppb) for two hours. You will be "blinded" to the exposure condition, which means that you we will not tell you whether you are being exposed to clean air, ozone, or nitrogen dioxide. Your exposure sessions will take place in a specially designed

stainless steel exposure chamber that measures approximately 4.8 x 5.8 x 3.2 meters. The chamber has a window and a door that can be opened at any time. Chamber conditions will be at a comfortable temperature and relative humidity. A trained member of the study team will be seated outside the chamber observing you at all times. Additionally, you will be able to speak to the member of the study team that is observing you at any time. During the exposure, your heart will be monitored and the amount of oxygen present in your blood will be monitored by placing a device (pulse oximeter) on your finger. During the course of the two-hour exposure, you will exercise at 15-minute intervals at the pace determined during your "Training Day" visit. Between exercise intervals you will rest for 15 minutes. While exercising, your breathing rate and volume may be measured intermittently. We may adjust your exercise level according to your breathing rate during the exposure. If it appears you are experiencing significant discomfort or breathing/heart problems, the exposure will be terminated immediately. **In addition, you may elect to terminate the exposure at any time for any reason.** If you do so, you will be paid for your participation up to that point, but will be ineligible for further participation in the study and any payments you would have received for future participation.

**Following the exposure, you will:**

- Have a breathing test (spirometry). You will breathe through a filter into the machine as described in the "Training Day" section. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have a reading of your heart rhythm taken with the Holter monitor. After these readings are taken the Holter monitor will be removed.
- Have up to 25ml of blood drawn (less than two tablespoons).
- Have the stiffness of your arteries tested using pulse wave analysis.
- Be assessed and discharged by the nursing staff.

**Follow-up (Day #3):** You will be asked to eat a normal breakfast and arrive at the EPA medical station at 8:00 AM. You will need to wear comfortable clothes.

**During the follow-up, you will:**

- Have your vital signs checked.
- Have a breathing test (spirometry). You will breathe through a filter into the machine as described in the "Training Day" section. We will coach you, and you will be asked to take a full breath in and then blow it out as hard and fast as you can. We will ask you to do this several times.
- Have a reading of your heart rhythm taken with the Holter monitor, and then have the Holter monitor removed.
- Have up to 25ml of blood drawn (less than two tablespoons).
- Have the stiffness of your arteries tested by pulse wave analysis.
- Be assessed and discharged by the nursing staff.

**Storage of Samples Following Completion of This Study**

If there are blood samples that we acquire from you remaining after completion of this study, we would like to store them for future studies. Having this option allows up to make the best use of the samples that we collect from you. You will be given a separate opportunity to provide or decline consent for us to store your samples after completion of this study.

**What are the possible benefits from being in this study?**

You will not benefit personally from being in this research study; however, this research is designed to benefit society in general by gaining new knowledge. Every American is exposed to these pollutants, thus this study has the potential to contribute to strategies that are aimed at protecting millions of people from the effects of these air pollutants.

**What are the possible risks or discomforts involved from being in this study?**

There may be uncommon or previously unknown risks. You should report any problems to the researcher and the medical staff.

This study might involve the following risks and/or discomforts to you:

1.) Moderate exercise on a stationary bicycle or treadmill has the potential, although minimal, risk of occasional muscle soreness, cramps or general fatigue. These discomforts are temporary and not harmful. Additionally, moderate exercise, which will be required as a part of the research study, has the potential to induce abnormal heart function. Irregular heartbeats can happen during regular daily activities and most irregular heartbeats are harmless; however, they have the potential to be life threatening under certain circumstances. Heart rate and rhythm will be monitored continuously while you are in our facility. Exercise levels will be adjusted (if necessary) to keep your heart rate below 80% of the age related maximum. Exercise will be terminated at any time at your request or if the investigator/medical staff observes signs of distress. During exercise, you will be monitored by direct observation and via closed-circuit television. You will also be wearing telemetry and a pulse oximeter for your safety.

2.) Blood sampling will be performed by well-trained personnel, and involves the risk of mild discomfort with the infrequent possibility of lightheadedness (feeling dizzy), fainting, infection, or developing a bruise.

3.) Breathing tests (spirometry) may cause you to cough or become dizzy during the test. If these symptoms occur, they are usually only temporary. Also, the forceful breathing during this test can cause wheezing or shortness of breath to occur.

4.) There are no risks associated with monitoring your heart by ECG or blood oxygen by pulse oximetry; however, preparing your skin for placement of ECG electrodes and removing the electrodes may cause some decolorization, irritation, itching, or burning in some people. If this occurs you should notify the nursing staff. Additionally, men may need to have small areas of their chest shaved to allow for proper electrode placement.

5.) There are no known risks associated with pulse wave analysis. There may be mild pressure

caused by the tonometer pressing down on the radial artery, but blood flow will not be stopped. There may be a red, minor indentation in the skin over the radial artery, but this usually fades within a few minutes to hours.

6.) During air pollution exposure, you may experience some minor degree of airway irritation, cough, and shortness of breath or wheezing. If these symptoms occur they typically disappear two to four hours after exposure, but may last longer for particularly sensitive people. Ozone exposure also induces an inflammatory reaction that may last for 24 hours after the exposure and may increase the chance of you catching a cold. In addition, there may be uncommon or previously unknown risks that might occur. Very recently some studies have suggested that elderly people, particularly those with underlying cardiovascular disease, are at increased risk for getting sick and even dying during episodes of high ozone pollution. While we cannot exclude the possibility that you may have an adverse reaction to breathing these pollutants, other human exposure studies have utilized 300ppb ozone (the level that you will be exposed to in this study), or more, for many years. The total amount of ozone that you will be exposed to during the two-hour period is equivalent to what you would be exposed to in a city at the current eight-hour national standard. Additionally, the levels of nitrogen dioxide (you will be exposed to 500ppb of nitrogen dioxide in this study) that you will be exposed to are lower than those that have been observed around an operating gas stove. Sequential exposure, which will be conducted during this study, may increase the effects of air pollutant exposure. Additionally, very small changes in heart rhythm have been observed in previous studies where people were exposed to 300ppb ozone and 500ppb nitrogen dioxide individually. Sequential exposure to these two air pollutants may result in minor changes in your heart rhythm; however, the potential risk to your health is small. One of the goals of this study is to evaluate the differences between the response of individuals to single and sequential air pollutant exposure. Your symptoms will be closely monitored during the exposure and the study terminated if more than modest respiratory symptoms occur during the exposure. Further, you should report any problems to the researchers. In the unlikely event that you develop medically significant symptoms, the exposure will be terminated and the appropriate medical intervention will be provided if required. A physician is always available to respond to an emergency and full resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. If you experience a significant drop in your lung function, the on-call physician will evaluate you before you are discharged. You should not engage in heavy levels of exercise for 24 hours before and after the exposure period.

7.) If you have any tendency to become uncomfortable in small closed spaces, it is possible that you may become uncomfortable during the chamber exposure. You will be taken to the exposure chamber during the "training day" to allow you an opportunity to see where you will exercise and what the chamber looks like. The chamber is similar in size to a full-size living room and has a private bathroom inside. You will be in constant visual contact with the investigator that is monitoring you during the exposure. Additionally, you will be able to verbally communicate with the investigator at all times.

**The following are policies and procedures to minimize risk during the study:**

1.) During this study, skilled personnel in an adequately-equipped facility will be observing you at all times. You may, without penalty, stop the test at any time by telling the monitor.

2.) A physician will be on call at all times during the study to deal with any medical problems

that may arise.

3.) Every test has some degree of risk and there may be some risks associated with this experiment which are unforeseeable. To minimize these risks, you should not participate in the study if you have an acute illness.

4.) Since irregular heartbeats have the potential to pose a health risk we will be screening all potential study participants as a part of their physical exam in an attempt to prevent individuals that may be at a higher risk of dangerous abnormal heart function from being enrolled in the study. You should be aware that approval for enrollment in this study does not mean that you are not at risk for abnormal heart function that has the possibility of resulting in injury or death.

5.) You may terminate your participation in the experiment at any time and for any reason that you wish without penalty. You will be paid an amount for participation prorated for the time and procedures in which you took part. You should understand that you are not under any obligation to complete any test procedures that you find objectionable or uncomfortable.

6.) The investigator may terminate your participation in the experiment at any time. If this happens, you will be entitled to payment for your time commitment and any experimental activities up to that point.

7.) Your participation in the study may be postponed if your pre-exposure lung function shows airflow obstruction, if you develop a cold, or if you exhibit symptoms of respiratory disease or distress.

**What if we learn about new findings or information during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**Will my genetic information be used in this study?**
With your permission, DNA from a collected blood sample will be genotyped for specific genes related to adverse health effects associated with air pollution exposure. Unwillingness to have samples genotyped will NOT exclude you from participating in the study. If you do not wish for your cells to be used for genotyping but do wish to participate in the study sign the section under ***Subject's Agreement to Participate in the Research Study Without Genotyping Consent*** located at the end of this document.

A Federal law called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. GINA does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect you against discrimination based on an already-diagnosed genetic condition or disease.

**How will information about you be protected?**
You will be given a study code number. All electronic documents will only have that number. The paper records that the coordinators and medical doctors use may have your name. Your information can be linked to your personal information by the study number; however, only

study personnel have access to your personal information. Paper records that use your name are kept in a locked file cabinet in the EPA Medical Station of the Human Studies Facility. The Medical Station is locked when not attended by study staff, and the EPA Human Studies Facility has limited access to authorized individuals only, 24 hours/day for 7 days/week. Samples used for genetic analysis will be securely stored at the EPA Human Studies Facility.

Participants will not be identified in any report or publication about this study.

There also exists the possibility that two subjects will be performing the study at the same time in the same exposure room. If such conditions were to occur, you would only be paired with someone of the same gender. We will respect your privacy by not identifying you by your last name while in the presence of the other volunteer. If you are comfortable with performing the study with another individual, initial the box to the right of this paragraph. If you are not comfortable with performing the study with another volunteer, do not initial the box and you will not be paired up with another subject.

| Initial/Date |
| --- |
| |

**What will happen if you are injured by this research?**

All forms of medical research, diagnosis, and treatment involve some risk of injury or illness. Despite our high level of precaution, you may develop an injury or illness due to participating in this study. If you develop an injury or illness determined by the on duty physician to be due to your participation in this research, the EPA will reimburse your medical expenses to treat the injury or illness up to $5000. If you believe your injury or illness was due to a lack of reasonable care or other negligent action, you have the right to pursue legal remedy. The Federal Tort Claims Act, 28 U.S.C. 2671 *et. seq.*, provides for money damages against the United Sates when personal injury or property loss results from the negligent or wrongful act or omission of any employee of the EPA while acting within the scope of his or her employment. Signing this consent form does not waive any of your legal rights or release the investigator, the sponsor, the institution, or its agents from liability for negligence. If a research related injury or illness occurs, you should contact the Director of the E.P.A. NHEERL Human Research Protocol Office at 919-966-6217.

**What if you want to stop before your part in the study is complete?**

You may withdraw from this study at any time. If you choose to withdraw, you will receive compensation for your participation up to that point.

The investigators have the right to stop your participation at any time. This could be because you have had an unexpected reaction, failed to follow instructions or because the study has been stopped. After you enroll, if you are dismissed by the investigator for failure to follow instructions you will receive compensation for your participation up to that point.

If you are dismissed by the investigators after enrollment in the study but prior to completion for reasons that are not your fault (involuntary reasons) you will be compensated for your participation through the day that you are dismissed in addition to 50% of the amount indicated for any remaining day(s) in that particular scheduled three-day exposure series.

We may need to cancel your study activity due to adverse weather conditions, equipment failure, or other unforeseen events. In the event the investigators must cancel a scheduled study visit with less than 72 hours prior notice, you will be paid 50% of the amount listed below for the remaining day(s) in the exposure series. If an exposure series has been started, but is cancelled prior to its completion, you will be compensated at the full rate for the day in progress and for 50% of the amount listed below for the remaining day(s) in that particular exposure series. If a cancellation occurs as a result of inclement weather, equipment malfunction, or similar problem then we will do our best to reschedule your exposure series.

**Will you receive anything for being in this study?**

Total compensation for completion of the entire study will be approximately $2,216. In addition, you will be reimbursed for reasonable travel expenses and for parking costs while at the research facility. Money received by participants in research studies is normally treated as ordinary income by taxing authorities and we will report payments made to you to the Internal Revenue Service (IRS) as required by law. This summary is to emphasize the importance of all the visits, and to signify the importance of your time and commitment to the research study. Additionally, you will have already been compensated for your screening and physical exam prior to being enrolled in this study.

Compensation for study visits will be as follows:

**Training Day**

- $36

**Exposure Series #1**

- Day #1: $235
- Day #2: $235
- Day #3: $50
- Total: $520

**Exposure Series #2**

- Day #1: $235
- Day #2: $235
- Day #3: $50
- Total: $520

**Exposure Series #3**

- Day #1: $235
- Day #2: $235
- Day #3: $50
- Total: $520

**Exposure Series #4**

- Day #1: $235
- Day #2: $235
- Day #3: $50
- Total: $520

**Study Completion Bonus**

- $100

**Total Compensation for Completion of the Entire Study:** $2,216

You should understand that your participation is voluntary. You may terminate your participation in the study at any time without penalty. If you voluntarily elect to withdraw from the study at any time or you fail to maintain compliance with eligibility requirements or follow directions of the investigators, you will be paid for that portion of the study that has been completed.

The investigators also have the right to stop your participation in the study at any time. In the event the investigators must cancel a scheduled study visit with less than 72 hours prior notice, you will be paid 50% of the amount listed above for the remaining day(s) in the exposure series. If an exposure series has been started, but is cancelled prior to its completion, you will be compensated at the full rate for the day in progress and for 50% of the amount listed above for the remaining day(s) in that particular exposure series.

Cancellations could occur due to adverse weather conditions, equipment failure, and other unforeseen events. When feasible, canceled visits will be rescheduled.

**Will it cost you anything to be in this study?**

It will not cost you anything to be in this study; however, if you are deemed not eligible to participate in the study for medical reasons, we may suggest that you seek follow-up care from your own health care provider for abnormalities discovered during the screening history, physical examination, or the study. **Such care is entirely at your own expense. The US EPA will not provide reimbursement for any follow-up care.**

We will give you parking coupons to cover the cost of parking. If you live beyond Chapel Hill/Carrboro you will be reimbursed for mileage at the U.S. Government mileage rate in effect at the time.

**Who is sponsoring this study?**

This research is funded by the United States Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study; however, the researchers do not hold a direct financial interest in the sponsor or in the product being studied.

**What if you have questions about this study?**
You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

**What if you have questions about your rights as a research participant?**
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu. Additionally, you may contact the Director of the EPA NHEERL Human Research Protocol Office at 919-966-6217.

**IRB Study # 13-0459**

**Title of Study:** Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.

**Principal Investigator:** Shaun McCullough, Ph.D.

**Participant's Agreement**:

I have read the information provided above in the Adult Consent form for IRB study #13-0459. I have asked all the questions I have at this time. I voluntarily **AGREE** to participate in this research study and I voluntarily **AGREE** to be genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

_____ _____

Signature of Research Participant Date

_____

Printed Name of Research Participant

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Member Obtaining Consent

_____ _____

Signature of Second Research Team Member Present for Consent Date

_____

Signature of Second Research Team Member Present for Consent

**IRB Study # 13-0459**

**Title of Study:** Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.

**Principal Investigator:** Shaun McCullough, Ph.D.

**Participant's Agreement**:

I have read the information provided above in the Adult Consent form for IRB study #13-0459. I have asked all the questions I have at this time. I voluntarily **AGREE** to participate in this research study and I voluntarily **REFUSE** to be genotyped for any genes decided by the study investigators to be related to adverse health effects associated with pollution exposure.

_____ _____

Signature of Research Participant Date

_____

Printed Name of Research Participant

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Member Obtaining Consent

_____ _____

Signature of Second Research Team Member Present for Consent Date

_____

Printed Name of Second Research Team Member Present for Consent

**University of North Carolina at Chapel Hill**
**Consent Checklist**

**Checklist Version Date:** 30 June 2014
**IRB Study #** 13-0459
**Title of Study**: Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.
**Principal Investigator**: Shaun McCullough, Ph.D.
**Principal Investigator Department**: Environmental Protection Agency (EPA)
**Principal Investigator Phone number**: 919-843-8031
**Principal Investigator Email Address**: mccullough.shaun@epa.gov
**Co-Investigators:** Robert Devlin, Ph.D.; David Diaz-Sanchez, Ph.D.; Michael Schmitt, MSPH; Ana Rappold, Ph.D.; Andrew Ghio, M.D.; Martin Case, BS; Maryann Bassett, R.N.; Tracey Montilla, R.N.; Julie Wood, R.N.; Jaime Mirowsky, Ph.D.; Juliette Kahle, Ph.D.; Wayne Cascio, M.D., Emma Bowers, M.S., Carissa Best, B.S., David Morgan, B.S., Katelyn Lavrich, B.S.

**Faculty Advisor**: Robert Devlin, Ph.D.
**Faculty Advisor Contact Information**: devlin.robert@epa.gov

**Funding Source and/or Sponsor:** United States Environmental Protection Agency (EPA)

_____ I have read the consent form titled, "*Consent to Participate in a Research Study*" for the study titled, "*Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.*"

_____ A member of the study team has reviewed the consent form titled, "*Consent to Participate in a Research Study*" for the study titled, "*Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers*" with me.

_____ I was given the opportunity to ask a member of the study team questions about the study and my involvement in the study.

_____ I have been made aware that the purpose of this research study is to determine if sequential exposure to the air pollutants nitrogen dioxide and ozone results in greater physiological changes than exposure to either pollutant alone.

_____ I understand that I will undergo controlled exposure to the air pollutants nitrogen dioxide (at a concentration of 500ppb) and ozone (at a concentration of 300ppb) during the course of this study.

_____ I understand that there are risks associated with my participation in this study. A study team member has discussed potential risks associated with participation in this study, and the measures that will be taken by the study team to reduce risk, with me.

_____ A member of the study team has discussed genotyping with me and I have been given the opportunity to either opt-in or opt-out of being genotyped as a part of this study.

_____ I understand that I have the ability to terminate my involvement in the study at any time for any reason and that I am under no obligation to complete the study.

_____ A member of the study team has reviewed the form, "*Consent for Storing Biological Specimens with Identifying Information*" with me and explained how, with my consent, blood samples taken from me during the course of this study will be stored.

_____ I understand that I can submit a written request at any time, during or after the completion of this study, to the Principle Investigator asking for my specimens to be destroyed.

_____ I have been made aware that if I choose to end my involvement in the study prior to completion of my study-related visits/activities, I will receive compensation at 100% of the indicated rate through the last day that I participated in the study.

_____ I have been given contact information for the Principal Investigator and the Medical Station at the EPA Human Studies Facility.

_____ I understand that I can ask a member of the study team or the medical staff a question regarding the study and my involvement in the study at any time.

_____ I have had an opportunity to ask a member of the study team any questions that I have up to this point regarding the study and my involvement in the study.

_____

**Title of study:** Effects of sequential exposure to nitrogen dioxide and ozone in healthy adult human volunteers.

**Principal investigator:** Shaun McCullough, Ph.D.

**Subject's Agreement:**

I verify that, to the best of my knowledge and belief, all of the information contained in the consent form was read and understood by me prior to signing it. I further certify that I have read the information above and by initialing at each statement and signing below I acknowledge that I agree and understand each statement above. I have asked all the questions I have at this time.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Member Obtaining Consent

_____ _____

Signature of Second Research Team Member Present for Consent Date

_____

Printed Name of Second Research Team Member Present for Consent