WILDLAND FIREFIGHTER WORK TASK-RELATED SMOKE EXPOSURES AT PRESCRIBED BURNS AND THEIR EFFECT ON PROINFLAMMATORY BIOMARKERS AND URINARY MUTAGENICITY

by

ANNA MARIE ADETONA

(Under the Direction of Luke Peter Naeher)

ABSTRACT

Objectives: 1) To characterize smoke particles on personal air filters, 2) determine differences in smoke particles according to work tasks, 3) estimate inhaled dose of smoke particles using estimated ventilation rate data, 4) assess cross-work shift (before work shift to after work shift) changes in proinflammatory responses according to work tasks, and 5) characterize occupational smoke exposures using urinary mutagenicity among wildland firefighters. **Methods:** Repeated measures on twelve subjects with the United States Forest Service were collected on prescribed burn and non-burn days during January-July 2015. Personal particulate matter ($PM_{2.5}$), carbon monoxide (CO), accelerometer data, and before-, after-, and morning-after- work shift dried blood spots (DBS), blood smears, and urines were collected. Accelerometer measurements were used to estimate ventilation rate and inhaled dose of $PM_{2.5}$. Light absorbing carbon (LAC) was measured as a surrogate for black carbon (BC) and divided by $PM_{2.5}$ concentrations to calculate mass absorption efficiencies. DBS were analyzed for inflammatory mediators using the Meso Scale Discovery assay and leukocyte populations were assessed on blood smears. Urine was analyzed for mutagenic potency using the Ames assay and for malondialdehyde (MDA) using High-

Performance Liquid Chromatography. **Results:** Firefighters conducting lighting (lighters) had an estimated 1.3 times higher, though not significant, inhaled amount of $PM_{2.5}$ compared to those managing fire boundaries (holders). Lighters had a three-fold higher exposure to LAC and significant cross-work shift increases in interleukin-8, C-reactive protein, and serum amyloid A, compared to holders. Positive associations were observed between interleukin-8 and segmented-neutrophil (p=0.0179), and mass absorption efficiency (p=0.0080), respectively. Though not significant, lighters had a two-fold higher cross-work shift increase in crude urinary mutagenicity compared to holders and non-burn day exposures. Positive associations were found between creatinine-adjusted urinary mutagenicity and MDA (p=0.0905), CO (p=0.0459), and mass absorption efficiency (p=0.1333), respectively. **Conclusion:** Healthy seasonal wildland firefighters conducting lighting at prescribed burns had higher acute proinflammatory responses compared to other tasks. Data suggest that intermittent diesel exposures contribute to acute health responses, while urinary mutagenicity may serve as a suitable measure of integrated occupational smoke exposures of wildland firefighters.

INDEX WORDS: Exposure assessment, Wood Smoke, Firefighter, Prescribed burns, Biomarkers, Inflammation, Urinary Mutagenicity, Black carbon, Particulate matter.

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DEDICATION

In loving memory of and in the words spoken of my dad—Timothy J. Hejl:

"TGBTG: To God Be The Glory!"

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CHAPTER 1

INTRODUCTION

PROBLEM STATEMENT

The International Agency for Research on Cancer (IARC) has recently listed wood smoke as a probable (Group 2A) human lung carcinogen.[1] Accounts of wildfires across the United States (U.S.) and around the world are expected to escalate as projected by the current increasing trends over recent decades.[2, 3] Increasing fire intensity both in size and frequency may be influential factors contributing to the greater number of people being exposed to wildfire smoke emissions. Downwind and close-to-source inhalation exposures of fine particulates from wildland fires is becoming a primary public health concern in the general public and wildland firefighter communities. Increased cardiorespiratory morbidity and mortality in the general public have been shown to increase during and immediately after wildfire events, and while the literature is limited, cardiovascular related morbidities or mortalities are found to be associated with wildfire events in the general population.[4-8] A meta-analysis study found a significant association with exposure to biomass (including wood smoke) and chronic obstructive pulmonary disease (COPD) and chronic bronchitis in the general population.[9] Occupational wood smoke exposure may be associated with adverse health outcomes such as reduced respiratory lung function and increased respiratory symptoms.[10, 11]

Wildland firefighters are exposed to high levels of wood smoke and associated pollutants due to the nature of their job and extended exposure periods throughout the year. Frequency of exposure is inadvertently high and workdays may last well over the normal 8-hour work shift.

Effective respiratory protection is not used nor are they feasible during wildland firefighting because of the high physical demand of work. There is also no commercially available respirator that would be protective for wildland firefighters.

PURPOSE OF RESEARCH

One of the many data gaps impeding the understanding of the underlying health risks of wood smoke exposure is the lack of health studies among firefighters and the characterization of their occupational exposures. It is suggested that wildland firefighters are not only exposed to a mixture of pollutants found in wood smoke but significant exposures may be attributed to emissions from non-wood smoke sources such as diesel exhaust. Improved exposure assessment approaches capable of distinguishing among the sources and estimating internal dose are needed for determining accurate dose-response relationships in epidemiological studies. Results from my master's thesis suggest that firefighting work tasks could substantially influence particulate composition and toxicity of inhaled exposures.[12] **Therefore, my dissertation project sought to characterize work task-related occupational smoke exposures and assess their association with select biomarkers of exposure and effect, with the intention of advancing the understanding of underlying mechanisms involved in acute health effects seen during wildland firefighting.** My dissertation research has five overarching objectives, of which results are presented in three separate manuscripts, respectively (Note: Manuscript 1 addresses main objectives 1-3 listed below):

1) Characterize smoke particles on personal air filters from wildland firefighters, (Manuscript 1)

2) Determine differences in smoke particles on personal air filters according to work tasks, (Manuscript 1)

3) Estimate inhalation dose of smoke particles using estimated ventilation rate data,
(Manuscript 1)

4) Assess cross-work shift (before work shift to after work shift) changes in proinflammatory responses according to work tasks,
(Manuscript 2)

5) Characterize occupational smoke exposures of wildland firefighters using urinary mutagenicity as a biomarker of integrated exposures.
(Manuscript 3)

OUTLINE OF DISSERTATION

This dissertation consists of three manuscripts, all of which are products from a pilot funded project on occupational smoke exposures of wildland firefighters working at prescribed burns, which are predetermined controlled set fires often used for the enhancement of ecosystems and for the reduction of understory growth. The study location was at the United States Department of Energy's Savannah River Site (SRS), Aiken, SC, USA. Chapter 1 states the problem and overall purpose of the research while also providing a general summary of each chapter presented herein. Chapter 2 consists of a background and literature review pertinent to the research. Chapter 3 presents a manuscript that will be submitted for publication to the *Journal of Exposure Science and Environmental Epidemiology*. This paper focuses on the characterization of differences in fine particulates and estimated pulmonary ventilation rate with respect to work tasks of wildland firefighters (Main objectives: 1, 2 and 3). Chapter 4 comprises of a manuscript that is prepared for submission to the *Journal of Occupational and Environmental Medicine*. This paper presents the health endpoints involving proinflammatory responses from occupational exposures in the same firefighter cohort as in Chapter 3 (Main objective: 4). Chapter 5 consists of a third manuscript to

be submitted to *Inhalation Toxicology*, which explores the effect of wood smoke exposure on oxidative stress and urinary mutagenicity of wildland firefighters from the same cohort (Main objective: 5). For this third manuscript, a subset of the data is presented herein. To date, this manuscript includes results on the data of the matched pre-post work-shift (paired) urine samples thus far analyzed (44% on burn days [n=20 of 45 total] and 56% on non-burn days [n=9 of 16 total]).

Chapter 6 provides an overall summary and conclusion of this dissertation and gives remarks on possible future directions. Lastly, two appendices are included which consist of supplemental material. Appendices A-D, respectively, comprise of copies of the Institutional Review Board (IRB) the University of Georgia approved consent form, baseline questionnaire, and daily exposure-work task and morning-after questionnaires given to subjects from the study reported in Chapters 3, 4, and 5. Appendix B, presents an abstract of a recently published paper in *Inhalation Toxicology*.[13] I was a contributing co-author on this paper. My role consisted of conducting an extensive systematic literature review of the papers using five major databases, including PubMed and MEDLINE Web of Knowledge, on wood smoke exposure and its health effects among the general population and wildland firefighters. I critiqued papers based on an evidence analysis protocol[14] and extracted data from the literature for future risk assessment purposes. My work on this review paper aided me in the development of my dissertation project as it allowed me to critically review current and previous publications surrounding the topic of wood smoke and its health effects and also note where research gaps exist.

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CHAPTER 2

LITERATURE REVIEW

OVERVIEW

Worldwide, catastrophic wildland fires[i] are increasing not only in frequency but in size and severity due to persistent multi-year drought conditions combined with increased fuel loads.[1, 2] In the United States (U.S.), annual costs due to structural loss caused by wildland fires has greatly increased over the past sixty years and is anticipated to rise. Since 1983, data collected on wildfire frequency by the National Interagency Fire Center (NIFC) report that 72,000 wildfires occur on average per year in the U.S., although other agencies suggest this estimate to be even higher.[3] In 2015, wildland fires swept over 10 million acres across the U.S. Preplanned controlled fires (prescribed burns)[ii] are used as preventive measures against the occurrence of large catastrophic wildfires.[4] In 2015, combined local, state, and government U.S. agencies conducted over 37,000 prescribed burns, covering nearly 3 million acres.[5] Escalating costs of wildland fire suppression are projected to be over $1.8 billion in the U.S. by 2025.[6]

Smoke exposure from wildfires and prescribed burns is a concern for fire personnel and the general public. The growing wildland-urban interface has created even a greater risk of wildfire smoke exposure to communities and surrounding populations.[7] Wood smoke is composed hundreds of pollutants, many of which are classified as harmful to human health.[8, 9] The presiding

[i] Wildland fires or wildfires are herein defined as unplanned fires caused by lightning, accidental or intentional humans actions, escaped prescribed burns, or volcanoes.

[ii] Prescribed burns are herein defined as fires intentionally ignited by trained personnel under an approved plan with specific objectives for land management purposes and are generally low intensity and low severity fires consuming mostly forest floor or understory growth.

health risk-related component of wood smoke is notably particulate matter (PM) with an aerodynamic diameter of ≤ 2.5 μm ($PM_{2.5}$).[8] Although the chemistry is complex, wood smoke $PM_{2.5}$ mainly consists of organic carbon and black carbon components and some inorganic species.[8, 10] Smoke from wildland fires, including prescribed burns, contribute to almost 20% of the total $PM_{2.5}$ ambient air emissions in the U.S. (Note: Data available from the U.S. Environmental Protection Agency's [U.S. EPA] national emissions inventory database for $PM_{2.5}$ was used to calculate percent of $PM_{2.5}$ attributed from wildland fires in 2014).[11] Ambient $PM_{2.5}$ emissions after wildland fire events have been associated with cardiorespiratory morbidity and mortality in the general population.[12-19] It is estimated that the global mortality attributable to smoke from wildland fires is 339,000 deaths annually.[20]

As more studies elucidate the association between wood smoke and adverse health effects in the general population, concern for wildland firefighter health has risen to the attention of many. Cardiorespiratory protection for personnel either combating wildland fires or managing prescribed burns is not mandated and clear occupational wood smoke exposure standards have yet to be established for wildland firefighters. Moreover, other additional exposures that are injurious to humans may be seen while conducting prescribed burns. Exposures to diesel and gasoline from fire engine trucks, mule utility vehicles, bulldozers, and particularly fueled drip-torches are typically seen during prescribed burning. PM is also a combustion product of fossil fuels such as coal, oil, gasoline, and diesel.[21] Air pollution PM products from fossil fuel-related combustion sources have been found to be harmful to health.[22] The International Agency for Research on Cancer (IARC) has classified diesel exhaust as a known human carcinogen (Group 1) and gasoline exhaust as a possible human carcinogen (Group 2B).[23]

PM toxicity can change from source to source and depends on the size and composition of the particles.[24] Oxidative stress and inflammation in the airways are two major mechanisms of toxicity from exposure to wood smoke particles.[9] Inhalation of diesel or gasoline combustion byproducts can act in a similar fashion in the body.[25, 26] However, the impact of combined exposures to wood smoke and diesel/gasoline is not clearly known.

The following literature review is intended to provide readers with an in-depth but succinct review of the available literature on 1) the composition and toxicity of wood smoke and diesel/gasoline, 2) biomarkers and exposure monitoring, 3) current practices of prescribed burning, and 4) health effects among wildland firefighters.

COMPOSITION AND TOXICITY OF WOOD SMOKE, DIESEL AND GASOLINE

Wood Smoke

The presiding components of wood smoke can be summarized into seven major classes: 1) inorganic gases (i.e. carbon monoxide [CO], nitrogen dioxide [NO_2]), 2) hydrocarbons (i.e. 1, 3-butadiene, n-hexane, benzene, polycyclic aromatics hydrocarbons [PAHs] such as benzo[a]pyrene), 3) oxygenated hydrocarbons (i.e. methanol acetic acid, methylphenols, hydroquinone, aldehydes like acrolein and formaldehyde), 4) chlorinated organics (i.e. dioxin, methylene chloride), 5) trace metals, 6) free radicals, and 7) PM.[8] To date, the components of most concern include respirable PM ($PM_{3.5/4}$; PM with an aerodynamic diameter of $\leq$ 3.5 or 4 μm) or fine PM ($PM_{2.5}$), acrolein, formaldehyde, carbon monoxide, nitrogen dioxide, and benzene.[9] Therefore, this review specifically addresses health effects associated with these components while others are discussed in brief.

Particulate Matter

PM, noted as the best single indictor of the health hazards of biomass combustion sources, is composed of a number of solid particles and liquid droplets including acids (such as nitrates and sulfates), organic chemicals, metals, and soot[iii] or dust particles.[27] Black carbon (BC) is also a constituent of both course and fine PM and is formed by the incomplete combustion of fossil fuels, biofuels, and biomass.[28] Precise chemical definitions of BC, brown carbon (BrC), elemental carbon (EC), and organic carbon (OC) are not clearly established and differ among disciplines, therefore, this dissertation relies on the general terminology provided in the U.S. Environmental Protection Agency's report on BC to the U.S. Congress.[28] BC and EC are often used interchangeably where EC is regarded as the non-organic, refractory portion of the total carbon and as an indicator for BC.[28]

Carbonaceous components of particles are often distinguished by their specific light-absorbing properties or refractory nature.[28] Although there is no universally accepted standard measurement, two primary ways of classification is through light-absorption (optical techniques) or thermal-optical methods (refraction techniques by measuring the inertness of the carbon at high temperatures).[28] Light-absorption carbon (LAC) is distinguished between BC and BrC using light-absorption methods, while apparent elemental carbon (ECa)[iv] and apparent organic carbon (OCa) are particles distinguished through the latter method (Figure 2.1).[28] Simply stated, carbonaceous PM can be analyzed by its physical or chemical properties, respectively. In this dissertation, our method of analyzing carbonaceous PM was through the use of optical measurements of apparent BC (BCa). We used a Smoke Stain Reflectometer (Diffusion Systems, Ltd.) which is an instrument

[iii] Soot is the agglomeration of EC and OC particles.
[iv] The addition of the term "apparent" specifies that the carbonaceous PM species is an estimate of concentration.

that is able to provide an estimation of the BC concentration in collected air PM (in our study $PM_{2.5}$) on air filter samples by reflectance technique using a monochromatic incandescent light (detector with a visible spectrum photopic response, i.e. human eye response).[28, 29]

Wood smoke particles consists of roughly 5-20% EC (indicator for BC), while the OC fraction is typically at least 50%[9] but can vary significantly due to differing fuel types and combustion conditions.[8] The ratio of OC:BC can vary depending on the soot mixture composition and combustion conditions (i.e. smoldering versus flaming) where very dark soot particles (i.e. PM from diesel exhaust) has a low OC:BC ratio. When the OC fraction increases, the apparent color of soot particles shifts to brown or yellow in appearance (i.e. PM from smoldering biomass).[28] Levoglucosan, is another component of wood smoke and is the most abundant organic compound in PM from wood smoke.[9] It is a sugar anhydride and a pyrolysis product of the combustion of cellulose and often used as a unique tracer of wood smoke exposure.[30]

Size and varying chemical composition of particles can determine their behavior and toxicity in the respiratory system.[31] Particles may be the same physical size but different in shape and density, thereby exhibiting different behavior within the respiratory tract.[32] Hygroscopic particles will absorb water as they travel down in the humid environment of the respiratory tract, thus growing in size while still airborne within the airways which can influence their regional deposition.[32] Smaller particles ($PM_{2.5}$) can penetrate deep into the lungs, deposit into the alveolar spaces, making it difficult for the body's clearance mechanism to remove them.[33] If not removed by coughing, sneezing, or mucociliary expulsion, resident phagocytic macrophages or dendritic cells may uptake the particles thereby eliciting an immune response.[32]

Wood smoke PM has been shown to have adverse health effects. Acute exposures to $PM_{2.5}$ are thought to induce inflammation and cytotoxicity.[34, 35] An *in vitro* study exposed monocytes

and pneumocytes to 10-40 μg/cm^2 wood smoke PM and traffic PM over 12, 40 and 64 hours to determine pro-inflammatory cytokine release (Tumor Necrosis Factor [TNF]-α, interleukin [IL]-1, IL-6, IL-8) and viability.[35] Findings showed that wood smoke PM induced a cytokine response and reduction in cell number of monocytes and pneumocytes.[35] The study also found that toxic and pro-inflammatory responses were associated with the organic fraction of the PM suggesting its effect to be source-dependent. Traffic PM was found to induce a higher cytokine release than wood smoke PM, while wood smoke PM was found to have a greater effect on the decrease in viable cells.[35] Associated adverse health effects with chronic exposures to wood smoke derived PM are less known, however, the mechanisms of toxicity are thought to involve a complex network of inflammation and oxidative stress responses.[36]

Acrolein and Formaldehyde

Pollutants like acrolein and formaldehyde found in wood smoke have been classified as irritants to the respiratory tract.[37, 38] The U.S. EPA has classified formaldehyde as a probable human carcinogen while not enough evidence is available for the assessment of human carcinogenic potential of acrolein.

Carbon Monoxide and Nitrogen Dioxide

Gases like CO and NO_2 have also been associated with adverse health effects. Prolonged CO exposure can result in tissue hypoxia through the formation of carboxyhemoglobin (COHb) in the blood. CO affinity for hemoglobin in the blood is up to 240 times that of oxygen.[39] Headaches, dizziness, and impaired cognition may occur after prolonged exposure.[39] NO_2 exposure has been shown to affect lung function and induce airway hyper-responsiveness.[40]

Benzene

The IARC and the U.S. EPA has classified benzene as a known human carcinogen for all routes of exposure. Although benzene levels in wood smoke have been found to be low,[41-43] chronic low level exposures are of concern. Non-cancer effects such as immunological, neurological, and hematological effects have been reported in humans exposed to chronic low levels of benzene.[44, 45]

Others

Wood smoke may also contain pollutants in lesser amounts, however, concern for human health remains for those such as dioxins and furans (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans [PCDD and PCDF]),[46-48] with 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) considered the most potent of the congeners since its classification by the U.S. EPA as a probable human carcinogen (Group B2). Human exposure studies suggest that exposure to TCDD by inhalation has been associated with chloracne, lung cancer, soft-tissue sarcomas, lymphomas, and stomach carcinomas.[49] Of note, other inhalation exposures to pollutants found in wood smoke such as benzo[a]pyrene (BaP) and 1,3-butadiene are found to have mutagenic and carcinogenic effects.[8, 9, 50]

Diesel and Gasoline

Components of diesel and gasoline combustion are similar to those of wood smoke. Diesel exhaust is a complex mixture of pollutants composed of a particulate and gaseous phase. Similar to wood smoke combustion by-products, components of diesel and gasoline exhaust varies based on different combustion conditions and efficiencies. Most particles fall within the smaller range of inhalable particles, having a mass median diameter of 0.05 to 1.0 μm.[32] The particulate component of diesel exhaust includes a mixture of BC (as the major constituent), OC, sulfates, metals and

trace elements.[28] Diesel exhaust particles (DEP) have a carbonaceous core with a large surface area per gram of mass capable of adsorbing hydrocarbons like PAHs and nitro-PAHs or other inorganic/organic compounds which can penetrate into lower portions of the respiratory tract.[32] Its soluble components (i.e. transition metals) also have the potential to penetrate the lung epithelium and gain entry into circulatory system.[51] The gaseous phase consists of combustion products, hydrocarbons, and PAHs, some of which are also found in the particle phase. While diesel exhaust is higher in nitric oxides and PM, diesel exhaust tends to have lower CO, hydrocarbons and CO_2 levels compared to gasoline exhaust.[32] Compared to wood smoke derived $PM_{2.5}$, diesel derived $PM_{2.5}$ contains 75% of EC (range from 33-90%) (indicator for BC) of the $PM_{2.5}$ chemical composition,[52] as opposed to the 5-20% EC seen in wood smoke[8]. Exposure to BC has been associated with cardiovascular and respiratory health effects.[53, 54] In present day gasoline formulations, chemicals including benzene, toluene, ethylbenzene, and xylene (the BTEX chemicals) are of most concern for human health.[55] Whole gasoline exhaust has been classified as possibly carcinogenic to humans (Group 2B) by IARC because of its chemical composition of benzene (Group 1), ethylbenzene (Group 2B), and naphthalene (Group 2B). Although benzene exposures have been shown to be associated with blood cancers, a meta-analysis based study recently reported that low occupational benzene vapor exposures (associated with gasoline formulations) did not have any strong or consistent associations with lymphatic/hematopoietic cancers.[56]

Many papers report on the short-term and long-term health effects of traffic-related exposures.[23, 57-63] A human crossover study reports findings of positive associations between short-term diesel exhaust exposure and changes in DNA methylation of circulating mononuclear cells in individuals who have asthma.[57] A human chamber study exposed eighteen healthy volunteers

to diluted diesel exhaust (300 μ/m^3 of PM_1) and to filtered air (2 μ/m^3 of PM_1) during two 3 hour sessions and found significant increases in inflammatory markers (monocytes and total leukocyte counts in peripheral blood samples) and decreases in lung function (peak expiratory flow [PEF]) after diesel exposures.[61] IARC documents that sufficient evidence exists to link diesel engine exhaust exposures to increased risk for lung cancer. Further, a recent paper deciphering health effects associated with long-term $PM_{2.5}$ exposure (from fossils fuel combustions processes like coal burning and diesel traffic) found that such exposures were significantly associated with increases in ischemic heart disease mortality among the U.S. general population.[63]

BIOMARKERS AND EXPOSURE MONITORING

Biomarkers

Biological markers (biomarkers) can be useful indicators of xenobiotic exposures and of signaling events or responses in biological systems. In environmental health research, a biomarker is traditionally defined as a "cellular, biochemical, or molecular alteration" that can indicate an individual's exposure, response (effect), or susceptibility to a xenobiotic agent.[64, 65] These changes can be observable in biological media such as human tissues, cells, or fluids.[64] In this dissertation review, pertinent biomarkers of exposure and effect to wood smoke exposure are discussed, while others are summarized briefly. A biomarker of exposure is a biological indicator which reflects a current or past exposure, represents the extent of an exposure especially at low levels, and is specific to the exposure of concern.[66] A biomarker of effect is defined as a biological response indicator devoted to determining the effect of the xenobiotic exposure to an individual. These effects could be present at the whole organism, organ, tissue, cellular, or subcellular levels and should indicate the presence and magnitude of biological responses to exposure to the xenobiotic.[66]

Measurable biomarkers of effect within an individual can be associated with an established disease or possible adverse health outcome.[64, 66]

To date, many potential biomarkers for wood smoke exposure and biomarkers of wood smoke-associated health effects are incompletely validated or otherwise non-specific. Factors that affect the validity of a biomarker include: the capability to decipher the magnitude of an exposure, the presence of a dose-responses relationship, information on its levels in unexposed populations, inter- and intra-individual variability, and confounders affecting its expression.[66] Previous studies have used urinary hydroxylated PAHs (OH-PAHs) (i.e. 1-hydroxy-pyrene, 2-hydroxy-naphthalene), urinary methoxyphenols, urinary levoglucosan, exhaled CO, and carboxyhemoglobin as biomarkers of wood smoke and traffic related exposures among various populations (i.e. structural and wildland firefighters, bus drivers, general public).[30, 67-70] Additionally, urinary mutagenicity assessed by the *Salmonella* mutagenicity assay (Ames assay)[71] has been used as a biomarker of exposure to complex mixtures such as wood smoke and ambient air combustion emissions.[33, 72-74] This assay may be used to characterized potential genotoxic exposures.[71, 75] Two non-specific biomarkers of effect, inflammatory and oxidative stress markers, have been widely used in previous epidemiological, clinical, *in vitro* and *in vivo* studies characterizing the effects of wood smoke and/or traffic-related exposure.[25, 31, 34, 35, 76-84] Both inflammation and oxidative stress underlie the development of cardiorespiratory diseases such as asthma, chronic obstruction pulmonary disease (COPD), and others.[85-87]

Urinary Mutagenicity

This dissertation explored the use of urinary mutagenicity as a biomarker of occupational smoke exposures of wildland firefighters. The Ames mutagenicity test, a short-term *in vitro* assay, was used to evaluate the mutagenic potency of the urine collected from firefighters exposed to

pollutants at prescribed burns. Wildland firefighters can be exposed to occupational pollutants intermittently over a burn season/year, however, their exposures may also be cumulative over the course of a firefighter's career. Though non-specific, urinary mutagenicity of wildland firefighters may be a useful biomarker of exposure used not only in the evaluation of acute genotoxic exposures but also in the assessment of long-term risk for cancer.

A mutagen is defined as an agent that induces increases in the frequency of the occurrence of mutations[v]. Mutagenicity therefore is herein defined as mutations caused by mutagens on DNA and chromosome which are typically heritable changes.[66] Mutations can either be advantageous or detrimental to an organism. Mutations may be categorized as base-pair substitutions, frame-shift mutations, insertions, deletions, or complex mutations which combines more than one mutation event.[66] If inherent repair mechanisms fail to correct or delete the mutation, the mutation may then be passed on from parent cell to daughter cell (heritable mutation).[66] If uncorrected mutations occur in the coding regions (i.e. genes) or noncoding (i.e. regulatory regions of genes) of the DNA, a cascade of events could lead to altered gene expression patterns and formation of nonfunctional/altered proteins and enzymes, ultimately influencing cellular processes leading to the possibility of carcinogenesis.[66] Carcinogenesis is a multistage process (i.e. initiation, promotion, malignant conversion, progression, metastasis), however, DNA mutations can play an important role in the initiation step of carcinogenesis.[33]

Several previous studies document associations between urinary mutagenicity and associated internal and external exposures. For instance, a recent study on a Guatemalan population exposed to wood smoke found that found that urinary mutagenicity was associated exhaled CO measurements.[73] A study on Brazilian charcoal workers reported a significant dose-

[v] A mutation is defined herein as a permanent change in the coding region of the DNA sequence or a heritable change in the sequence of an organism's genome.[55]

response association between wood smoke exposure estimated levels and urinary mutagenicity of workers. Although studies are limited, positive associations between urinary mutagenicity and adverse health outcomes have also been reported in the literature. For example, one study found a positive association between urinary mutagenicity and risk for colon cancer after the consumption of fried meats.[88]

Inflammatory Biomarkers

Inflammation is essential in survival and acts as a protective response while serving two main purposes, 1) to rid the host of damaged or necrotic tissue, and 2) to protect against foreign invaders.[89] Innate immunity is the body's first line of defense against a pathogen or foreign body exposure. Physical barriers like the skin, mucous membranes, cells (i.e. pattern recognition molecules, polymorphonuclear cells, monocytes, macrophages, natural killer cells, eosinophils, etc.), and chemical barriers (i.e. lipids, enzymes, pH barriers, etc.) consist of the body's innate immune system.[89] Primary soluble mediators such as cytokines and chemokines are also released during an innate response and can thereby trigger humoral and cell-mediated responses. Cytokines, chemokines, and interferons are critical in the interactions dictated by them—cellular activation, initiation, termination of intracellular signaling events, proliferation, differentiation, migration, and others.[89] Events following an insult include the release of these chemotactic factors, increased blood flow, increased capillary permeability to allow for cellular infiltration, and acute resolution of damage tissue or persistence of the response (ultimately contributing to disease).[89, 90] Thus, a pro-inflammatory response is often initiated after exposure to a cellular stressor. Resolution of an inflammatory response will often consist of removal of leukocytes by the lymphatics or by apoptosis to make sure on-going acute inflammatory response is terminated.[89] When an

inflammation response cannot be resolved, damaged to tissue and disease development can follow.[89]

Inflammation in the body may be considered as a non-specific biomarker of effect. The course of an inflammatory event may be triggered by a number of reasons and is a common mechanism of action in the progression of many diseases, including cancer.[66] Inflammatory biomarkers have been used extensively as biomarkers of effect associated with exposures to wood smoke and traffic-related pollutants such as diesel and gasoline exhaust. For instance, an *in vitro* study assessing the pro-inflammatory effects of wood smoke and traffic-derived particles on a co-culture of human monocyte and pneumocyte cell lines showed that wood smoke particles derived from different combustion conditions elicited a proinflammatory mediator response (release of Interleukin [IL]-6, IL-8, and Tumor-necrosis-factor-α [TNF-α]) and cytotoxicity.[24] The study also reported that traffic derived particles induced a higher release of IL-6 than most of the wood smoke derived particles after 12 hours of exposure.[24] Results from this study also suggest that the organic fraction of the particle composition impact particle toxicity.[24] Another study explored the effects of wood smoke exposure on airway inflammation in ovalbumin-induced mice (with preexisting lung inflammation). Results from this study showed that short-term exposure to wood smoke was positively associated with an increase in ovalbumin-induced bronchoalveolar lavage eosinophil cells and serum Interferon-gamma (INF-γ) levels when allergen challenge (ovalbumin-aerosol exposure) preceded 300 $\mu g/m^3$ wood smoke exposures.[80] Human experimental studies have also been conducted to assess the inflammatory effects of wood smoke and traffic-derived particles.[62, 78, 79] A randomized, single-blinded cross-over intervention study comparing traffic-impacted and wood smoke-impacted areas found evidence of increases in C-reactive protein (CRP: an acute-phase protein and established marker of inflammation) and indoor $PM_{2.5}$ concentrations among

healthy adults (in traffic-impacted polluted areas).[62] A study conducted in 2004-2005 assessed inflammation in healthy non-smoking forest firefighters employed by the British Columbia Forest Service.[78] Biological monitoring included lung function tests (spirometry), sputum and blood sampling to assess lung inflammation and systemic inflammation before a fire season, during a fire season, and immediately after the end of active duty.[78] Results from this study showed significant increases in serum IL-6, IL-8, and monocyte chemotactic protein-1 following fire-fighting.[78] Significant associations were also found between changes in sputum macrophages (with phagocytosed particles) and circulating band-neutrophil cells, indicating lung and systemic inflammatory responses after acute forest fire smoke exposures.[78]

In this dissertation, the proinflammatory biomarkers measured in blood samples from our study population included cytokines (IL-6, IL-1β, and TNF-α), a chemokine (IL-8), cellular adhesion molecules (Inter-Cellular Adhesion Molecule 1 [ICAM-1] and Vascular Cell Adhesion Molecule-1 [VCAM-1]), and acute phase proteins (CRP and Serum Amyloid A [SAA]). Because cytokines and chemokines are responsible for the cell-to-cell communication during an inflammatory event, and are not only involved in the recruitment of adhesion molecules but also leukocytes to the cite of insult (i.e. IL-8 is involved in the recruitment of neutrophils to vascular endothelium and extravasation into inflamed tissues),[91, 92] we also used blood smears to assess 5-point differential leukocyte populations (band-neutrophils, segmented-neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

Oxidative Stress Biomarkers

Oxidative stress is defined as "a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage"[93] or where the production of reactive-oxygen species (ROS) or free radicals exceed that of the antioxidant species that are involved in the neutralization

of free-radicals.[94] Many xenobiotics (singularly or as mixtures) can produce oxidative stress in the body and therefore oxidative stress markers are considered as non-specific biomarkers of toxic responses. Biomarkers of oxidative stress can include decreases in antioxidant concentrations or increases in oxidative metabolites.[66] For instance, increases in lipid peroxidation products such as malondialdehyde (MDA), protein oxidative products such as protein carbonyl levels or glutamine synthetase activity, or decreases in endogenous antioxidants like ascorbic acid, reduced glutathione may be indicative of oxidative stress in the body.[66] Oxidative damage of proteins and DNA may also occur and result in measurable levels (i.e. 3-nitrotyrosine or 8-hydroxy-2'-deoxyguanosine [8-OHdG], respectively).[66] Consistent associations between combustion-derived PM exposures in human experimental studies and increased levels of oxidative damage (to DNA and lipid) products have been found in the literature.[25, 95] Oxidative damage has been identified as one of the primary toxicological mechanisms by which PM has been shown to induce adverse health effects.[9]

In this dissertation, urinary MDA was the only biomarker of oxidative stress measured in our study population. Mentioned previously, MDA is a product of lipid peroxidation of polyunsaturated fatty acids (oxidative degradation of lipids where free radicals steal electrons from the lipids in cell membranes).[66] Although a previous study reported no significant increases in MDA concentrations after wood smoke exposure among wildland firefighters,[96] results from a human chamber exposure study indicate that wood smoke exposures could induce lipid peroxidation.[97]

In the future (contingent on funding), aliquot urine samples from the same study population reported in this dissertation may be analyzed for 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane) and 8-OHdG. Isoprostanes are prostaglandin-like compounds produced by the reaction of free radicals with arachidonic acid and are well established markers of oxidative stress.[66] Particularly, 8-

isoprostane has been used extensively in previous studies as a reliable *in vivo* marker of oxidative stress.[98-100] Additionally, 8-OHdG has been one of the most studied DNA oxidation products.[66, 101] Available literature indicates that levels of 8-isoprostene and 8-OHdG increase after household wood smoke exposure among Peruvian women.[102] Additionally, a previous study's findings on wildland firefighters suggest that increases in oxidative damaged nucleobases such as urinary 8-oxo-dG (8-OHdG) levels can be sustained during the burn season and effected by length of the firefighting career (with higher levels observed for firefighters with fewer number of years as a firefighter).[96]

Exposure Monitoring

While biomonitoring (the use of biomarkers) is a useful tool in exposure assessment and in the understanding of mechanisms involved in disease development and/or prevention, it is best used in combination with other exposure assessment methods such as questionnaires, environmental and personal monitoring measurements (i.e. what amount of a xenobiotic exposure concentration is present to an exposed individual), graphical information systems, and others.[68] Traditional personal exposure monitoring consists of testing the air that a person would breathe regardless of where the person moves throughout the day. Ideally, a portable sampling device is attached to as close as feasible to the breathing zone of the subject and worn for a designated period either during the length of a work-shift or 8-hour day. Sampling devices can include the use of passive diffusion monitors for measuring volatile chemicals or small air pumps that can be connected as a sampling train using tubing with attached glass sorbent tubes or cyclone devices ready to capture particulates of a certain size on pre-loaded air sample filters. Once the samples are collected, they are analyzed and the amount of exposure is quantified. A time-weighted average (TWA) (average exposure concentration over the time the sampling devices was worn by the

subject) or ceiling limit (maximum amount or concentration of a chemical that a worker can be exposure to) may be calculated.[103] The application of exposure monitoring in occupational settings allows governmental agencies to estimate harmful work exposures, set, and enforce applicable exposure standards to improve the safety and health of those in the workplace.

Although personal exposure monitoring can yield results that help establish permissible exposure standards, there are a few notable uncertainties that are difficult to tease out when correlating exposures to adverse health effects. PM exposure concentrations outside of the body can be estimated using air monitors/pumps; however, the uncertainty of inhalation rate of an individual is not considered. Differences in exertions during activities throughout a workday, physical fitness, age, differences in body weight, may all contribute to varying inhalation rates of a given individual.[104] Epidemiology studies suggest that such population characteristics could influence the amount of chemical inhaled by the subject.[104-106] Exposure measurements either may be over or under estimating dose of inhaled exposures depending on the subject's characteristics. Therefore, it has been proposed that risk assessment estimations can be improved if exposure assessment not only measures exposure but could also estimate an inhaled dose of an exposure with the use of monitors capable of such estimations.[107] A recent experimental study explored the application of accelerometer data in the estimation of ventilation rates among twenty-two healthy human subjects who were asked to conduct a variety of ranging activities.[107] Results from this study suggest that predicted ventilation rates using triaxial accelerometers within personal exposure monitors could allow for calculations of real-time estimates of a potential inhaled dose of a pollutant.[107] Therefore, this dissertation sought to apply a similar method to account for subjects' ventilation rate and thereby estimate an inhaled dose of PM.

CURRENT PRACTICES OF PRESCRIBED BURNING

Wildland firefighters often perform various work tasks during prescribed burning. Tasks can include ignition of understory growth ("lighting"), management of fire boundaries ("holding"), extinguishing of smoldering fires after burning ("mop-up"), direct extinguishing of creeping fires or flanks of fire ("direct-attack"), and other tasks involving chainsaw, debris removal work, etc.[41, 108] Before prescribed burns, dozer-lines (fire lines constructed by a bulldozer) are usually placed around pre-determined boundaries of a designated burn area.[108]

Lighting and holding are the primary tasks conducted during burns. Wildland firefighters who perform lighting use a drip torch that contains a mixture of lighter fluid consisting of three-part diesel and one-part gasoline. Firefighters hike the designated burn area and lay parallel strips of fire measured in chain lengths (a unit of linear measurement equal 66 feet)[108] on the forest floor. In the case of large burns, trained personnel may use helicopters to ignite the forest understory by aerial ignition methods (i.e. "ping-pong" method; plastic spheres containing potassium permanganate and ethylene glycol distributed by a dispensing device).[109] During holding, firefighters use tools such as shovels, mule trucks, water hoses, and fire engines to keep the fire from burning outside boundaries.

Supervisor positions such as a "Burn Boss" and "Firing Boss" are assigned for each burn who are personnel responsible in overseeing the overall work and making decisions if management tactics should be altered during the course of a burn.[41] Constant monitoring of weather parameters including wind direction, wind speed, temperature, humidity, understory fuel load, and fire behavior is essential in proper management during prescribed burns.

HEALTH EFFECTS AMONG WILDLAND FIREFIGHTERS

Although prescribed burning is a key tool in land management, occupational exposures to various pollutants while performing a prescribed burn may be a concern for wildland firefighter health. Protective measures to reduce occupational exposures are often minimal and respiratory protection is not frequently used and self-containing breathing apparatuses (SCBA) are not feasible during wildland firefighting.[8] $PM_{2.5}$ in the ambient air concentrations (immediate vicinity) of wildland firefighters typically exceed the lowest occupational exposure limit of 3000 $\mu g/m^3$ recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). In addition, $PM_{2.5}$ exposure levels among wildland firefighters are certainly above the current 24-hr U.S. EPA's ambient air-quality standard of 35 $\mu g/m^3$.[9] Maximum short-term exposure to constituents of wood smoke, such as acrolein (i.e. 0.129 ppm)[41] and formaldehyde (i.e. 1.46 ppm)[41] and others, reported among wildland firefighters, exceed the National Institute of Occupational Health and Safety (NIOSH) recommendation ceiling limits (i.e. 0.1 ppm for formaldehyde and 0.1 ppm for acrolein).[9]

Typical occupational hazards include burns injuries, heat stress, dehydration, fatigue, slips, trips, and falls during wildland firefighting, however, acute health effects of wood smoke exposures among wildland firefighters have been observed. Decline in lung function measures across work shifts have been reported by a few studies, while some report negative findings.[110-113] Increased respiratory symptoms, acute airway inflammation, acute systemic inflammation, oxidative stress (limited data), and arterial stiffness (limited data) have been observed in a number of studies on wildland firefighters.[9, 77, 78, 96, 112, 114, 115] A study on seasonal wildland firefighters found increased respiratory symptoms in 65% (n=33 of 52) of the study population.[78] Significant increases in sputum granulocytes, circulating white blood cells and band cells, and serum IL-6 and

IL-8 levels were observed following one day of firefighting.[78] Estimated respirable PM reported by this study was relatively high (i.e. peak concentration: 2000 μg/m^3) during firefighting events.[78] Another study measured arterial stiffness (through the use of an aortic augmentation index) and urinary oxidative stress biomarkers (i.e. 8-isoprostane and 8-OHdG) in wildland firefighter hotshot crews.[115] Results from this study showed that mean augmentation index % was higher in wildland firefighters with higher oxidative stress scores, after controlling for smoking status.[115] Moreover, 8-OHdG levels were significantly higher in subjects who were recently exposed to wood smoke (self-reported by participants) compared to those who were not.[115] However, literature is still vastly lacking on how these acute effects may be linked to disease development.

Studies are sparse on the chronic health effects seen among wildland firefighters. However, a recent paper on a cross-sectional survey study investigated self-reported health effects of 499 wildland firefighters.[116] The study distributed questionnaires geared toward gathering information on employment, health, and demographics.[116] Findings from this study showed that career length was associated with hypertension, with firefighters of 10-19 years of experience and ≥ 20 years of experience having significantly higher blood pressure levels than those with < 10 years of experience.[116] Furthermore, significant associations between greater number of years as a wildland firefighter and self-reported two subclinical cardiovascular risk factors and biomarkers of musculoskeletal health were observed.[116] The authors of this study also noted that estimates may be under-reported, as individuals who experience serious health events (i.e. strokes or myocardial infarctions), are not expected to be current wildland firefighters and therefore were not included in the study survey.[116] Additionally, there is evidence that continuous occupational wood smoke exposures may impact lung function over time with declines in lung function measures being observed in wildland firefighters across one or two burn season periods (over several months).[9,

[110-112, 114, 117] However, it remains to be determined whether these declines in lung function declines would persist over many years.[9]

SUMMARY

In summary, wildland firefighters are likely exposed to a mixture of toxic constituents found in wood smoke and also other non-wood smoke occupational exposures such as diesel or gasoline exhausts during prescribed burning. Currently, no occupational wood smoke exposure limits exist for wildland firefighters. The chief inhalation hazards of their exposures include $PM_{2.5}$, acrolein, formaldehyde, CO, NO_2, and benzene. The organic fraction and/or speciation of PM, such as the characterization of BC, will be an important factor in distinguishing among the sources of exposures during firefighting, while improved exposure assessment approaches capable of estimating internal dose are needed for determining accurate dose-response relationships in epidemiological studies. Current literature, though limited, suggest that occupational firefighter smoke exposures are associated with acute lung and systemic inflammation, and perhaps oxidative stress and decreased lung function. Remaining gaps include the long-term health effects of occupational wood smoke exposures among firefighters.

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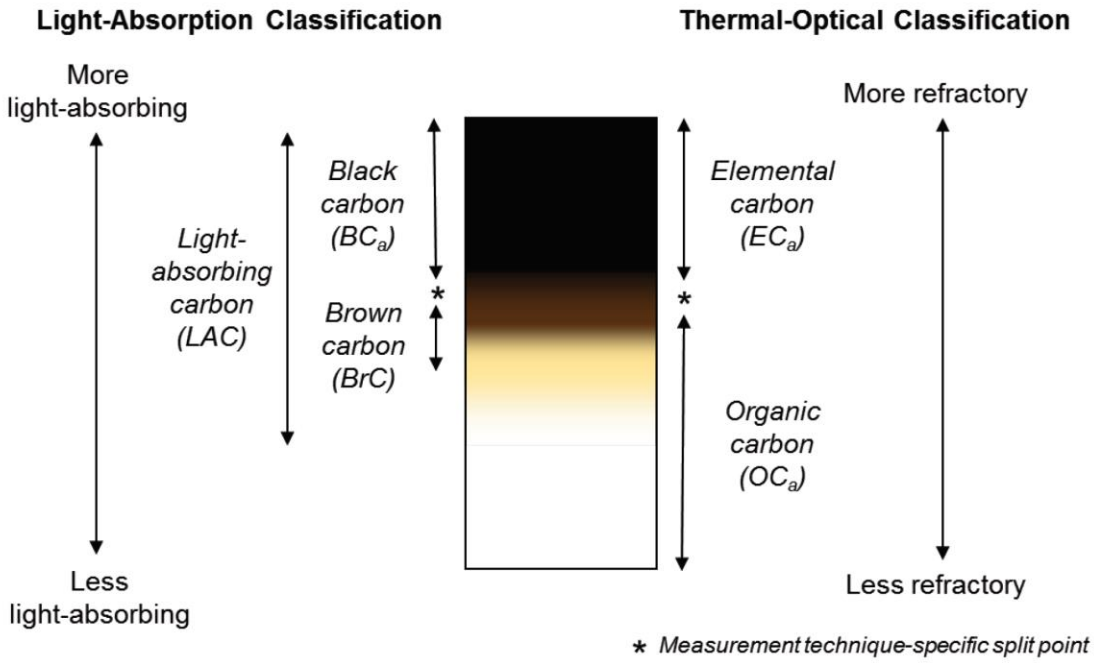

Figure 2.1. Measurement of the Carbonaceous Components of Particles. (**Adopted from U.S. EPA: Report to Congress on Black Carbon, Figure 5-1)**[28] Black carbon can be characterized in two primary ways, by measuring its light-absorbing properties or characterizing its particle refractory nature. Abbreviations: BCa, Apparent Black Carbon; ECa, Apparent Elemental Carbon; OCa, Apparent Organic Carbon; Light-Absorbing Carbon, LAC.

CHAPTER 3

DIFFERENCES IN FINE PARTICLES AND ESTIMATED PULMONARY VENTILATION RATE WITH RESPECT TO WORK TASKS OF WILDLAND FIREFIGHTERS: A REPEATED MEASURES STUDY [1]

[1] Adetona AM, Adetona O, Chartier RT, Paulsen M, Simpson C, Rathbun SL, & Naeher LP. To be submitted to *Journal of Exposure Science and Environmental Epidemiology*.

ABSTRACT

Background: Wildland firefighters are exposed to a mixture of chemicals found in wood smoke and emissions from non-wood smoke sources such as diesel.

Objectives: We investigated exposure, including inhaled dose, and compositional differences in particulate matter associated with work tasks of wildland firefighters.

Methods: Repeated measures on ten professional firefighters and two volunteers were collected on prescribed burn and non-burn days. Personal monitoring consisted of real-time and gravimetric $PM_{2.5}$, carbon monoxide, and accelerometer measurements to estimate ventilation rate and inhaled dose of $PM_{2.5}$. Light absorbing carbon was measured as a surrogate for black carbon.

Results: No difference in $PM_{2.5}$ concentration was observed between firefighters managing fire boundaries (holders) and those conducting lighting (lighters) (p=0.1743; n=16, 338 [95% CL: 174, 654] µg/m^3; n=32, 240 [95% CL: 134, 430] µg/m^3, respectively). However, we found that lighters had an estimated 1.3 times higher, though not significant, inhaled amount of $PM_{2.5}$ compared to holders. Contrary to $PM_{2.5}$ concentrations, lighters had nearly a three-fold higher exposure to light absorbing carbon versus holders.

Conclusion: Our findings indicate the importance of using dose as a metric of inhalation exposure in occupational or other settings, and show that other non-wood smoke occupational exposures while firefighting may significantly contribute to PM exposures.

INTRODUCTION

In many regions, wildland firefighters routinely conduct prescribed burns for ecological benefits and as preventive measures against the occurrence of catastrophic wildfires. During prescribed burning, firefighters often perform two primary work tasks: lighting and holding. Lighting is typically performed by hand using a diesel-gasoline fueled drip torch. In the case of large burns, helicopters may be used to ignite the forest understory. During holding, firefighters use water hoses and mule trucks to keep the fire from burning outside designated boundaries.

Although prescribed burning is a key tool in forest management, exposures to various pollutants during prescribed burns are a concern for health.[1] Protective measures to reduce occupational exposures are often minimal and respiratory protection is not frequently used nor feasible during wildland firefighting.[2]

Previous studies report that work tasks at prescribed burns can result in significantly different levels of exposure.[3-6] Results from these studies also suggest that the exposures of wildland firefighters to pollutants at prescribed burns may be substantially impacted by non-wood smoke sources.[5, 6] It is possible that soot from drip torches and exhaust from fire engines play a significant role in differing particulate matter (PM) levels between work tasks. Distinguishing PM sources can help to characterize occupational exposures more accurately for the development of suitable occupational health standards for wildland firefighters.

Wood smoke consists of a number of components including inorganic gases, oxygenated hydrocarbons, carcinogenic persistent organic compounds, and PM.[1] Notably, respirable PM, is identified as one of the primary health hazard indicators of wood smoke exposure.[2] Black carbon (BC) is a component of respirable PM and formed during the process of incomplete combustion of fossil fuels or biomass.[7] Different amounts of BC may be associated with specific PM sources

and distinguishing wood smoke PM exposure from other sources such as diesel exhaust[8, 9] will be a unique component to quantify according to work tasks at prescribed burns.

Differences in exertion related to varying activities throughout a workday contributes to the amount of pollutant(s) inhaled by the individual. Therefore, it has been proposed that risk assessment estimations can be improved if exposure assessment not only measures personal PM exposures but also estimate inhaled dose of PM with the use of monitors capable of such estimations.[10]

A previous study piloted the use of a dual functioning personal aerosol monitor, capable of measuring $PM_{2.5}$ in real-time and gravimetrically, and its ability to predict pulmonary ventilation rate using on-board accelerometer measurements in an experimental setting.[10] Results indicated that linear regression could be used to predict ventilation rate from accelerometer data over a range of activities. Moreover, the research suggests that future studies could apply such information to predict potential dose of PM.

In 2015, we set out to investigate whether occupational exposure to fine PM, or its components, differs with respect to work tasks at prescribed burns. Although we anticipated that wildland firefighters performing holding would have higher exposure to $PM_{2.5}$ (particulate matter with aerodynamic diameter of ≤ 2.5 microns) and CO based on results of previous studies,[5, 6] we hypothesized that firefighters lighting, with anticipated higher activity levels, will have higher ventilation rates and estimated inhaled dose of $PM_{2.5}$. We also hypothesized that personal air samples from firefighters performing lighting will contain a significantly higher fraction of BC due to the use of fueled drip torches.

METHODS

Study Design and Population

Repeated measures were collected from ten wildland firefighters employed by the United States Forest Service-Savannah River (USFS-SR), South Carolina and two individuals certified to work as volunteer firefighters on prescribed burns. Subjects were in general good health, currently non-smokers, not pregnant, and at least 18 years old. Subjects signed an informed consent form processed through Institutional Review Board at the University of Georgia, Athens, Georgia. Subjects were monitored for occupational exposures to $PM_{2.5}$ and CO on days when prescribed burning was conducted and working days when no prescribed burning occurred during the months of January-July 2015 at the United States Department of Energy's Savannah River Site (SRS) in Aiken, South Carolina. Vegetation at the 800 km^2 site is comprised of pines and mixed hardwoods.[11]

Questionnaires

Baseline questionnaires were administered to capture pertinent health information (e.g. age, weight, height, pre-existing health conditions, smoking status, etc.). Subjects also responded to additional questionnaires each sampling day and answered questions pertaining to primary work task performed during the work-shift (defined as comprising > 50% of the duration of a work-shift) and cofounding exposure (e.g. second-hand cigarette exposure). On burn days, two primary tasks were performed: lighting and holding. Activities on non-burn work days consisted of office work, patrolling, engine maintenance, and field prep work were collectively categorized as "Non-burn day Work Tasks". Duration of work-shifts were recorded by field technicians as well as information on burn size in acres, time, and date of burn.

$PM_{2.5}$ Collection

Personal real-time $PM_{2.5}$ measurements and gravimetric $PM_{2.5}$ samples were collected in the breathing zone of the subjects during their work-shifts using the MicroPEM^{TM} (RTI International, Research Triangle Park, NC, USA). The MicroPEM has a dual capability for measuring continuous $PM_{2.5}$ concentrations by nephelometry at 780 nm, and measuring integrated $PM_{2.5}$ off-line gravimetrically. Gravimetric $PM_{2.5}$ samples were collected on pre-weighed 25-millimeter polytetrafluoroethylene (PTFE) membrane filters with a porosity of 3.0 micrometers (Pall Life Sciences, Ann Arbor, MI, USA) loaded into the MicroPEMs. All filters were equilibrated in a climate-controlled laboratory (20°C; 40% Relative Humidity) for 48 hours prior to pre-weighing at the Department of Environmental Health Science, University of Georgia. MicroPEMs were zero-calibrated using a low-pressure drop HEPA filter and were set to run for 20 seconds for every minute (33% duty cycle) at a flow rate of 0.4 L/min using a mass flowmeter (TSI 4100 flowmeter; TSI Incorporated, Shoreview, Minnesota, USA) and computer software (MicroPEM Docking Station version 3.2, RTI International, Research Triangle Park, NC, USA). Raw (not filter-corrected) real-time time-weighted average (TWA) $PM_{2.5}$ concentrations were calculated by averaging the measurements over the sample duration. Filter-corrected real-time $PM_{2.5}$ concentrations were calculated by obtaining a filter-specific correction factor (CF) (Gravimetric $PM_{2.5}$ Concentration/Average Real-Time $PM_{2.5}$ Concentration) and multiplying the real-time data for that individual sample by its respective CF. We averaged the 95th percentile of the filter-corrected real-time $PM_{2.5}$ data to calculate peak exposure concentrations.

Gravimetric Analysis

Loaded filters were removed from the MicroPEMs and stored in a climate-controlled laboratory (20°C; 40% Relative Humidity) for 48 hours before they were subsequently weighed at

the Department of Environmental Health Science, University of Georgia using a Cahn C-35 microbalance (sensitivity of ±1.0 μg; Thermo Electron, Waltham, MA, USA) and stored in a -20ºC freezer until further analyses. The average pre-weight (twice weighed) of the filter subtracted from its average post-weight (twice weighed) was used to determine the total weight of the $PM_{2.5}$ collected on the filter. Work-shift TWA gravimetric $PM_{2.5}$ concentrations were calculated by dividing the mass of $PM_{2.5}$ collected by the total volume of air sampled. All TWA gravimetric $PM_{2.5}$ concentrations were adjusted by subtracting the mean weight change of field blanks from each sample weight before calculating the final $PM_{2.5}$ concentrations. Eleven field blanks, accounting for about 15% of the total number of filter samples collected had a mean weight change of 1.5 μg (Standard Deviation [SD]: 0.8 μg). All gravimetric analyses followed United States Environmental Protection Agency (USEPA) specifications.[12] $PM_{2.5}$ concentrations are in $\mu g/m^3$.

Reflectance Analysis

After gravimetric analysis, PTFE filters were shipped to the University of Washington for measurement of light absorbing carbon (LAC) by reflectance analysis. LAC of PM samples on filters was used as a surrogate for BC. The reflectance of all sample filters was determined using the Evans Electroselenium Limited smoke stain reflectometer (Model 43D, Diffusion Systems Ltd, London, United Kingdom), which measures the reflection of the light incidence in percent (linear scale; 0 to 100 % reflectance). The instrument was recalibrated after each sample using the standard calibration plate with reference white and gray spots provided by the instrument's manufacturer. The equation for calculation of absorption coefficient according to ISO 9835[13] is

$$a = \left(\frac{A}{2V}\right)\ln\left(\frac{R_b}{R_s}\right) \qquad \textbf{(1)}$$

where a is the absorption coefficient (m^{-1}), A is the area of the sample (m^2), V is the volume sampled (m^3), R_b is the reflectance of a field blank filter, R_s is the reflectance of a sample filter. Absorption coefficients were calculated essentially as described in the ISO 9835[13], except that modifications to the calculation were necessary due to not having pre-exposure measurements for each sample filter and each field blank filter. Therefore, R_b in equation (1) was determined as the average reflectance value for the eleven field blank filters (Average % reflectance of the blank: 69.3%; SD: 0.9%; Percent coefficient of variation [% CV]: 1.3%). Absorption coefficients were multiplied by 10^5 to make the values more comprehensible.[13] In addition, we calculated $PM_{2.5}$ mass absorption efficiencies in $\times 10^{-5} m^2/\mu g$ (absorption per unit concentration of total $PM_{2.5}$) by dividing the absorption coefficients by gravimetric $PM_{2.5}$ concentrations

Precision of replicate measurements of reflectance on twenty-eight filters was determined (SD: 0.2; % CV: 0.5%). For samples with replicate measurements of reflectance, replicate values were averaged.

CO Measurements

Real-time CO measurements were collected in the breathing zone, collocated with $PM_{2.5}$ measurements, using Dräger Pac III single gas monitors equipped with CO sensors (DrägerSafety Inc., Pittsburgh, PA, USA). To calibrate the sensor, the zero point was set using pure nitrogen gas and a span value set using 100 ppm CO certified gas standard before the study began. CO sensors were programmed to log data in 30-second intervals. TWA CO concentrations, reported in ppm, were calculated by averaging the CO measurements over the duration of sampling.

Estimated Pulmonary Ventilation Rate

The MicroPEM's triaxial accelerometer activity measurements were used to estimate minute ventilation rate (L/min) according to the linear regression equation published by Rodes et

al. (i.e. $V = m \times ACCEL + b$ where V is ventilation rate, m is the experimentally determined slope, *ACCEL* is the composite variable computed from the triaxial accelerometer measurements, and b is the intercept).[10] Average minute ventilation rate over the work-shift was then calculated for each person-day. The accelerometer composite was determined by the square root of the sum of the squares of accelerometer data in x, y, and z axes, where (x, y, z) are in units of g (gravity).[10] Rodes et al. regressed the accelerometer data against a gold standard-derived ventilation rate using the Oxycon Mobile metabolic measurement system (Carefusion, Yorba Linda, CA, USA). MicroPEM accelerometer data were not calibrated against ventilation rates of firefighters in the current study. Therefore, we adopted the intercept of 10.7 L/min reported by Rodes et al. as the median resting ventilation rate.[10] We also took a conservative approach to estimate the slope for the equation by setting the 95th percentile average ventilation rate by age, gender, and activity level reported by the USEPA in the Exposure Factor Handbook against the average peak accelerometer composite (i.e. USEPA 95th percentile average ventilate rate = $m \times$ *peak ACCEL* + 10.7).[14] Lighters had, on average, a peak accelerometer composite of 0.4 whereas holders and non-burn day activities yielded 0.2. To account for the added weight of backpacks and drip torches used by firefighters lighting fires, we used USEPA's average ventilation rate at the 95th percentile for high intensity activities (Metabolic Equivalents [METS] > 6.0). Since holding and non-burn work day activities had similar peak accelerometer composites, we used USEPA's average ventilation rate at the 95th percentile for light intensity activities ($1.5 < METS \leq 3.0$). A new slope was calculated for each person-day. The averaged absolute value of (1-accelerometer composite) data were used as the *ACCEL* variable in the slope-adjusted equations to determine the estimated ventilation rates in real-time. To calculate an average ventilation rate, a sum of the estimated ventilation rates in real-time was divided by the number of time points measured.

Inhaled mass of $PM_{2.5}$ (µg) was calculated by multiplying $PM_{2.5}$ exposure concentrations by the total volume of air inhaled which was obtained from the estimated ventilation rates. $PM_{2.5}$ dose (µg/kg body weight) was calculated by dividing the amount of inhaled $PM_{2.5}$ by the subjects' body weight.

Statistical Analyses

Histograms, scatter, and matrices of scatterplots of the residuals of the variables from fitted models were created to assess heteroskedasticity and normality of the data. Log-transformations were applied to TWA gravimetric and filter-corrected real-time peak (average of the 95th percentile) $PM_{2.5}$, CO, absorption coefficient, ventilation rates and estimated inhaled exposure data to normalize these outcome variables. Mass absorption efficiencies were arcsine square root transformed[15] to achieve normality (All mass absorption efficiencies were between 0 and 1). All transformed variables were back transformed after statistical analyses before reporting results.

After TWA gravimetric $PM_{2.5}$ concentrations were blank corrected, some filter weights fell below or were at zero (ten samples), resulting in negative $PM_{2.5}$ concentrations. These samples were either collected on non-burn work days, from one sample from a firefighter performing aerial ignition, or during the shortest burn session of the summer burn season. Therefore, the limit of detection (LOD; Cahn C-35 Microbalance sensitivity of +/- 1.0µg) divided by the square root of two was used in such situations before $PM_{2.5}$ concentrations were log-transformed. Several TWA CO concentrations were zero, all of which were collected on non-burn work days. These were replaced by one half of the smallest positive observed value (0.00068 ppm) before log-transformations since the reading range for the CO instrument is between 1 and 2000 ppm. Two absorption coefficient values fell below zero (both collected on non-burn work days) and therefore

were replaced with the LOD divided by the square root of two (LOD: 0.076 $\times 10^{-5}$/m absorption coefficient, assuming a 1 m^3 nominal sample volume).

Primary analyses consisted of using linear mixed-effects models to test the effect of work task on TWA gravimetric $PM_{2.5}$, filter-corrected real-time peak (average of the 95th percentile) $PM_{2.5}$, CO, absorption coefficient, ventilation rates, and estimated inhaled exposure/dose. Subject and date of sample collection were treated as random effects in the model to account for longitudinal within-subject correlation among the data and for possible heterogeneity in meteorological and other variable conditions from day to day, respectively. The exponential correlation structure was used to assess temporal correlation in the data because repeated measurements were collected at irregularly spaced times.

Covariates including season (winter or summer), acres burned, and sample duration (work-shift at the fire-line in minutes) and day type (burn or non-burn work day) were first tested individually in the models. Possible covariates were then evaluated based on the forward elimination procedure with only significant covariates included in the final model.

It should be noted that the effect of sample duration did not apply when the dependent variable was ventilation rate or inhaled/dose $PM_{2.5}$. A simple linear regression was also conducted to test the association between absorption coefficients and gravimetric $PM_{2.5}$ concentrations. All statistical analyses were conducted using SAS v.9.4 (SAS Institute, Cary, NC, USA). Statistical significance for all analyses was set at p-value < 0.05 and adjusted using the Bonferroni method when doing multiple comparisons (with p-value < 0.025 being the adjusted significant level).

RESULTS

Study Population

The mean age of the subjects was 33 ± 5.4 years, ranging from 26 to 43 years. The average body mass index was 27.0 ± 4.8 kg/m^2 and 75% of the subjects were male. None of the firefighters used any form of respiratory protection during the study. Fifty-four person-day samples were collected from 12 subjects (10 career firefighters and 2 volunteers certified to work on prescribed burns) working on 7 prescribed burn days during the study period. Twenty-one person-day samples were collected from 8 subjects during 3 non-burn work days. Information about burn size and length of work-shift are provided in Table 3.1.

Five $PM_{2.5}$ samples were excluded from statistical analyses due to random pump failures. An additional $PM_{2.5}$ sample was excluded for gravimetric analyses due to poor O-ring seal and a probable leak, however, real-time $PM_{2.5}$ data was still collected. One CO sample was not collected due to equipment loss in the field.

Burn Day versus Non-Burn Work Day

The unadjusted geometric means by day type (burn or non-burn work day) for TWA gravimetric $PM_{2.5}$ samples, raw (not filter-corrected) real-time $PM_{2.5}$, CO measurements, and absorption coefficients are provided in Table 3.1. For comparison, Table 3.1 also lists unadjusted exposure measurements collected during prior studies conducted at SRS. Data fit to the covariate-adjusted mixed effect model showed geometric mean gravimetric $PM_{2.5}$ concentration depended on day type (p=0.0001). Adjusted gravimetric $PM_{2.5}$ concentrations were significantly lower on non-burn work days compared to burn days (35.1 [95% Confidence Limits: 15.9, 77.3] µg/m^3; 259.4 [156.1, 431.1] µg/m^3, respectively). Likewise, CO concentrations were lower on non-burn work days compared to burn days (p<0.0001; 0.005 [0.002, 0.016] ppm; 0.8 [0.4, 1.8] ppm,

respectively). Adjusted absorption coefficients were lower on non-burn work days compared to burn days (p<0.0001; 0.7 [0.3, 1.8] $\times 10^{-5}$/m; 47.3 [25.7, 87.2] $\times 10^{-5}$/m, respectively). Adjusted mass absorption efficiencies also differed by day type, with non-burn work days significantly lower than burn days (p=0.0001; 0.03 [0.0001, 0.10] $\times 10^{-5} m^2/\mu g$; 0.25 [0.17, 0.35] $\times 10^{-5} m^2/\mu g$, respectively).

Other Covariates

There was no seasonal or burn size effect on geometric mean gravimetric $PM_{2.5}$ concentrations (p=0.8367 and p=0.2629, respectively). In addition, the results indicated that there was no significant temporal correlation in the data (p=0.7202). However, results suggested geometric mean of gravimetric $PM_{2.5}$ depended on sample duration (p=0.0953) (with increasing gravimetric $PM_{2.5}$ concentration with increasing sample duration), which was included in the final mixed model with our main effect of work task. Similarly, the above findings held true when CO and mass absorption efficiency were used as the dependent variable.

Effect of Work Task

To further characterize non-burn work days based on subjects' self-reported exposures, we created two categories: "Non-burn day Exposures" or "Non-burn day Office". "Non-burn day Exposures" included tasks where firefighters reported experiencing likely higher PM exposures such as exhaust from engines or dust during field prep work. "Non-burn day Office" also included one person-day when a firefighter forgot to report his primary work task.

On burn days, subjects' work tasks included holding, lighting while on foot using a drip torch, and lighting by helicopter. Only two firefighters performed ignition by helicopter (two person-days) and were included in the lighting category (Ventilation rates for the two subjects were calculated assuming low activity level tasks). Only four person-days self-reported having

spent 50% of the work-shift holding and 50% lighting. These samples were included in the lighting category since a significant amount of time was spent using a drip torch. Therefore, burn day work tasks performed by the subjects were categorized into two main groups, "Holding" and "Lighting".

Overall, we found that the gravimetric $PM_{2.5}$ concentration depended on work task (p=0.0004). Gravimetric $PM_{2.5}$ concentration was not significantly different between holding and lighting (p=0.1743) (Figure 3.1a). Gravimetric $PM_{2.5}$ concentration for work tasks designated as "Non-burn Day Exposures" was significantly lower compared to non-burn day office work (p=0.0241) (Figure 3.1a).

Filter-corrected real-time peak (average of the 95^{th} percentile) $PM_{2.5}$ concentration depended on work task (p<0.0001), with lower peak exposure on non-burn day compared to burn day tasks (Non-burn day Office: 216 [78, 602] $\mu g/m^3$; Non-burn day Exposures: 106 [41, 270] $\mu g/m^3$; Lighting: 2578 [1389, 4785] $\mu g/m^3$; Holding: 2295 [1115, 4727] $\mu g/m^3$). No statistical difference was observed between lighting and holding (p=0.7038). Interestingly, we found that the peak exposures covering 5% of the sample work-shift length for lighting resulted in an average of 63% (Range: 41-84%) of the total $PM_{2.5}$ exposure during a given work-shift, while it resulted in 45% (Range: 20-77%) of the exposure for holding, 28% (Range: 13-89%) of the non-burn day exposure, and only 21% (Range: 11-32%) of the non-burn day office exposure.

Likewise, CO concentration was dependent upon work task (p<0.0001) and was significantly higher for holding compared to lighting (p=0.0055) (Figure 3.1b). CO exposures did not differ between non-burn day work task exposures and office (p=0.5628) (Figure 3.1b).

Both particle light absorption coefficient and mass absorption efficiency depended on the effect of work task (p<0.0001) (Figures 3.2 and 3.3, respectively). Contrary to $PM_{2.5}$ and CO concentrations, lighting had nearly a three-fold higher mean light absorption coefficient compared

to holding (p=0.0006) (Figure 3.2). No difference was observed between non-burn work day exposures and office tasks (p=0.8291) (Figure 3.2). Lighting had over five times higher mass absorption efficiency compared to holding (p<0.0001) (Figure 3.3). Although the mean mass absorption efficiency was 1.8 times higher for non-burn day exposures compared to non-burn office tasks, the difference was not significant (p=0.2449) (Figure 3.3).

We found a highly significant positive correlation between log-transformed light absorption coefficients and gravimetric $PM_{2.5}$ concentrations (Figure 3.4) (Linear mixed-effect model results: p<0.0001). The positive relationship appears to depend on work tasks with lighting having a higher slope compared to holding (y= –0.7865 + 0.9229x, R^2=0.64, and y= – 0.8810 + 0.7071x, R^2=0.81, respectively) and an apparent clustering of data points according to work task can be seen (Figure 3.4).

Ventilation Rates and Inhaled Dose Estimations

As expected, we found that the ventilation rate depended on work task (p<0.0001) (Figure 3.5). Lighters had 55% higher mean ventilation rate compared to holders (p<0.0001) (Figure 3.5). Non-burn day work tasks involving reported exposures did not have a significantly different mean ventilation rate compared to non-burn day office tasks (p=0.4666) (Figure 3.5). Inhaled $PM_{2.5}$ depended on work task (p=0.0216) (Figures 3.1c). Though not statistically significant, lighters had higher inhaled gravimetric $PM_{2.5}$ compared to holders (p=0.4315) (Figure 3.1c). Likewise, $PM_{2.5}$ dose depended on work task (p=0.0186) (Figure 3.1d). Similar to the estimated amount of inhaled exposure, firefighters lighting appeared to have higher gravimetric $PM_{2.5}$ dose compared to those holding, although this difference was not statistically significant (p=0.4957) (Figure 3.1d).

DISCUSSION

Combustion derived smoke including wood smoke consists of hundreds of chemicals with PM identified as its primary health hazard.[1, 2] Establishing methods to characterize PM exposure and its sources are therefore important to develop suitable occupational health standards. Increased efforts and novel approaches to identify and quantify components of PM, such as BC, are needed to determine associated health effects with respect to varying PM composition. Moreover, physical exertion and pulmonary ventilation rate will impact how much of a pollutant is inhaled by an individual during a given activity.

Results from past studies suggested that work tasks of wildland firefighters during prescribed burns may influence changes in PM constituents.[5, 6] Other non-wood smoke occupational exposures while firefighting, such as exposures to exhaust from fire engines and soot from drip torches, may also significantly contribute to PM exposure.

Results from previous studies conducted at SRS[5, 6, 16] showed overall higher exposures on burn days compared to our current study. Personal exposures collected from wildland firefighters working at prescribed burns in 2011 resulted in unadjusted geometric mean gravimetric $PM_{2.5}$ and CO of 608 (95% CL: 481, 767) $\mu g/m^3$ and 3.9 (95% CL: 3.2, 4.5) ppm respectively (Table 3.1).[6, 16] Furthermore, similar magnitudes of exposure as in 2011 were observed in 2008 and 2009 (Table 3.1).[5] Lower exposure concentrations seen in our current study could be due to shorter burn durations and use of enclosed vehicles to conduct holding operations as opposed to mule trucks that firefighters had used in previous years. In addition, the average burn size during our study was at least 2.5 times smaller.

Although average CO concentrations were higher during holding, average $PM_{2.5}$ concentrations in our study were not significantly different between holding and lighting, as

opposed to what had been observed in previous studies.[4, 6] These findings may be due to the use of fire truck engines to manage fire boundaries during holding as opposed to the unenclosed mule utility vehicles used in previous years.

Several studies have quantified BC using aethalometers[8, 9, 17, 18]; however, few studies have measured LAC contribution to $PM_{2.5}$ by reflectance.[19, 20] Although there are limited comparisons, our study appears to have considerably higher (in some instances 25 times higher) LAC measurements from $PM_{2.5}$ at prescribed burns compared to $PM_{2.5}$ samples that were collected in highly polluted neighborhoods in New York City[19] and in traffic- and wood smoke-impacted cities in Vancouver, British Columbia, Canada[20]. To our knowledge, our study is the first to differentiate PM LAC by work tasks conducted at prescribed burns. The method of measuring LAC on filters enabled us to measure a surrogate of BC in a non-destructive manner. While exposures to $PM_{2.5}$ and CO during lighting were lower compared to holding, it was apparent that BC content was significantly higher on personal air filters from firefighters lighting (Figure 3.2). We also regressed light absorption coefficients against gravimetric $PM_{2.5}$ concentrations to determine if the mass absorption efficiency differed by work task (Figure 3.4). Our results showed higher mass absorption efficiencies, indicative of higher BC content, during lighting (Figure 3.3). Visual differentiation of PM collected on personal air sample filters further confirmed our analytical results; PM on filters collected from firefighters conducting lighting appeared black, while PM from holding appeared brown in color. Although less understood, BC may drive responses seen in toxicological studies. Animal and *in vitro* studies indicate that health effects may be different for particles from different combustion sources and that the amount of BC might be a particularly important indicator of PM's ability to elicit adverse biological effects.[21, 22]

Our estimated ventilation rates accounted for gender, age, and activity levels among work tasks. We also accounted for the effect of load burden on the ventilation rates of the lighters since they use a backpack weighing approximately 11-20 kg (25-45 lbs.) and carry a fuel torch (~6.8 kg when full [15 lbs.]) while hiking during the majority of their work-shift. For firefighters conducting lighting we estimated a mean ventilation rate ± standard error of 29 ± 1.0 L/min. By utilizing the USEPA Exposures Factor Handbook[14] average ventilation rate at the 95th percentile, our method in calculating ventilation rates in this study yielded conservative yet realistic estimates. Our study's estimates are comparable to results in the few studies reporting ventilation rates among subjects performing arduous activities. A study, exploring the effect of carrying a 15 kg (33 lbs.) weighted backpack on lung function during treadmill walking among healthy men, calculated a mean ventilation rate of 38 ± 2.1 L/min at a walking pace of 4.3km/h (2.5 mph) on a treadmill inclined at 10%.[23] Results from an older study assessing the effects of work rate on energy expenditure and physiological responses of firefighters building fire-lines with a rake hoe, showed that raking even at a slow pace resulted in a mean ventilation rate of 41 ± 8 L/min (Range: 24-59 L/min).[24]

Lastly, we observed no significant difference in gravimetric $PM_{2.5}$ exposure concentrations between lighting and holding, although holders had a 1.4 times higher $PM_{2.5}$ exposure concentration compared to lighters; however, after adjusting for ventilation rates, we found that lighters had an estimated 1.3 times higher, though not significant, inhaled amount of gravimetric $PM_{2.5}$ compared to holders. Such differences in inhaled amount of exposure and dose of exposure may have even been further increased if firefighters had worked longer shifts at prescribed burns or had our approach of estimating ventilation rates not been conservative. Furthermore, a significant amount of the total exposure (63% and 45% for lighting and holding, respectively) was attributed to only 5% of a given work-shift on burn days. These results have implications for

mitigation strategies, as reduction in exposure might be achieved through temporary avoidance or use of mask coupled with alarm-activated sensors to alert firefighters of high exposures as suggested in Edwards et al.[25]

Limitations

Additional weight-load while performing lighting and its effect on ventilation rate could not be directly captured in this study, therefore we had to account for it using an assumed effect. Furthermore, no physiological modeling was conducted in the calculation of dose. However, using slope-adjusted linear equations enabled us to estimate credible ventilation rates according to activity level, gender, and age. We believe these estimates to be realistic, albeit conservative, during prescribed burning. Substantial uncertainty may exist in individual-level accelerometry-based estimates of ventilation rates when subjects perform multiple fire operations resulting in alternating body burdens throughout a day. Rodes et al. noted that for high intensity tasks (METS >10), the relationship between accelerometry and ventilation rate can be non-linear, although when and how non-linearities become apparent is still unclear.[10] To better estimate ventilation rates, future studies may consider doing an actual in-field subject calibration of the accelerometer by having subjects, or a subset thereof, perform scripted activities while directly measuring ventilation rate. Additionally, light absorption coefficients measured in the current work could not be converted into units of mass concentration since the conversion factors are source specific and no transformation was available for the PM filters used in our study.

CONCLUSION

Our study is the first to apply accelerometer data to estimate ventilation rates and inhaled $PM_{2.5}$ dose of wildland firefighters. We observed no difference in gravimetric $PM_{2.5}$ exposure concentrations between two primary work tasks conducted at prescribed burns; however, after

adjusting for ventilation rates we found that lighters had an estimated 1.3 times higher, though not significant, inhaled amount of gravimetric $PM_{2.5}$ compared to holders. Even with some uncertainty, the ability to account for ventilation rate to estimate inhaled dose instead of the traditionally used air exposure concentration would result in more accurate assessment of the associations between exposures and biological responses. Future studies looking at PM exposure-health responses may consider adopting similar methods, as adjusting estimated exposures using ventilation data will be widely applicable to many settings. Lastly, our results show that other non-wood smoke occupational exposures while firefighting, such as exposures to exhaust from fire engines and soot from drip torches, may also significantly contribute to PM exposures of firefighters at prescribed burns.

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Table 3.1. Unadjusted Geometric Means for Exposure Monitoring and a Comparison between Previous Exposure Assessment Studies Conducted at Savannah River Site[a]

Exposure	Geometric Mean (95% Lower, Upper CLs)[a]	Minimum	Maximum	N
2015 (Present Study)				
Samples from Burn Days				
Overall Gravimetric $PM_{2.5}$ ($\mu g/m^3$)[b]	240 (179, 321)	11[c]	1859	48
Winter[d]	266 (185, 382)	11[c]	1859	29
Summer[e]	205 (122, 345)	48	1086	19
Overall Raw Real-Time $PM_{2.5}$ ($\mu g/m^3$)[b]	175 (139, 221)	8	787	49
Winter[d]	192 (140, 261)	8	787	30
Summer[e]	153 (105, 222)	33	680	19
Overall CO (ppm)[b]	0.9 (0.6, 1.2)	0.02	9.0	53
Winter[d]	1.2 (0.8, 1.7)	0.04	9.0	33
Summer[e]	0.6 (0.3, 1.1)	0.02	6.8	20
Overall Absorption Coefficient (surrogate for BC) ($10^{-5}m^{-1}$)[b]	46.5 (33.0, 65.5)	1.6	339.2	48
Winter[d]	41.0 (27.5, 61.0)	1.6	127.8	29
Summer[e]	56.4 (29.2, 108.6)	4.5	339.2	19
Overall Mass Absorption Efficiency (BC to $PM_{2.5}$) ($10^{-5}m^2/\mu g$)[b]	0.25 (0.19, 0.31)	0.03	0.87	48
Winter[d]	0.18 (0.13, 0.23)	0.04	0.52	29
Summer[e]	0.36 (0.23, 0.49)	0.03	0.87	19
Overall Burn Size (acres)[b]	280	38	1000	
Winter[d]	350	111	1000	
Summer[e]	161	38	392	
Overall Duration of Work Shift at Fire Line (h)[b]	4.5	1.9	9.3	
Winter[d]	5.3	3.2	9.3	
Summer[e]	3.1	1.9	4.4	
Samples from Non Burn Days[f]				
Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	42 (30, 60)	14[c]	158	21
Raw Real-Time $PM_{2.5}$ ($\mu g/m^3$)	13 (9, 17)	3	31	21
CO (ppm)	0.006 (0.002, 0.014)	0	0.8	21
Absorption Coefficient (surrogate for BC) ($10^{-5}m^{-1}$)	0.8 (0.5, 1.1)	0.1[g]	3.3	21
Mass Absorption Efficiency (BC to $PM_{2.5}$) ($10^{-5}m^2/\mu g$)	0.03 (0.01, 0.04)	0.002	0.15	21
Duration of Work Shift (h)	6.2	3.9	7.8	
2011 (Adetona 2011)[g]				
Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	608 (481, 767)			41
Raw Real-Time $PM_{2.5}$ ($\mu g/m^3$)	920 (779, 1089)			37
CO (ppm)	3.9 (3.2, 4.5)			58
Duration of Work Shift at Fire Line (h)	6.2	1.5	10.2	
2009-2008 Overall (Adetona et al. 2013)[h]				
Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	530 (476, 591)	64	2068	130
CO (ppm)	1.5 (1.3, 1.7)	0.02	8.2	140
Burn Size (acres)	910	80	3300	
Duration of Work Shift at Fire Line (h)	5.5	2	11	
Duration of Work Shift (h)	7.9	3	13	
2003-2005 Overall (Adetona et al. 2011)				
Samples from Burn Day[i]				
Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	264 (221, 316)	5.9	2673	177
CO (ppm)[j]	1.0 (0.09, 11.6)	<1	14	134
Burn Size (acres)	697	1.0	2745	
Duration of Work Shift (h)	10.3	6.8	19.4	
Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	9.3	7	11.5	
Samples from Non Burn Days[k]				
Duration of Work Shift (h)	16 (12, 20)			35

Footnotes for Table 3.1

[a]Arithmetic means for burn size and work shift duration. Arcsine-square root back-transformed values for mass absorption efficiency.

[b]Samples from 4 burn days during winter (January through March) and 3 burn days during summer season (May through July).

[c]The LOD divided by the square-root of two was used to calculate the minimum $PM_{2.5}$ concentration.

[d]Samples from 4 burn days during winter season (January through March).

[e]Samples from 3 burn days during summer season (May through July).

[f]Samples from 3 non burn days during winter (January through March) and summer season (May through July).

[g]Minimum value is LOD divided by the square-root of two.

[h]Samples from 10 burn days collected during winter season (February through March).

[i]Samples from 30 burn days collected during winter seasons (January through March) from 2 years.

[j]Samples from 30 burn days collected during winter seasons (January through March) from 3 years.

[k]CO was measured only on burn days and in 2004 and 2005 alone; CO range concentrations retrieved from Adetona et al. 2013 as cited in.

[l]Samples from 7 non burn days collected during winter seasons (January through March) from 3 years.

[m]$PM_{2.5}$ range concentrations not reported for non burn day samples.

Note: Real-time $PM_{2.5}$ data presented above are raw, not filter corrected. *Abbreviations:* BC, Black Carbon; CLs, Confidence Limits; CO, Carbon Monoxide; h, hours; N, person-day samples; PM, Particulate Matter.

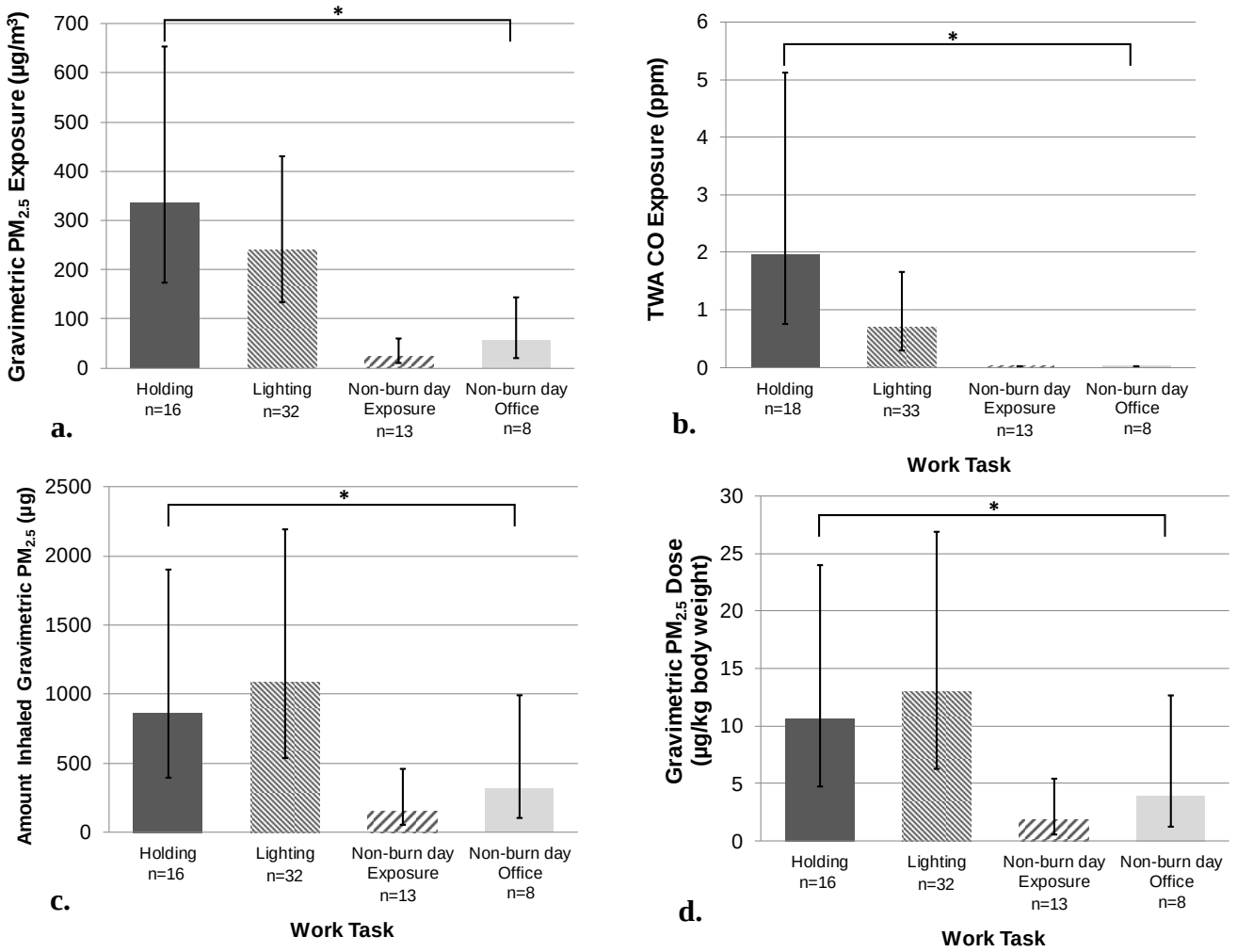

Figure 3.1 (a-d). Adjusted geometric mean derived exposures for gravimetric $PM_{2.5}$ and TWA CO (a-b), and estimated inhaled amount and dose concentrations of gravimetric $PM_{2.5}$ (c-d). Person-days are indicated as *n*. *Overall effect of work task is significant, $p<0.05$. *Abbreviations:* Time-weighted average, TWA.

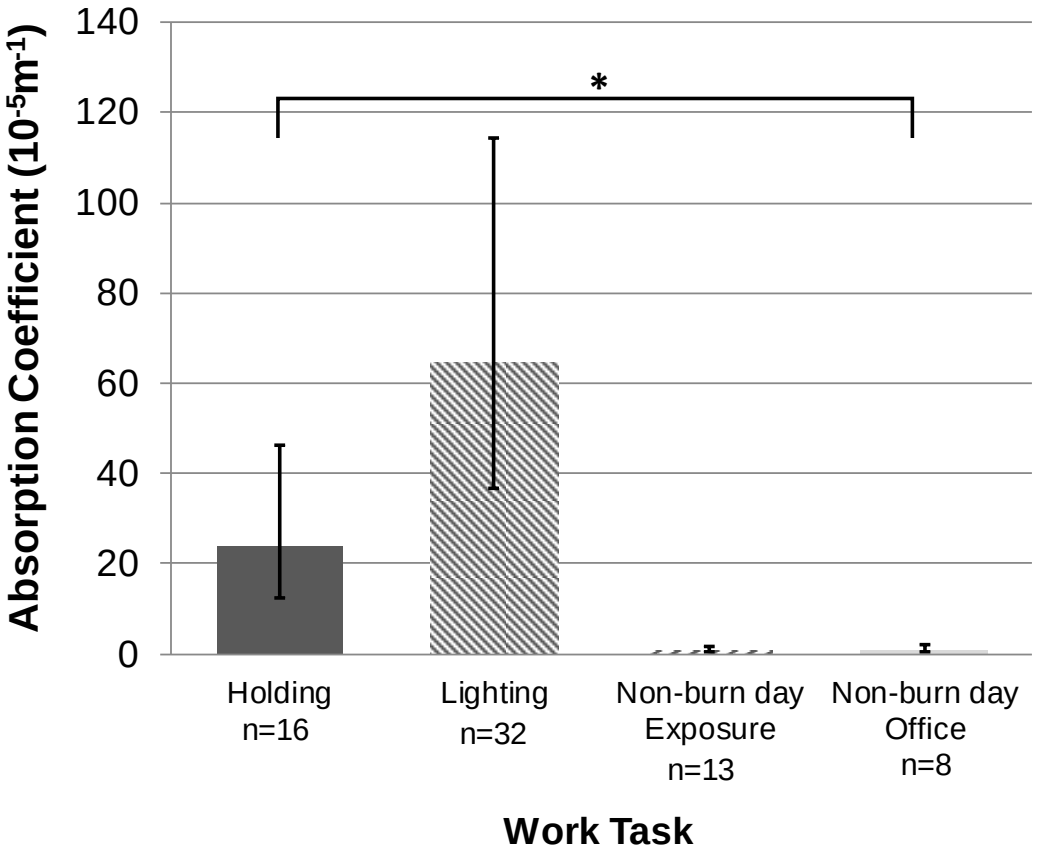

Figure 3.2. Adjusted geometric mean derived light absorption coefficients (surrogate for black carbon) by work task. Person days are indicated as *n*. *Overall effect of work task is significant, $p<0.05$.

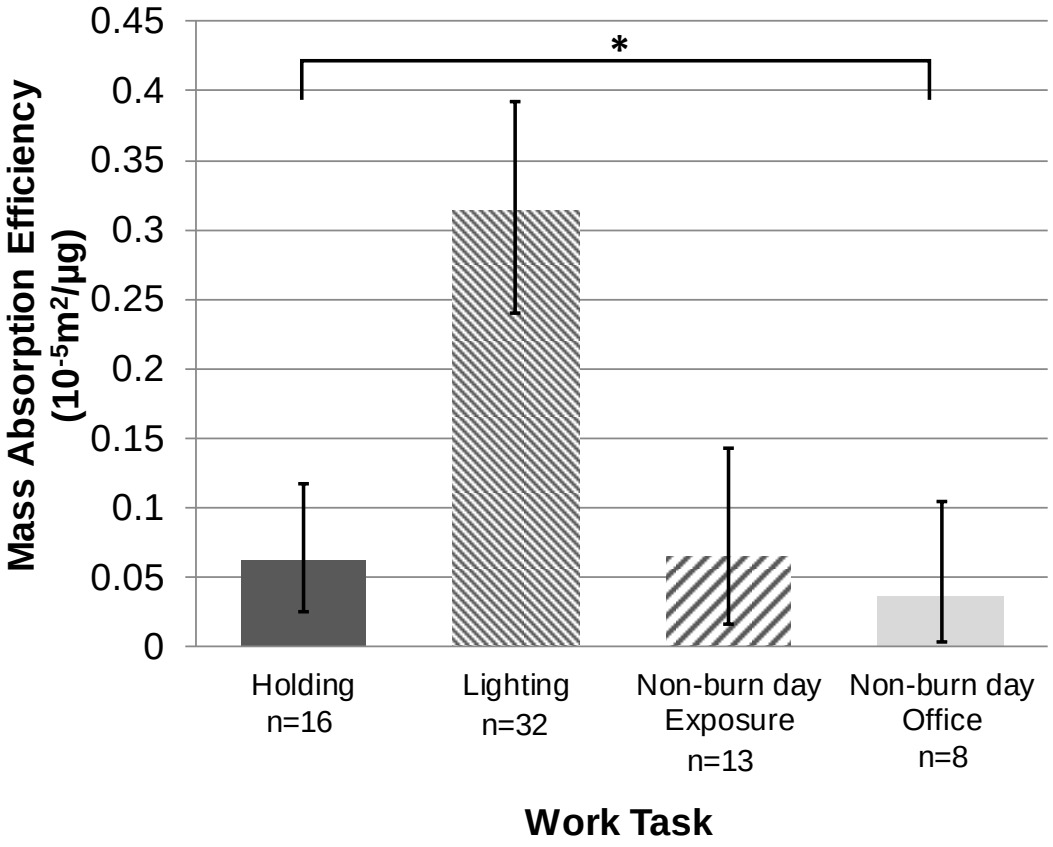

Figure 3.3. Adjusted geometric mean derived mass absorption efficiency (light absorption coefficient [surrogate for black carbon] divided by gravimetric $PM_{2.5}$ concentration) by work task. Person days are indicated as *n*. *Overall effect of work task is significant, $p<0.05$.

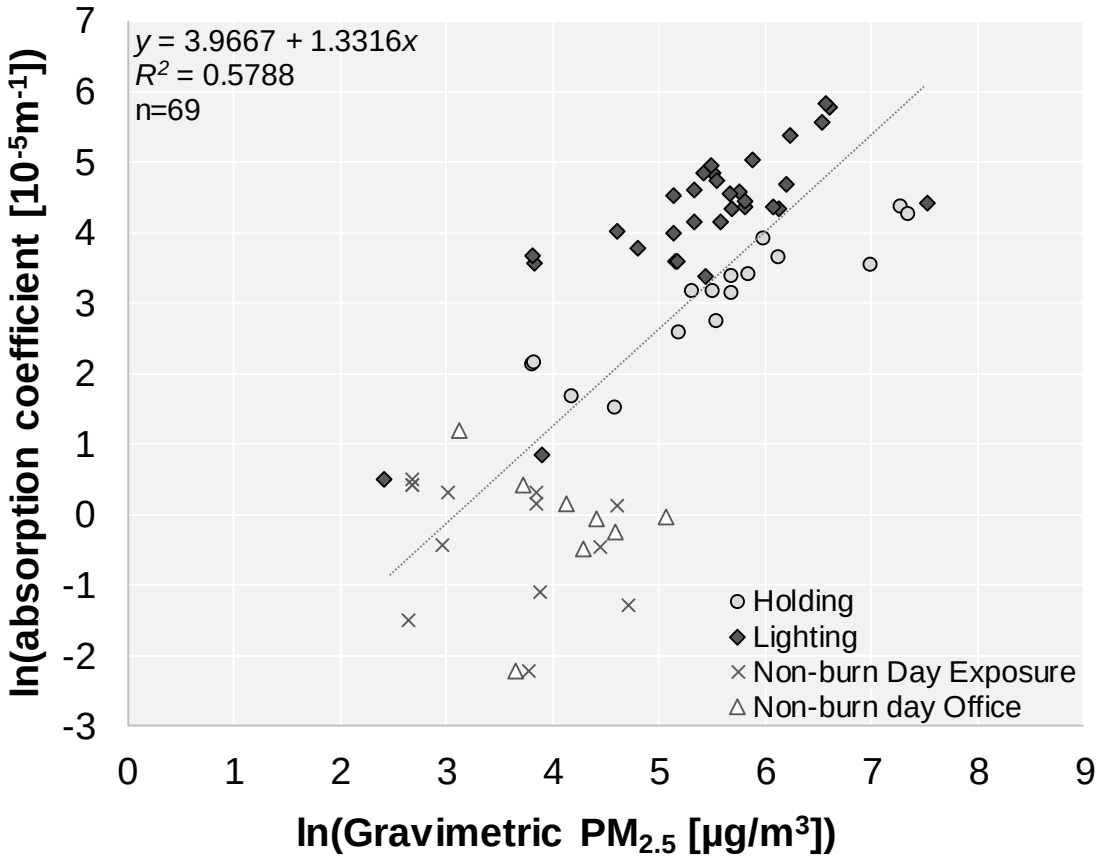

Figure 3.4. Correlation between log-transformed light absorption coefficients (surrogate for black carbon) and log-transformed gravimetric $PM_{2.5}$ concentrations. Person days are indicated as *n*. Linear mixed effect model results showed statistical significant correlation ($p<0.0001$).

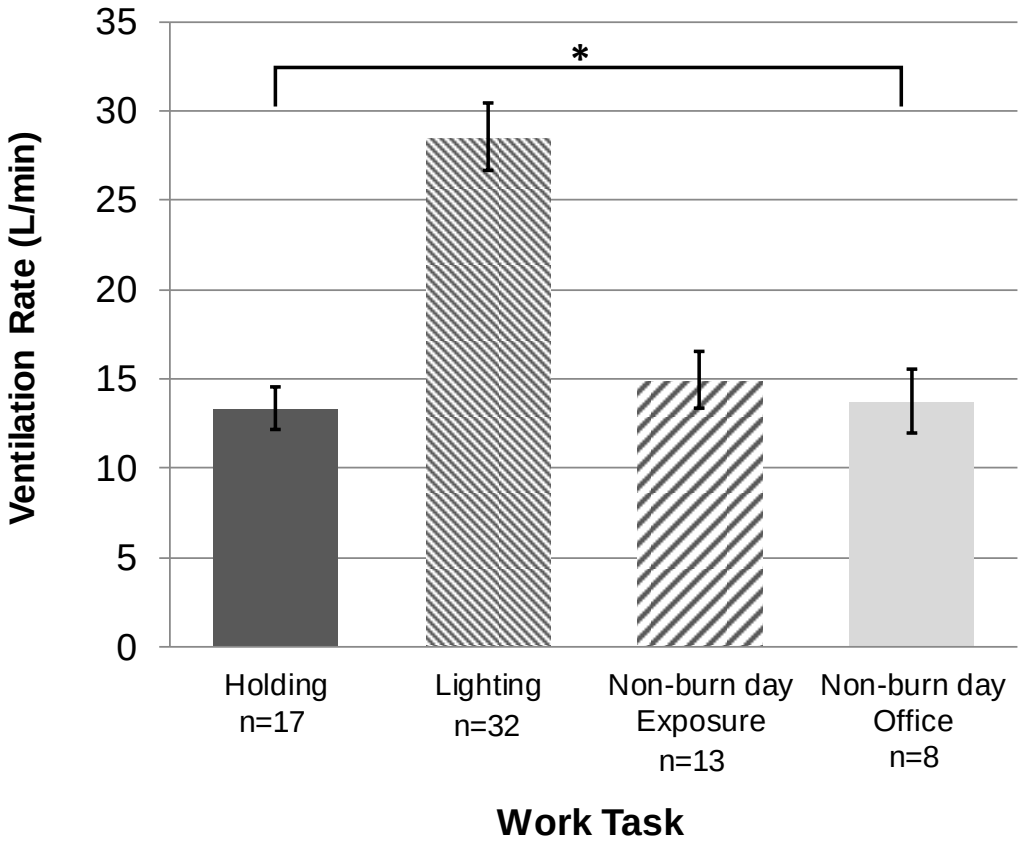

Figure 3.5. Adjusted geometric mean derived estimated ventilation rates by work task. Person days are indicated as *n*. *Overall effect of work task is significant, $p<0.05$.

CHAPTER 4

IMPACT OF WORK TASK-RELATED ACUTE OCCUPATIONAL SMOKE EXPOSURES ON SELECT PRO-INFLAMMATORY IMMUNE PARAMETERS IN WILDLAND FIREFIGHTERS [1]

[1] Adetona AM, Adetona O, Gogal RM, Diaz-Sanchez D, Rathbun SL, & Naeher LP. To be submitted to the *Journal of Occupational and Environmental Medicine*.

ABSTRACT

Objective: A repeated measures study was used to assess the effect of work tasks on select proinflammatory biomarkers in firefighters working at prescribed burns.

Methods: Ten firefighters and two volunteers were monitored for particulate matter and carbon monoxide on workdays, January-July 2015. Before and after work-shift dried blood spots were analyzed for inflammatory mediators using the Meso Scale Discovery assay, while blood smears were used to assess leukocyte parameters.

Results: Firefighters lighting with drip-torches had higher cross-work-shift increases in interleukin-8, C-reactive protein, and serum amyloid A compared to holding, a task involving management of fire boundaries. A positive association between interleukin-8 and segmented-neutrophil was observed.

Conclusion: Results from this study suggest that intermittent occupational diesel exposures contribute to cross-work-shift changes in host systemic innate inflammation as indicated by elevated interleukin-8 levels and peripheral blood segmented-neutrophils.

INTRODUCTION

Wood smoke is composed of hundreds of pollutants, including respirable particulate matter (PM), carbon monoxide (CO), nitrogen and sulfur oxides, aldehydes, polycyclic aromatic hydrocarbons, free radicals, and others, some of which are carcinogenic components (e.g. dioxins and furans).[1] *In vitro*, *in vivo*, clinical and epidemiological studies in humans suggest that various components in wood smoke and smoke emissions from other sources can affect the immune system.[2-7] Acute exposure to particulate matter has been shown to induce a neutrophilic airway inflammatory response resulting in prolonged systemic inflammation in municipal firefighters. [8] Systemic inflammation is also well recognized as an important process in the pathogenesis of cardiovascular and other chronic diseases.[9] After exposure, there is an increase in infiltrating immune cells at the site of insult as wells as shifts in select proinflammatory and anti-inflammatory mediators. For example, elevation in cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) are commonly observed during acute inflammation. After local inflammation ensues, the persistence of the initial antigenic insult can trigger a systemic response, which can be detected by screening inflammatory mediators in the peripheral blood.

A few human studies have reported inflammatory effects of wood smoke exposure, however the specific immune mechanisms associated with these observed health effects are still being explored. In two recent studies, IL-6 and IL-8 were used to assess systemic inflammation in wildland firefighters.[5, 10] These results showed that peripheral blood cytokine levels increased after wood smoke exposure; however, a dose response relationship between specific wood smoke constituents and inflammatory responses has yet to be clearly established.[5, 10]

Results from our previous pilot study suggest that the work tasks performed by wildland firefighters at prescribed burns influence cross-work-shift (pre-work-shift to post-work-shift) changes in the inflammatory biomarker IL-8.[10] Firefighters who lighted fires had the highest IL-8 response even though their PM and CO exposures were significantly lower than firefighters who performed holding, a task that involves maintaining the fire within pre-established boundaries.[10] It is suspected that this finding could be due to other occupational exposures during firefighting such as from drip-torches which are used to light fires and fueled by a 3-part diesel 1-part gasoline mixture. Studies show that acute exposure to diesel exhaust induces increases in IL-8 levels,[11, 12] and previous *in vitro* and animal studies suggest that the type of systemic response observed from particulate exposure may also depend on the type of combustion sources.[3, 4]

The purpose of the current study was to assess the effect of work tasks conducted during prescribed burning on inflammatory biomarkers collected from wildland firefighters, while also addressing some important limitations from our previous work.[10] We prospectively powered our current study based on a primary health endpoint measured from this prior study. We collected before and after work-shift blood samples from firefighters on workdays when prescribed burns were not conducted and were able to account for exercise using pulmonary ventilation rate estimations, as a number of inflammatory biomarkers may increase after rigorous physical activity.[13] In addition, data from our concurrent exposure study[14], regarding personal PM specific light absorbing carbon (used as a surrogate for black carbon [BC], a constituent of incomplete combustion of diesel and biomass burning[15]) was used in this study to better understand how source of exposure (i.e. soot from drip-torches) may influence inflammatory responses in firefighters. Blood was collected to generate blood smears for 5-point leukocyte differentials and dried blood spots to measure inflammatory mediators such as cytokines, acute phase proteins, and

cellular adhesion molecules in peripheral blood samples. Since IL-8 is a neutrophil chemokine, we predicted that blood smear leukocyte differentials would shift favoring an elevation in blood neutrophils. PM exposure samples were collected using a personal aerosol monitor capable of also collecting real-time accelerometry measurements that were used to estimate pulmonary ventilation rates and inhaled dose of PM.

We hypothesized that the effect of work task would be associated with cross-work-shift (before/pre to after/post-work-shift) changes in inflammatory biomarkers, and that cross-work-shift increases in inflammatory responses would be higher in firefighters igniting fires using drip-torches ("lighting") compared to firefighters performing other work tasks. Morning-after work-shift samples were also collected to further characterize the time-course of inflammatory events post exposure. We also predicted that there would be changes in leukocyte populations across work-shifts after prescribed burning. To our knowledge, this is the first study to use blood smears to assess changes in systemic differential leukocyte cell populations following wood smoke exposure. This research is useful for understanding the mechanisms involved in acute inflammatory responses due to such exposures. In addition, internal dose of PM exposure (previously calculated from our concurrent exposure study[14]) and its relationship with inflammation was uniquely explored.

METHODS

Study Population

This study was approved by the University of Georgia Institutional Review Board. Written informed consent was obtained from each subject before participation. A total of twelve healthy subjects, currently non-smokers, not pregnant, and 18 years of age or older, enrolled in the study. This included ten firefighters employed by the United States Forest Service-Savannah River

(USFS-SR), South Carolina and two volunteers certified to work on prescribed burns. Baseline questionnaires were administered along with daily work activity questionnaires to capture information pertaining to personal work history, length of firefighter career, health habits (i.e. exercise frequency, diet), disease history, allergy, medication, food, daily work tasks, and other factors that could be considered influences on exposure and/or on the inflammatory biomarkers of interest.

Study Design

A repeated measures design was used to collect samples from subjects working at prescribed burns and non-burn workdays during January-July 2015. We conducted personal monitoring to measure occupational exposure to particulate matter with an aerodynamic diameter of 2.5 microns and below ($PM_{2.5}$) and CO. During the active prescribed burning season from January to March, personal exposure measurements were obtained from subjects working at four prescribed burn days and two non-burn days. Measurements were also collected on three burn days and one non-burn day from May to July during the summer burn season. Subjects served as their own controls. Dried blood spot samples were collected during each sample day throughout the study period and blood smears were collected on a subset of sampling days (May to July). Work tasks performed by the subjects were categorized into four groups, "Holding", "Lighting", and "Non-burn day—Exposures" and "Non-burn day—Office." On burn days, subjects were employed in work tasks including holding where firefighters used fire engine trucks to monitor and maintain fire boundaries (also referred to as a "Holder"), and lighting either by hand using a drip-torch or by helicopter (both referred to as a "Lighter"). On non-burn workdays, subjects performed various tasks such as, patrolling areas where recent burns were conducted, field prep work, engine maintenance, etc. which were all classified either as non-burn day exposures (when subjects

reported experiencing occupational exposures to vehicle exhaust, diesel exhaust and/or dust) or office work. Work tasks were self-reported by subjects and responses were cross-checked with a technician's field notes. A primary work task was assigned accordingly if the subject had spent more than 50% of the duration of a work-shift conducting the task.

Exposure Monitoring

A detailed description of exposure monitoring methods has been reported previously.[14] In brief, personal gravimetric $PM_{2.5}$ samples were collected in the breathing zone of the subjects during the work-shifts using MicroPEMs (Research Triangle Park, NC, USA). In addition, real-time CO measurements were collected using Drager Pac III single gas monitors outfitted with CO sensors (DragerSafety Inc., Pittsburgh, PA, USA). Time-weighted average (TWA) concentrations were calculated for both $PM_{2.5}$ (reported in $\mu g/m^3$) and CO (reported in ppm). Estimated pulmonary ventilation rates and estimated dose of $PM_{2.5}$ used in this study were previously calculated using subject-specific accelerometry data collected by the MicroPEMs and linear regression models.[14]

Gravimetric Analysis

The gravimetric analytic method has been described elsewhere[14]. Briefly, gravimetric $PM_{2.5}$ samples, which were collected on pre-weighed 25-millimeter polytetrafluoroethylene (PTFE) membrane filters with a porosity of 3.0 micrometers (Pall Life Sciences, Ann Arbor, MI, USA), were weighed using the Cahn C-35 microbalance (sensitivity of ±1.0 μg; Thermo Electron, Waltham, MA, USA) and stored in a -20ºC freezer until further analyses.

Particulate Differentiation

$PM_{2.5}$ on sample filters were analyzed for light absorbing carbon. Analysis was performed by a method described elsewhere in detail.[14] Briefly, the PTFE membrane filters were analyzed for reflectance using the Evans Electroselenium Limited (EEL) smoke stain reflectometer (Model

43D by Diffusion Systems Ltd, London, United Kingdom). Absorption coefficients were determined using an adjusted equation provided in ISO 9835[16].[14] Absorption coefficients are reported in $10^{-5}m^{-1}$. A mass absorption efficiency ($\times 10^{-5}m^2/\mu g$) was also calculated by dividing the absorption coefficients by the $PM_{2.5}$ concentrations to quantify absorption (surrogate for BC) per unit concentration of total $PM_{2.5}$.[14]

Dried Blood Spot Collection and Multiplex Assay Analysis

Whole blood samples were collected from subjects immediately before a work-shift, immediately after a work-shift, and the following morning after a work-shift. Time of blood collection was recorded. A detailed collection protocol was described previously.[10] Briefly, single-use, permanently retracting sterile lancets (BD Genie 366582, 1.5 mm blades by 2.0 mm depth; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used to prick the subject's sterilized finger and drops of whole blood were collected on Whatman 903 Protein Saver Cards (GE Healthcare Life Sciences/Whatman, Piscataway, NJ, USA). Cards were allowed to dry overnight and subsequently packaged in low-gas permeable plastic bags with desiccants (AGM Container Controls, Inc., Tucson, AZ, USA) and humidity indicator cards (3M, Austin, TX, USA). Samples were transported and stored in a -20°C freezer until immunoassays were performed. Dried blood samples were analyzed using the Meso Scale Discovery multiplex assay system to analyze for IL-1β, IL-6, IL-8, TNF-α, and C-reactive protein (CRP), serum amyloid A (SAA), cellular adhesion molecules (inter-cellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) in two separate kits, respectively.[10] IL-1β, IL-6, IL-8, and TNF-α were reported in pg/mL while CRP, SAA, ICAM-1, and VCAM-1 were reported in ng/ml. For all assays both reliability (intra plate variability) and reproducibility (inter plate variability) were tested and

the coefficient of variation was between 5-10%. The above proinflammatory biomarkers are herein referred to as proinflammatory mediators.

Whole Blood Smears and Enumeration

In addition to dried blood spot samples, blood smears were made from finger sticks by placing one drop of whole blood (approximately 10-15μL) on a glass slide to allow for leukocyte differential counts. On few occasions, replicate blood smears were made if an adequate smear was not performed the first time. The slides were allowed to air dry and were stained with Wright Giemsa stain (Sigma-Aldrich, St. Louis, MO, USA). For each slide, 200 leukocytes were manually enumerated for the examination of a 5-point differential (neutrophils-segmented and -band, lymphocytes, monocytes, eosinophils and basophils). Cells were examined in a blinded fashion under 40x magnification using an Olympus CX31 Light Microscope (Olympus Optical Co., LTD) and were expressed as percent (%) out of 200 (unless otherwise noted). Smears were also examined for the presence of any abnormal red blood cell, platelets, or leukocytes. Overall staining quality of the blood smear and distribution of cells was examined at low power (10x magnification) and noted comments were recorded.

Statistical Analyses

All statistical analyses were conducted using Microsoft Excel 2016 or SAS v.9.4 (SAS Institute, Cary, NC, USA). Statistical significance for all analyses was set at $p < 0.05$ and adjusted using the Bonferroni method when doing multiple comparisons. Primary analyses consisted of using linear mixed-effects models to test the effect of work task on changes in proinflammatory mediators and leukocytes.

Initially, descriptive statistical analyses were performed. Histograms, scatter plots, and matrices of scatterplots of the residuals from fitted models were created to assess

heteroskedasticity and normality of the data. Log-transformations were applied to inflammatory mediators, TWA gravimetric $PM_{2.5}$, CO, absorption coefficients, and estimated inhaled dose of $PM_{2.5}$ to normalize these variables. Mass absorption efficiencies were arcsine-square root transformed to achieve normality. All transformed variables were back transformed before reporting results. Under a log-transformation, back-transformed estimates can be interpreted as geometric means. Some proinflammatory mediator concentrations fell below the lower limit of detection (LLOD) (in the case of 33 of 218 [~15%] IL-1β sample concentrations; LLOD: 0.18 pg/mL). The LLOD is reported herein as the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. In such instances, values were replaced with the LLOD divided by the square root of two. All IL-6 and TNF-α concentrations fell below their respective LLODs (0.26 and 0.37 pg/mL, respectively) and therefore were not statistically analyzed. In addition, matrices of scatterplots showed one point-outlier across all inflammatory mediator variables (the outlier was well below the absolute median ± 3σ for all proinflammatory mediators) and therefore was removed from the data before final statistical analyses. Leukocyte cell counts on replicate smears were averaged and the average used as the final value. Percentage of white blood cell types were transformed by taking the log odds of the value (p/1-p) to achieve normality. If zero values were present, a value of 0.025 (half of the lowest observed value above zero percent) was added to the proportion before transformation and the same value subtracted after back transformation.

For the proinflammatory mediators, we tested whether the difference between the means of log transformed post-work-shift and pre-work-shift inflammatory mediators was significantly different from zero, or equivalently when results were back-transformed, that the post- over pre-work-shift ratio was significantly different from one. Cross-work-shift changes are defined herein

as between log post-work-shift minus log pre-work-shift changes (post-work-shift/pre-work-shift ratios). Linear mixed models were fit to test whether there were cross-work-shift changes in percent leukocyte cell type using the differences in log odds values. We tested that the log odds post-work-shift minus the log odds pre-work-shift leukocyte cell type was significantly different from zero, or when results were back transformed, that the odds ratio was significantly different from one. Morning-after minus pre-work-shift paired samples were also compared as outlined above for both proinflammatory mediators and leukocytes.

Work task was used as the primary explanatory variable of interest in all linear mixed models. Non-burn day work tasks were collapsed into one category ("Non-burn day activities") due to small sample sizes in subcategories for cross-work-shift changes in leukocyte cell percentages. Random subject and date effects were included in the models to account for longitudinal within-subject correlation among the data. Other outcome variables were included individually in the linear mixed effect model to test whether they were associated with cross-work-shift differences in proinflammatory biomarkers. These included TWA $PM_{2.5}$ and CO, absorption coefficient, mass absorption efficiency, and estimated inhaled dose of $PM_{2.5}$. We tested for other possible cofounding factors such as gender, years of service as a firefighter, days between blood sample collections and last prescribed burn, age, body mass index (BMI), ventilation rate (surrogate for physical exertion/exercise), medication use, and allergies. Covariates were evaluated based on the forward elimination procedure. Only significant covariates were included in the final model. We also explored the relationships between proinflammatory mediators and leukocyte cell type, as we were specifically interested in assessing the relationship between IL-8 and segmented-neutrophils. In addition, we explored the possibility of a dose-response relationship between cross-work-shift proinflammatory biomarker changes and estimated dose of $PM_{2.5}$.

RESULTS

Descriptive Characteristics

A total of twelve subjects (nine men and three women) enrolled in the study. No subject reported using any form of respiratory protection during the study. Four (33%) of the subjects reported having allergies and four (33%) reported quitting smoking although several years prior. The mean age of the participating subjects was 33 ± 5.4 years, with a range from 26 to 43 years. The average BMI was 27.0 ± 4.8 kg/m^2. The average acres burned per day were 280 (Range: 38-1000 acres) and the length of work-shifts on prescribed burn days averaged 4.5 hr (Range: 1.9-9.4 hr). Non-burn day work-shifts averaged 6.2 hr (Range: 3.9-7.8 hr). Subjects were classified by years of experience as a wildland firefighter while the two volunteers, although certified to work on prescribed burns were non-career volunteer firefighters (<1 year of experience). Career firefighters reported 3.25 to 22 years of experience as wildland firefighters. Days between blood sample collection and last prescribed burn ranged from 1 to over 30 days.

A total of fifty-four paired dried blood spot cross-work-shift (pre- and post- work-shift) samples were collected from the twelve subjects on seven prescribed burn days during the study. Twenty-one paired dried blood spot cross-work-shift (pre- and post- work-shift) samples were collected from eight subjects during three non-burn workdays throughout the study period. Additionally, twenty-person day blood smear samples were collected from ten subjects (2 women, 8 men) on three burn days and eight blood smear samples were collected on one non-burn workday from each of the eight subjects (1 woman, 7 men). During the study, some subjects did not report to work the following day, which resulted in fewer morning-after work-shift samples. Unadjusted means of pre-, post-, and morning-after-work-shift inflammatory mediator concentrations and 5-

point differential leukocyte cells expressed as percentages collected on burn and non-burn workdays are presented in Tables 4.1 and 4.2, respectively.

Work Task Exposures

Adjusted geometric means of gravimetric $PM_{2.5}$ and TWA CO concentrations and 95% confidence limits (95% CL), respectively, by work tasks were 338 (174, 654) $\mu g/m^3$ and 2.0 (0.7, 5.1) ppm for holding, 240 (134, 430) $\mu g/m^3$ and 0.7 (0.3, 1.7) ppm for lighting, 24 (10, 61) $\mu g/m^3$ and 0.004 (0.001, 0.016) ppm for non-burn workday self-reported exposures, and 55 (21, 144) $\mu g/m^3$ and 0.003 (0.001, 0.012) ppm for non-burn workday office.[14] While $PM_{2.5}$ exposure concentrations did not differ significantly between holding and lighting (p=0.1743), there was marginally significant evidence that firefighters conducting lighting had 36% higher inhaled total $PM_{2.5}$ compared to when they were holding (p=0.0751; 1310 [561, 3054] µg; 841 [344, 2054] µg, respectively).[14] Lighting had nearly a three-fold higher mean absorption coefficient (surrogate for black carbon) compared to holding (p=0.0005; 60.7 [34.5, 107.0] $\times 10^{-5} m^{-1}$; 22.3 [11.6, 43.1] $\times 10^{-5} m^{-1}$, respectively).[14] No difference was observed between non-burn day exposures and office tasks (p=0.9807; 0.9 [0.4, 2.3] $\times 10^{-5} m^{-1}$, and 0.9 [0.4, 2.4] $\times 10^{-5} m^{-1}$, respectively) on non-burn days.[14] Lighting had over five times higher mean mass absorption efficiency compared to holding (p<0.0001; 0.32 [0.24, 0.40] $\times 10^{-5} m^2/\mu g$, and 0.06 [0.02, 0.12] $\times 10^{-5} m^2/\mu g$, respectively), while the mean mass absorption efficiency was 1.8 times higher for non-burn workday exposures compared to non-burn office tasks, although the difference was not statistically significant (p=0.2034; 0.07 [0.02, 0.15] $\times 10^{-5} m^2/\mu g$, and 0.4 [0.004, 0.11] $\times 10^{-5} m^2/\mu g$, respectively).[14]

Proinflammatory Mediators

Adjusted cross-work-shift (pre to post) changes of proinflammatory mediators according to work tasks performed on burn and non-burn workdays are presented in Figure 1a. Likewise,

adjusted pre to morning-after (pre to MA) cross-work-shift differences of proinflammatory mediators according to work tasks are presented in Figure 4.1b.

Overall, we found the IL-8 cross-work-shift (pre to post) changes depended on work tasks conducted on both non-burn and burn days (p=0.0004). As anticipated, IL-8 cross-work-shift (pre to post) changes were significantly different between firefighters lighting compared to those who were holding (p<0.0001), with lighters having over 1.3 times higher mean cross-work-shift increase in IL-8 compared to holders (1.36 [1.25, 1.47] and 1.02 [0.92, 1.14], respectively) (Figure 4.1a). Although not statistically different (p=0.1737), lighters had observably higher IL-8 mean cross-work-shift (pre to post) increase compared to non-burn day activities that included self-reported exposures (Figure 4.1a). In addition to IL-8, we found that SAA cross-work-shift (pre to post) changes marginally depended on work task (p=0.0611). Lighters had a SAA cross-work-shift (pre to post) increase that was 1.3 times that of the holders (p=0.0074) (Figure 4.1a). Although no overall work task effect was seen in CRP cross-work-shift (pre to post) changes (p=0.1036), lighters had 1.1 times higher CRP cross-work-shift (pre to post) increase compared to holders (p=0.0153) (Figure 4.1a). We observed the highest cross-work-shift (pre to post) increase in IL-1β for non-burn day exposure tasks compared to all other work tasks (p=0.0487) (Figure 4.1a). Lastly, we observed no significant cross-work-shift (pre to post) differences between work tasks in ICAM-1 or VCAM-1.

We also found that the adjusted geometric mean of CRP pre to morning-after work-shift changes depended on work tasks (p=0.0009) with lighters having a 1.6 times higher work-shift increase compared to holders (Figure 4.1b). In addition, corresponding increase in SAA appeared to depend on work tasks (p=0.0403; Bonferroni adjusted p-value < 0.0167); pre to morning-after work-shift increases were 1.4 times higher for lighters compared to holders (Figure 4.1b).

However, we did not find an overall effect of work task on pre- to morning-after work-shift changes in the remaining proinflammatory mediators.

5-point Leukocyte Differentials

The unadjusted arithmetic 5-point differential percentages of white blood cell type are presented for non-career (volunteer) and career firefighters across the sampling times in Figures 4.2a and b, respectively. Reference values[17] are also provided. Interestingly, eosinophils were elevated in several career wildland firefighters who had no reported history of allergies (Figure 4.2b) whereas the two career firefighter subjects with reported seasonal allergies had eosinophil levels within the human population reference range.

Adjusted logit-transformed mean cross-work-shift (pre to post) leukocyte changes by work tasks are provided in Figure 4.3a. Although not statistically significant (*p*-values not reported herein), results indicate that firefighters who conducted lighting had cross-work-shift increase in segmented neutrophils and decrease in lymphocytes compared to other work tasks (Figure 4.3a). Figure 4.3b visually depicts the pre- to morning-after work-shift changes according to work tasks while controlling for medication use.

Additionally, we found that adjusted logit-transformed mean cross-work (pre to post) shift changes in band neutrophil cells appeared to be higher on burn day tasks compared to non-burn day (*p*-values=0.0810). All other adjusted mean cross-work-shift changes in the reaming leukocyte cell types were not statistically different (data not shown).

Other Covariates and Significant Correlations

A significant effect of medication use (use within prior 24 hours) on pre to morning-after cross-work-shift changes in segmented neutrophils and lymphocytes was found (*p*=0.0044), with those who used medication having 1.8 times lower pre to morning-after cross-work-shift increases

in segmented-neutrophils compared to those who did not take medication (1.07 [0.87, 1.31] n=8, and 0.60 [0.45, 0.81], n=17, respectively). A reciprocal relationship was found between medication use and pre to morning-after cross-work-shift changes in lymphocytes (Medication: 1.70 [1.13, 2.55]; and No medication: 0.92 [0.67, 1.27]). The remaining tested covariates had no significant confounding effects (i.e. gender, years of service as a firefighter, days between blood sample collections and last prescribed burns, age, body mass index (BMI), ventilation rate [surrogate for physical exertion/exercise], and allergies) (results not presented herein).

We found a statistically significant positive correlation between IL-8 and segmented neutrophils (p=0.0179) (Figure 4.4). Additionally, a statistically significant positive correlation was observed between log-transformed cross-work (pre to post) shift changes in IL-8 and arcsine-square root transformed mass absorption efficiency (p=0.0080). Figure 4.5 depicts a scatter plot of the exposure-response relationship, using IL-8 as a representative proinflammatory mediator. Likewise, log-transformed cross-work (pre to post) shift changes in CRP and SAA had significant positive relationships with arcsine-square root transformed mass absorption efficiency (p=0.0574; and p=0.0312, respectively). No significant relationships were found between cross-work-shift (pre to post; and pre to morning-after) changes in proinflammatory biomarkers and the remaining outcomes variables (results not shown).

DISCUSSION

Concurrent exposure to wood smoke and diesel particles may be seen at prescribed burns where firefighters perform work tasks involving ignition of understory growth using diesel-gasoline fueled drip-torches. Wood smoke exposure by inhalation is thought to induce systemic inflammation through various cascading biological pathways involving oxidative stress in the airways and the induction of a local inflammatory response, which if prolonged can lead to

systemic changes measured in the vasculature.[1] Primary soluble inflammatory mediators such as cytokines and chemokines can be released during an innate response and can thereby induce other immune mediators and trigger activation, proliferation, differentiation, or migration of leukocytes. Specifically, IL-8 is involved in the recruitment of neutrophils to the site of insult.[18] During response to an acute insult (i.e. the lung), neutrophils demarginate in the blood stream and increase in number. Diesel exposures can illicit similar responses.[11, 12] Moreover, previous animal and human chamber studies have reported significant increased levels of CRP and ICAM-1 following diesel exhaust particle exposure.[19]

Results from our previous pilot study suggest that wildland firefighter work task influences cross-work-shift increases in inflammatory biomarker, IL-8.[10] Firefighters who lighted fires had the highest IL-8 response even though their PM exposures were significantly lower than firefighters who performed holding a task involving the maintenance of fire within pre-established boundaries.[10] Acute phase protein, CRP, was also found to be elevated in more than 75% of cross-work (pre to post) shift blood samples. We proposed that the impact of different smoke constituents (i.e. wood smoke vs. diesel exposure) could influence proinflammatory responses seen in the wildland firefighters. In 2015, we set out to investigate the effect of work tasks on select proinflammatory markers in firefighters working at prescribed burns.

We found that lighters had the highest IL-8 cross-work-shift (pre to post) response compared to other work tasks performed on burn and non-burn workdays. These results are consistent with our previous findings in 2011 showing that IL-8 cross-work-shift (pre to post) differences were significantly higher in lighters compared to holders ($p<0.0001$ [current study], and $p=0.0122$ [Hejl et al.][10], respectively). The lower cross-work-shift (pre to post) changes in IL-8 may be due to lower exposures and shorter work-shifts in the current study compared to the 2011

study (1.36 [1.25, 1.47]; *n*=34 [current study], and 1.70 [1.35, 2.13]; *n*=13 [Hejl et al.][10]). Personal exposures to $PM_{2.5}$ and CO of the wildland firefighters working at prescribed burns during the 2011 study were 2.5 and 4.3 times higher than those observed in the current study, respectively (2011 study: 650 [510, 828] $\mu g/m^3$; *n*=22, and 3.6 [2.6, 5.0] ppm; *n*= 23, from four burn days, respectively).[10] Work-shift hours at the fire-line (during active prescribe burning) in our current study[14] averaged 4.5 hours as opposed to 6.2 hours in 2011[10].

We found that firefighters that served as lighters had the highest SAA and CRP cross-work-shift (pre to post) changes compared to firefighters holding. The even greater increase in SAA and CRP from pre- to morning-after work-shift changes suggests there was a delay in select proinflammatory responses post work task related exposures. This is explainable as both CRP and SAA acute phase proteins are produced downstream of the release of cytokines and thus, likely to undergo a delayed systemic release.[20] In contrast, IL-8 immune response morning-after concentrations appeared to return to pre-work-shift blood levels as indicated by the pre-work-shift/morning-after work-shift ratio of 1.00 (0.92, 1.10).

The observed strong correlation between IL-8 and peripheral blood segmented neutrophils would suggest that acute diesel and wood smoke exposure evoke systemic innate inflammation in the host. Although we did not find significant correlations between inhaled dose of $PM_{2.5}$ exposure and cross-work-shift changes in the proinflammatory biomarkers, we found a positive significant correlation between IL-8 and mass absorption efficiency (Figure 4.5). Similarly, strong positive correlations of mass absorption efficiency were also seen with CRP and SAA, respectively. Such findings suggest that intermittent diesel soot exposures may contribute to more pronounced cross-work-shift changes in host systemic inflammation as indicated by elevated IL-8 levels and segmented-neutrophil number. Additionally, increased band neutrophil cell populations seen in

firefighters conducting burn day work-related tasks (holding and lighting) as compared to collective non-burn work tasks is suggestive of a systemic effect of occupational exposures to smoke emissions. Band neutrophils are immature segmented-neutrophils in that they are an intermediary step prior to complete maturation of segmented-neutrophils. An increase in band neutrophils can often indicate that the bone marrow has been signaled to release more leukocytes during an insult.[18]

Interestingly, we found a significant increase in IL-8 cross-work-shift (pre to post) for non-burn day work tasks involving occupational exposures related to combustion smoke. These results indicate that some occupational exposures on non-burn days might also elicit an immune response. Work tasks involving fire engine maintenance and field prep work using bulldozers is often performed on such days. Although there was an anomalous result regarding cross-work-shift changes in IL-1β with lower concentrations for holders and lighters compared to non-burn day exposures, results from *in vitro* studies suggest that IL-1β may not be involved in the pathway for the inflammatory response induced by combustion derived smoke particles.[4, 21]

In the present study, the leukocyte cell differentials of the study participants were within the reference range of the general population, however there appeared to be a numerically higher neutrophil number in our study population compared to the general population average.[17] Additionally, eosinophil counts were numerically higher for several career wildland firefighter subjects with no known history of allergies or parasitic infections. Immune functional assays from future firefighter blood samples could be employed to evaluate their sensitivity to the different burn products. For instance, radioallergosorbent (RAS) tests could be used to measure changes in Immunoglobulin E (IgE) levels to select allergenic antigen profiles as a result of diesel and/or wood smoke exposure. Non-career volunteer firefighters had a greater increase in neutrophils on

burn days compared to career wildland firefighters, indicating naivety of their immune system to the exposures. However, we did not see a significant firefighter career length effect on cross-shift changes in neutrophils or IL-8 in our linear mixed effect models, possibly due to the small sample size of this study.

Our study's findings are consistent with results from previous studies looking at the effects of smoke (diesel/wood smoke) emission components on systemic inflammation. One study on seasonal forest firefighters showed significant cross-work (pre to post) shift increases in serum IL-8 on days when the firefighters worked at wildfires compared to days when they did not (Estimated $PM_{3.5}$ exposures: peak levels of 2.8 mg/m^3 and 6-hours of levels less than 1 mg/m^3 during a given work-shift).[5] A human chamber study found that healthy volunteers had increased neutrophils in peripheral blood samples after only 1-hour exposure to diluted vehicle-produced diesel particles (exposure standardized by maintaining PM_{10} concentration at 300 $\mu g/m^3$).[19] Additionally, various *in vivo* models have shown that exposure to wood smoke particles induces airway inflammation characterized by an increase in cytokines and infiltration of immune cells, especially neutrophils.[22-25] A clinical study that exposed young healthy individuals to wood smoke particles saw an increase in systemic neutrophils.[6] Along with varying combustion conditions, such combined wood smoke and diesel particles may act additively or perhaps even synergistically in their overall toxic effect.[26]

The concentrations for IL-6 and TNF-α were observed to be below their respective LLODs. It is possible that the sensitivity of the multiplex instrument may have been a factor. For future studies, additional blood volume may be needed to measure these cytokines, as another study found significant changes in serum blood levels of IL-6 in firefighters after wood smoke exposure.[5]

Limitations

Although sample size was small, firefighters served as their own controls in this study improving the ability to detect differences. However, sample size may be increased, and control non-exposed subjects may be included in future studies for comparisons. We were also unable to capture time spent (i.e. > 50% of the day) during a specific work task on non-burn workdays in this current study resulting in possible exposure misclassification on non-burn days. This study primarily assessed the effect of acute occupational exposures on firefighter health. Future studies are needed to evaluate the chronic effects of occupational exposures among wildland firefighters.

CONCLUSION

Healthy seasonal wildland firefighters conducting lighting at prescribed burns had significantly higher cross-work-shift changes in three proinflammatory mediators—IL-8, CRP, and SAA—compared to holding. No significant correlation was found between internal dose of $PM_{2.5}$ exposure and inflammatory biomarkers; however, we did observe a significant exposure-response relationship between a surrogate black carbon measure and inflammation. To our knowledge, our study is the first to include blood smears to assess changes in differential white blood cell counts following occupational smoke exposure. This technique may serve as a useful and cost-effective supportive tool for the assessment of acute inflammatory responses due to related occupational smoke exposures. In conclusion, results from this study suggest that intermittent diesel exposures may contribute to more pronounced cross-work-shift changes in systemic inflammation among wildland firefighters during prescribed burns as indicated by elevated IL-8 levels and peripheral blood segmented-neutrophil number.

Disclaimer

The article has been reviewed and approved for publication by the U.S. Environmental Protection Agency. Approval does not signify that the contents necessarily reflect the views and policies of the Agency.

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Table 4.1. Unadjusted Means of Proinflammatory Mediator Concentrations by Day Type and Time of Sample Collection.

Day Type	Time of Sample	Avg Time	Proinflammatory Mediators					
			IL-1b (pg/mL)	IL-8 (pg/mL)	CRP (ng/mL)	SAA (ng/mL)	ICAM-1 (ng/mL)	VCAM-1 (ng/mL)
Burn	**Pre-Work Shift (n=54)**	08:14						
	Arithmetic Means (95% CL)		1.72 (1.12, 2.32)	2.84 (2.58, 3.11)	24.00 (15.75, 32.25)	38.13 (25.37, 50.88)	6.42 (5.33, 6.02)	11.14 (10.44, 11.83)
	Geometric Means (95% CL)		0.89 (0.65, 1.23)	2.72 (2.50, 2.95)	12.13 (8.87, 16.59)	22.27 (16.74, 29.63)	6.21 (5.79, 6.66)	10.85 (10.18, 11.56)
	Ranges		(0.13, 11.03)	(1.42, 7.67)	(2.15, 129.71)	(3.05, 260.99)	(3.97, 11.67)	(6.52, 16.52)
	Post-Work Shift (n=54)	15:35						
	Arithmetic Means (95% CL)		1.30 (0.75, 1.85)	3.51 (3.11, 3.92)	24.53 (15.66, 33.41)	39.97 (25.76, 54.18)	6.76 (6.22, 7.30)	11.55 (10.86, 12.24)
	Geometric Means (95% CL)		0.60 (0.43, 0.84)	3.09 (2.55, 3.74)	12.20 (8.90, 16.72)	22.33 (16.51, 30.19)	6.48 (5.98, 7.02)	11.27 (10.59, 11.99)
	Ranges		(0.13, 12.68)	(0.04, 9.18)	(2.07, 159.72)	(2.38, 300.67)	(2.49, 12.15)	(6.40, 17.49)
	Morning-After (n=50)	08:27						
	Arithmetic Means (95% CL)		1.21 (0.68, 1.74)	2.83 (2.57, 3.09)	26.70 (18.06, 35.34)	37.52 (26.68, 48.36)	6.37 (5.82, 6.92)	10.73 (10.05, 11.41)
	Geometric Means (95% CL)		0.65 (0.48, 0.89)	2.68 (2.42, 2.96)	14.39 (10.43, 19.85)	23.49 (17.55, 31.45)	6.14 (5.69, 6.62)	10.46 (9.80, 11.17)
	Ranges		(0.13, 12.20)	(0.59, 6.20)	(1.87, 111.86)	(3.58, 190.73)	(3.83, 12.77)	(6.76, 15.53)
Non-burn	**Pre-Work Shift (n=21)**	09:14						
	Arithmetic Means (95% CL)		0.79 (0.47, 1.12)	2.72 (2.46, 2.97)	19.09 (8.49, 29.69)	27.24 (16.48, 38.00)	6.25 (5.44, 7.07)	10.75 (9.69, 11.80)
	Geometric Means (95% CL)		0.55 (0.36, 0.84)	2.66 (2.42, 2.93)	10.02 (5.99, 16.76)	17.87 (11.40, 28.02)	6.04 (5.34, 6.82)	10.51 (9.51, 11.61)
	Ranges		(0.10, 2.99)	(1.89, 3.74)	(2.60, 75.28)	(3.32, 78.64)	(3.83, 10.66)	(7.22, 15.10)
	Post-Work Shift (n=21)	16:26						
	Arithmetic Means (95% CL)		1.24 (0.68, 1.81)	3.11 (2.84, 3.38)	19.45 (8.90, 30.00)	28.48 (17.48, 39.48)	6.69 (5.89, 7.49)	11.50 (10.39, 12.61)
	Geometric Means (95% CL)		0.73 (0.43, 1.23)	3.06 (2.81, 3.33)	10.36 (6.21, 17.30)	18.68 (11.85, 29.45)	6.50 (5.83, 7.24)	11.25 (10.21, 12.41)
	Ranges		(0.13, 4.36)	(2.23, 4.47)	(2.46, 75.40)	(3.18, 76.18)	(4.50, 11.06)	(7.91, 15.86)
	Morning-After (n=18)	09:15						
	Arithmetic Means (95% CL)		0.94 (0.41, 1.46)	2.66 (2.33, 2.99)	24.70 (12.02, 37.38)	49.41 (21.56, 77.26)	6.41 (5.51, 7.31)	10.28 (9.23, 11.33)
	Geometric Means (95% CL)		0.58 (0.36, 0.95)	2.59 (2.30, 2.91)	13.63 (7.60, 24.42)	25.26 (13.62, 46.84)	6.20 (5.44, 7.06)	10.08 (9.11, 11.16)
	Ranges		(0.13, 4.10)	(1.70, 4.46)	(2.53, 78.58)	(4.37, 174.49)	(4.09, 10.56)	(7.35, 13.94)

Abbreviations: n, person-day samples; 95% CL, lower and upper confidence levels. Note: Samples were collected on seven burn days and three non-burn days.

Table 4.2. Unadjusted Arithmetic Means of Five Point Differential Percent of White Blood Cells by Day Type and Time of Sample Collection.

Day Type	Time of Sample	Avg Time	% Neutrophils-Segmented	% Neutrophils-Band	% Lymphocytes	% Monocytes	% Eosinophils	% Basophils
			Avg ± SEM	Avg ± SEM	Avg ± SEM	Avg ± SEM	Avg ± SEM	Avg ± SEM
Burn	*Pre-Work Shift (n=20)*	*07:57*	47 ± 3	0.2 ± 0.1	41 ± 3	7 ± 0.5	4 ± 1	1 ± 0.2
	Post-Work Shift (n=20)	*14:19*	57 ± 2	1 ± 0.1	33 ± 2	6 ± 1	3 ± 1	1 ± 0.3
	Morning After (n=18)	*08:44*	46 ± 2	0.3 ± 0.1	42 ± 2	7 ± 1	4 ± 1	1 ± 0.2
Non-Burn	*Pre-Work Shift (n=8)*	*09:07*	44 ± 5	0.3 ± 0.2	44 ± 5	7 ± 1	3 ± 1	1 ± 0.2
	Post-Work Shift (n=8)	*16:04*	48 ± 4	0.1 ± 0.1	42 ± 4	6 ± 1	3 ± 1	1 ± 0.1
	Morning After (n=7)	*09:37*	44 ± 5	0.3 ± 0.2	45 ± 6	7 ± 1	3 ± 1	1 ± 0.2

Abbreviations: Avg, average; SEM, standard error of the mean; n, person days; Note: Samples were collected on 3 burn days and 1 non-burn day.

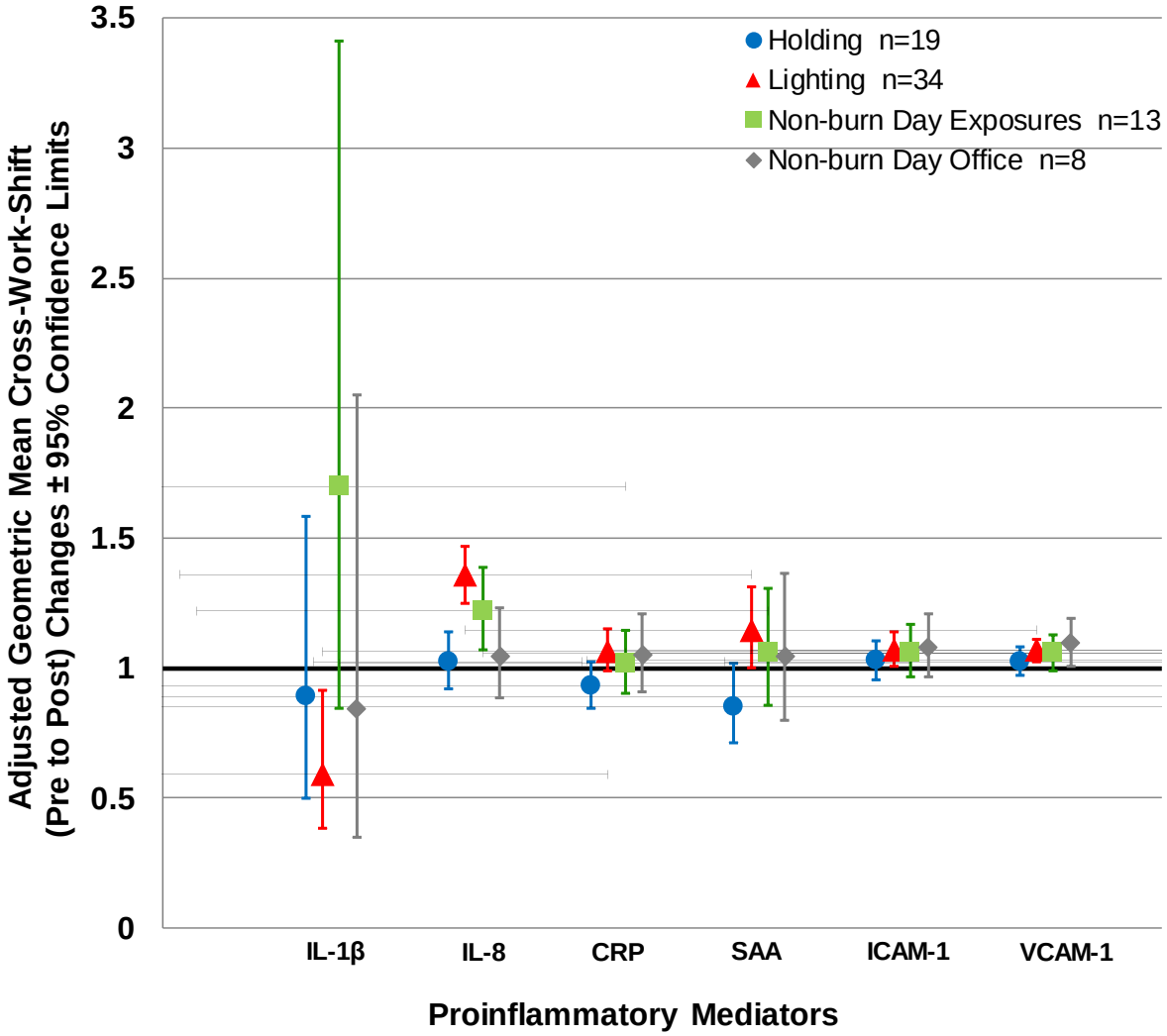

Figure 4.1 (a). Adjusted Geometric Mean Cross-work-shift (Pre to Post) Changes in Proinflammatory Mediators according to Work Task. Note: *n*= person-day pre-post paired samples; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios. Where 95% confidence limits do not cross the x-axis, proinflammatory mediator cross-work-shift changes are statistically different from 1 (*p*-values < 0.05, specific *p*-values not reported herein).

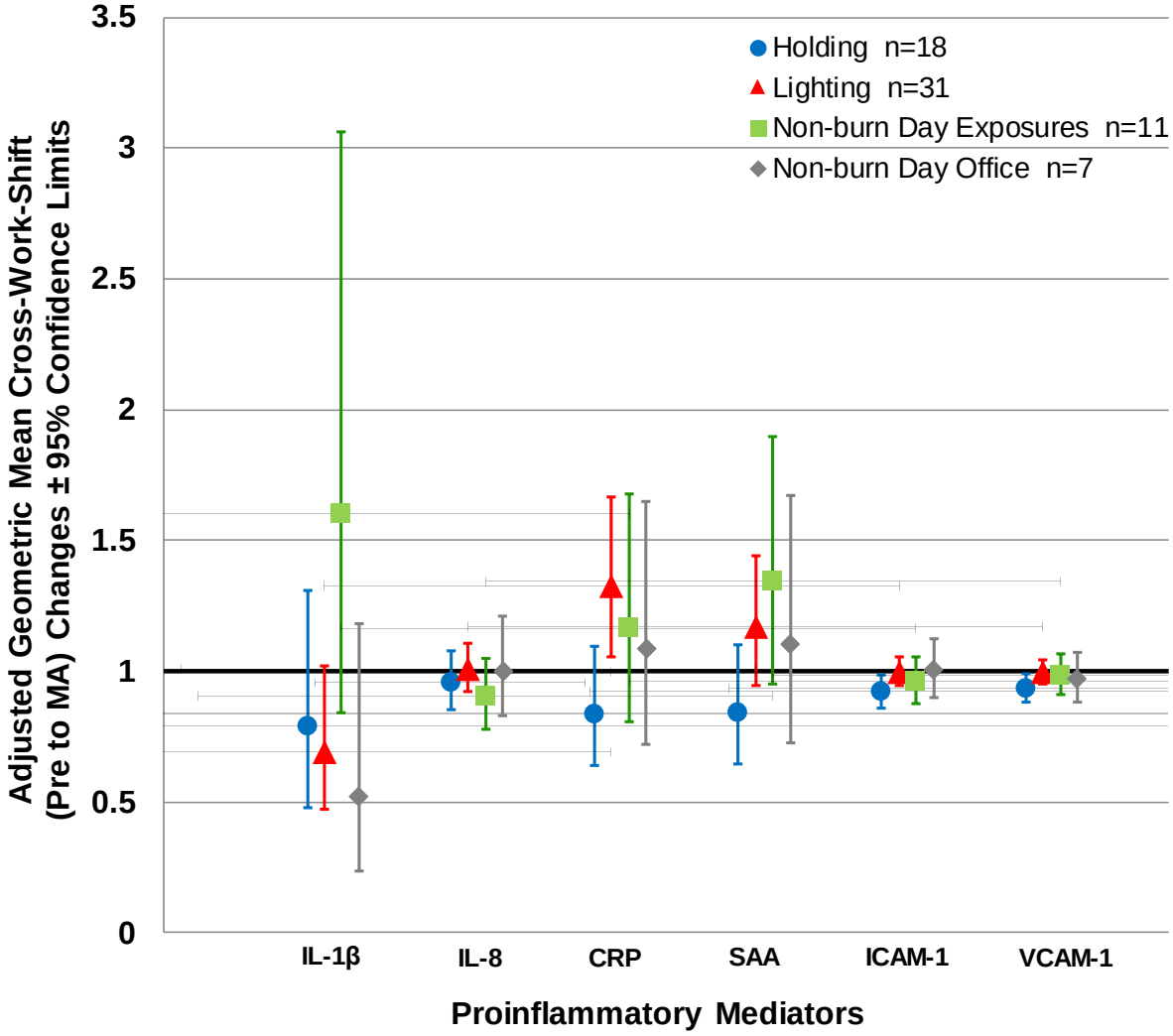

Figure 4.1 (b). Adjusted Geometric Mean Pre to Morning-after Cross-work-shift Changes in Proinflammatory Mediators according to Work Task. Note: *n*= person-day pre-morning-after paired samples; Cross-work-shift changes are reported as pre-work-shift/morning-after-work-shift ratios. Where 95% confidence limits do not cross the x-axis, proinflammatory mediator cross-work-shift changes are statistically different from 1 (*p*-values < 0.05, specific *p*-values not reported herein). Abbreviations: MA, morning-after.

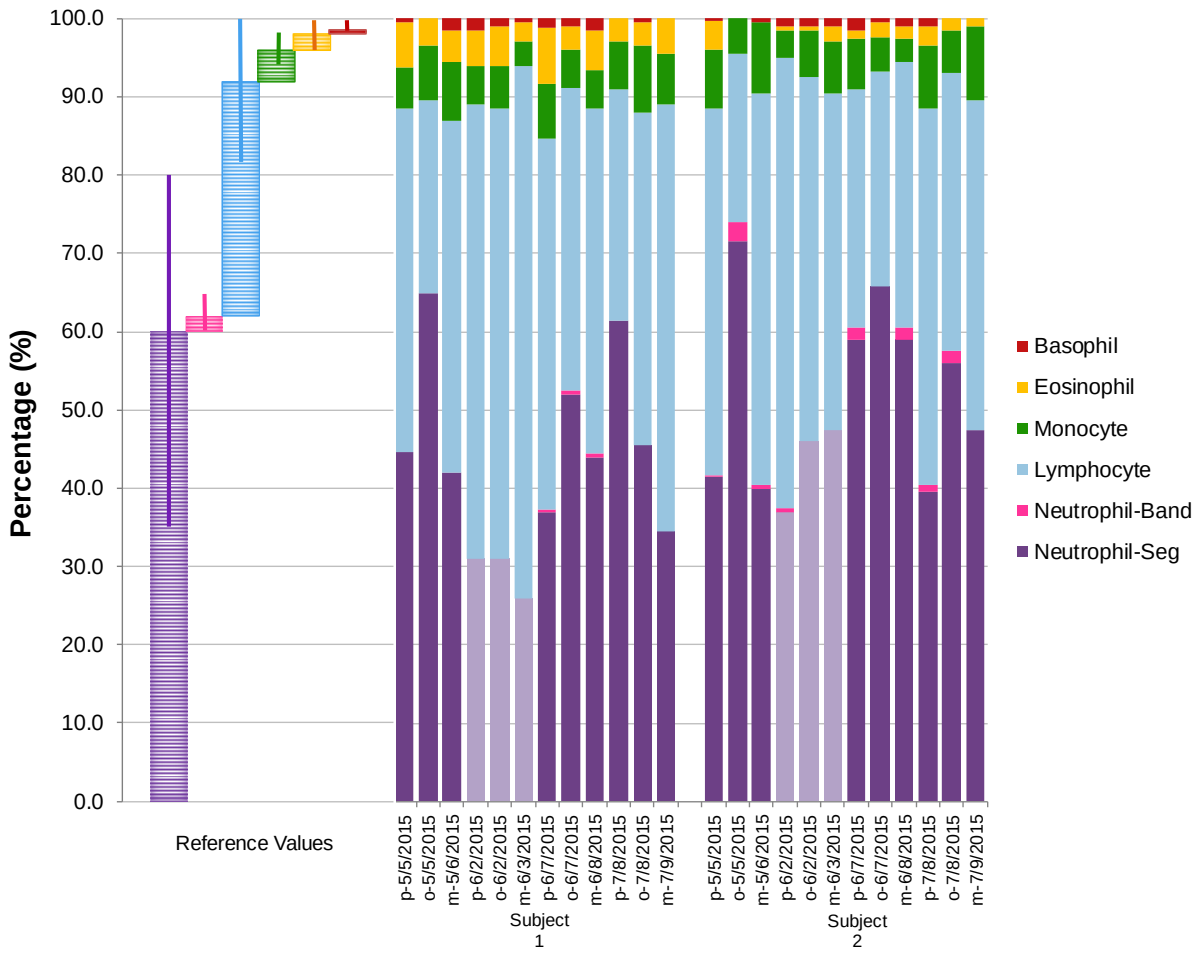

Figure 4.2 (a). Five-point Differential Percentage of White Blood Cell Type by Non-Career Volunteer Firefighter Subjects and Sampling Time. Note: Subject numbers 1 and 2 were non-career volunteer firefighters. Pre-work-shift, post-work-shift, and morning-after work-shift samples were taken and correspond to "p", "o", and "m", respectively. Non-burn workday samples are colored as light purple columns. Reference mean % values are provided along with corresponding range % values (Reference range represents 95% of the general population). Reference values and range % values were retrieved from Williams, James, and Roberts, 2014.[17]

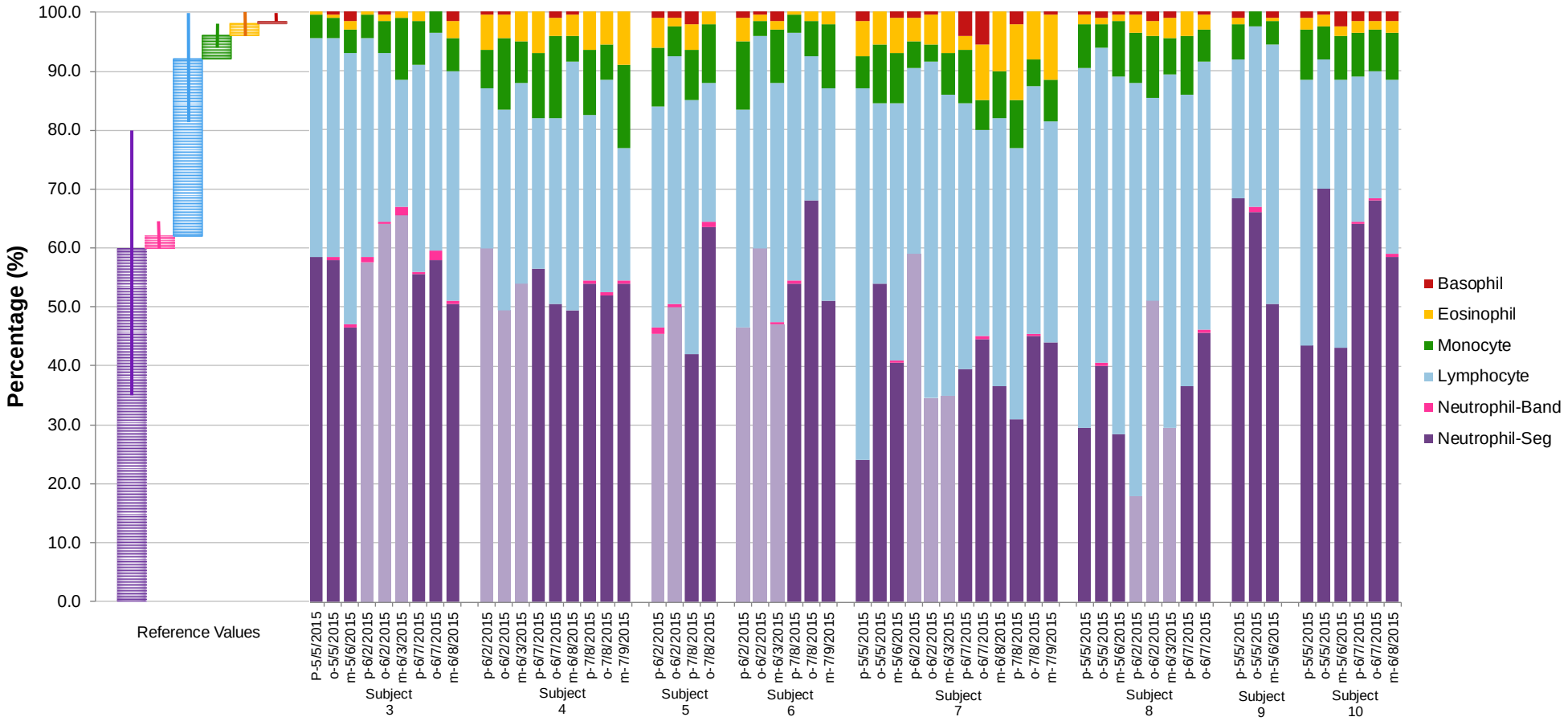

Figure 4.2 (b). Five-point Differential Percentage of White Blood Cell Type by Career Firefighter Subjects and Sampling Time. Note: Subject numbers 3-10 were career firefighters. Pre-work-shift, post-work-shift, and morning-after work-shift samples were taken and correspond to "p", "o", and "m", respectively. Non-burn workday samples are colored as light purple columns. Reference mean % values are provided along with corresponding range % values (Reference range represents 95% of the general population). Reference values and range % values were retrieved from Williams, James, and Roberts, 2014.[17]

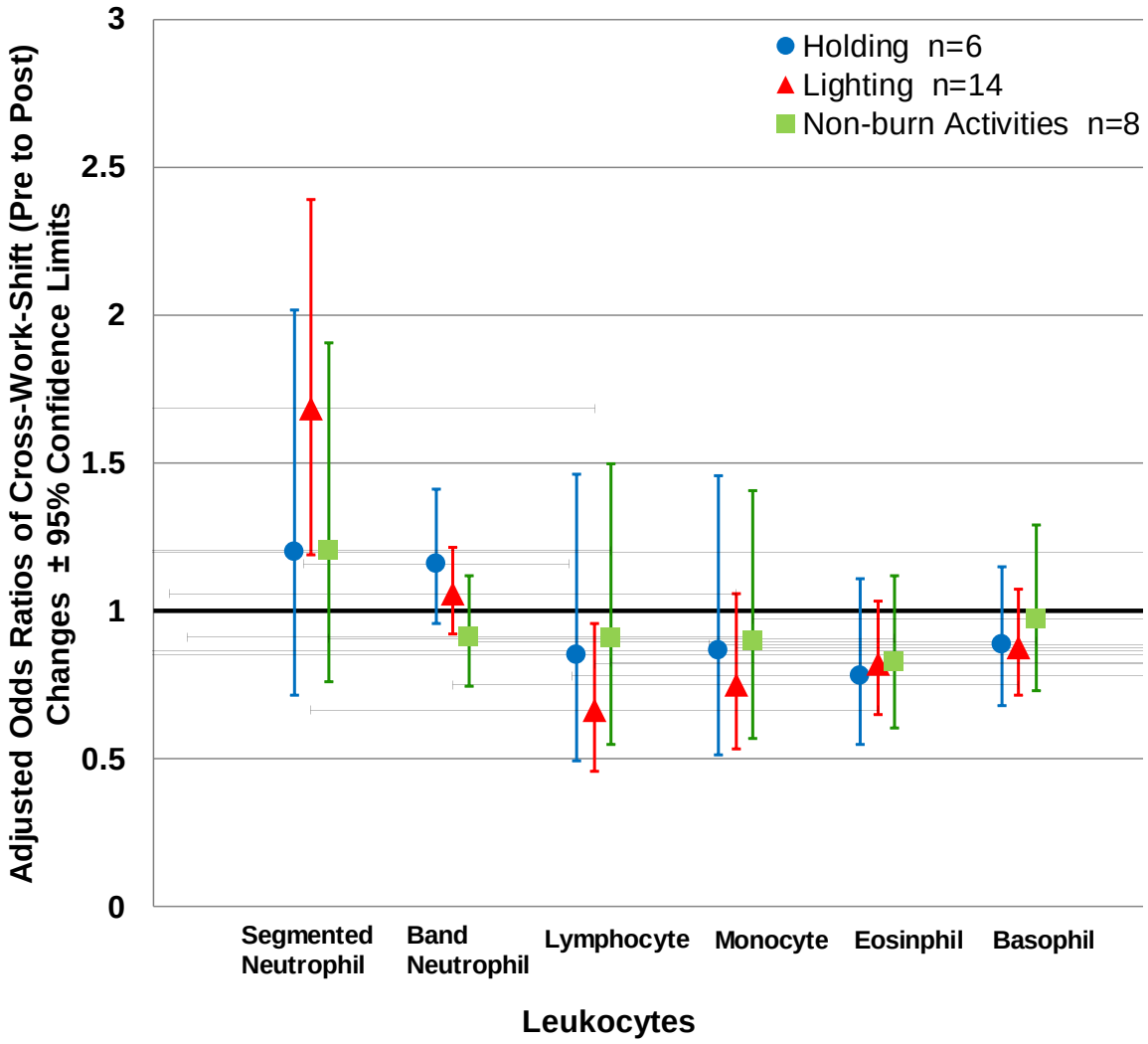

Figure 4.3 (a). Adjusted Odds Ratios of Cross-Work-shift (Pre to Post) Changes according to Work Tasks across Five-point Differential Percentages of White Blood Cell Type. Note: Non-burn day—Exposure and Office were collapsed in to one category; *n*= person-day pre-post paired samples; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes in leukocytes are statistically different from 1 (*p*-values < 0.05, specific *p*-values not reported herein). No *p*-value derived from the linear mixed models were statistically significant.

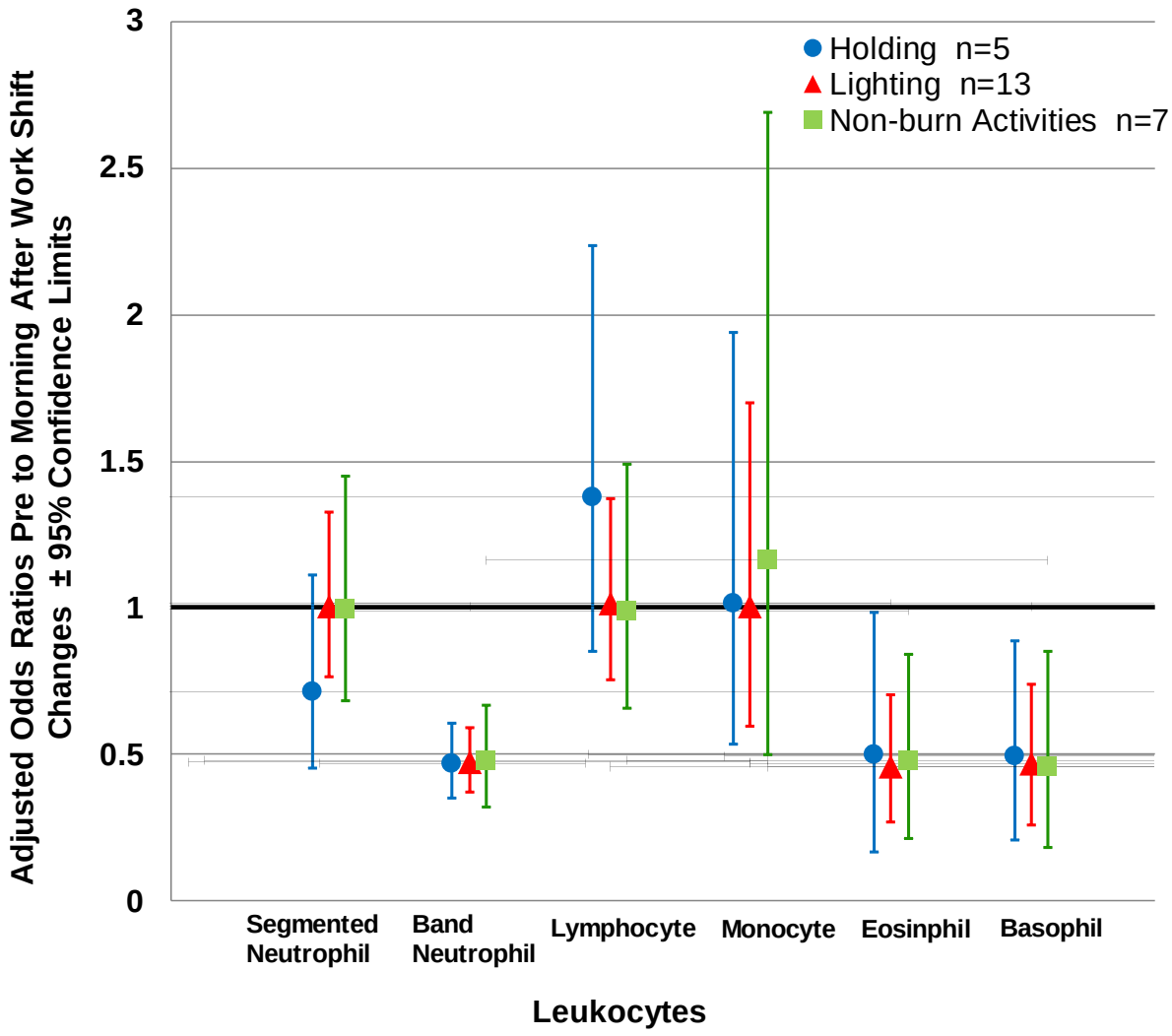

Figure 4.3 (b). Adjusted Odds Ratios of Pre to Morning-after Work-shift Changes according to Work Tasks across Five-point Differential Percentages of White Blood Cell Type. Note: Non-burn day—Exposure and Office were collapsed in to one category; *n*= person-day pre-morning-after paired samples; Cross-work-shift changes are reported as pre-work-shift/morning-after-work-shift ratios. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes in leukocytes are statistically different from 1 (*p*-values < 0.05, specific *p*-values not reported herein). No *p*-value derived from the linear mixed models were statistically significant. Model controls for medication use.

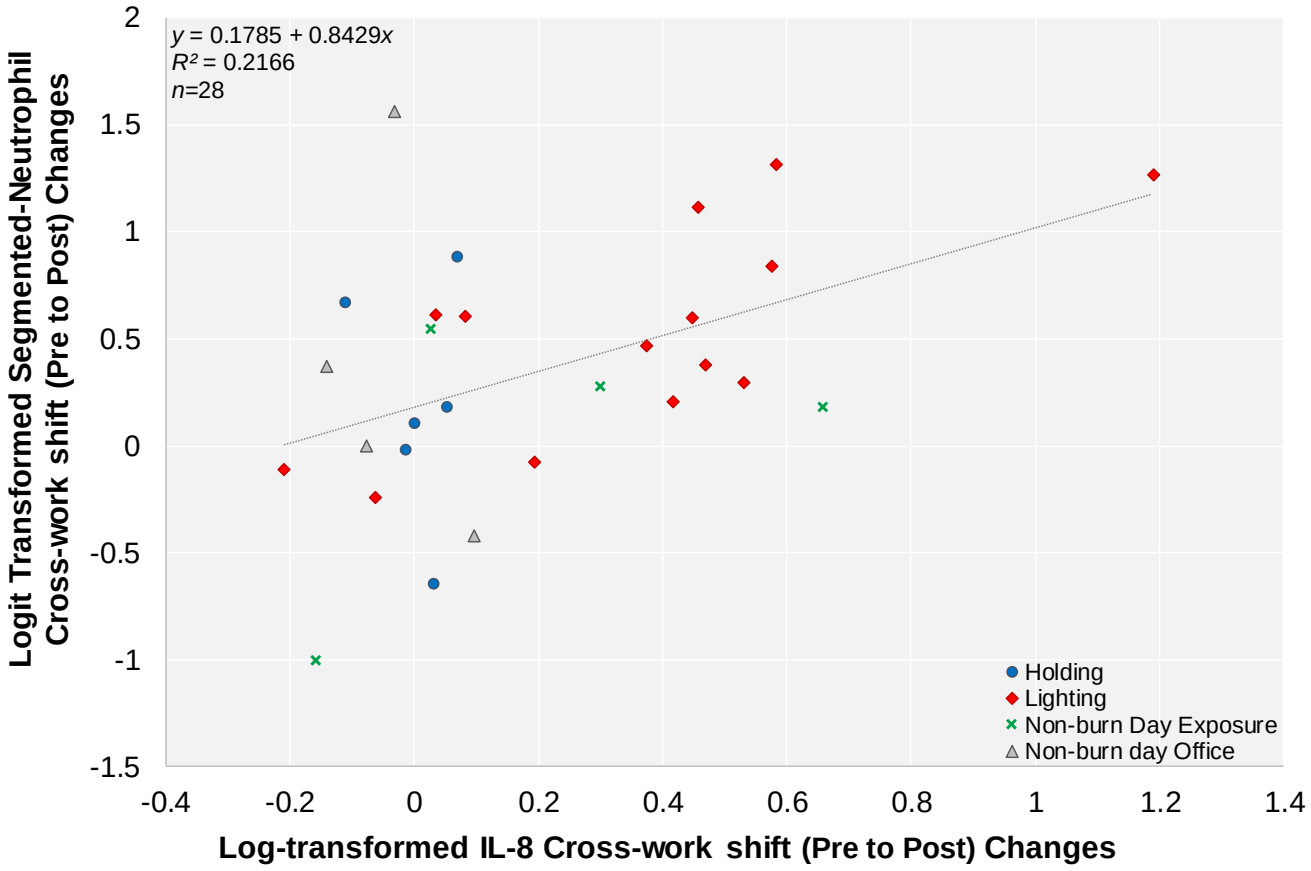

Figure 4.4. Correlation Between Logit-transformed Cross-work-shift (Pre to Post) Changes in Segmented Neutrophils and Log-transformed IL-8 Changes. Person days are indicated as *n*. Linear mixed effect model results showed statistical significant positive correlation (p=0.0179). Note: Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios.

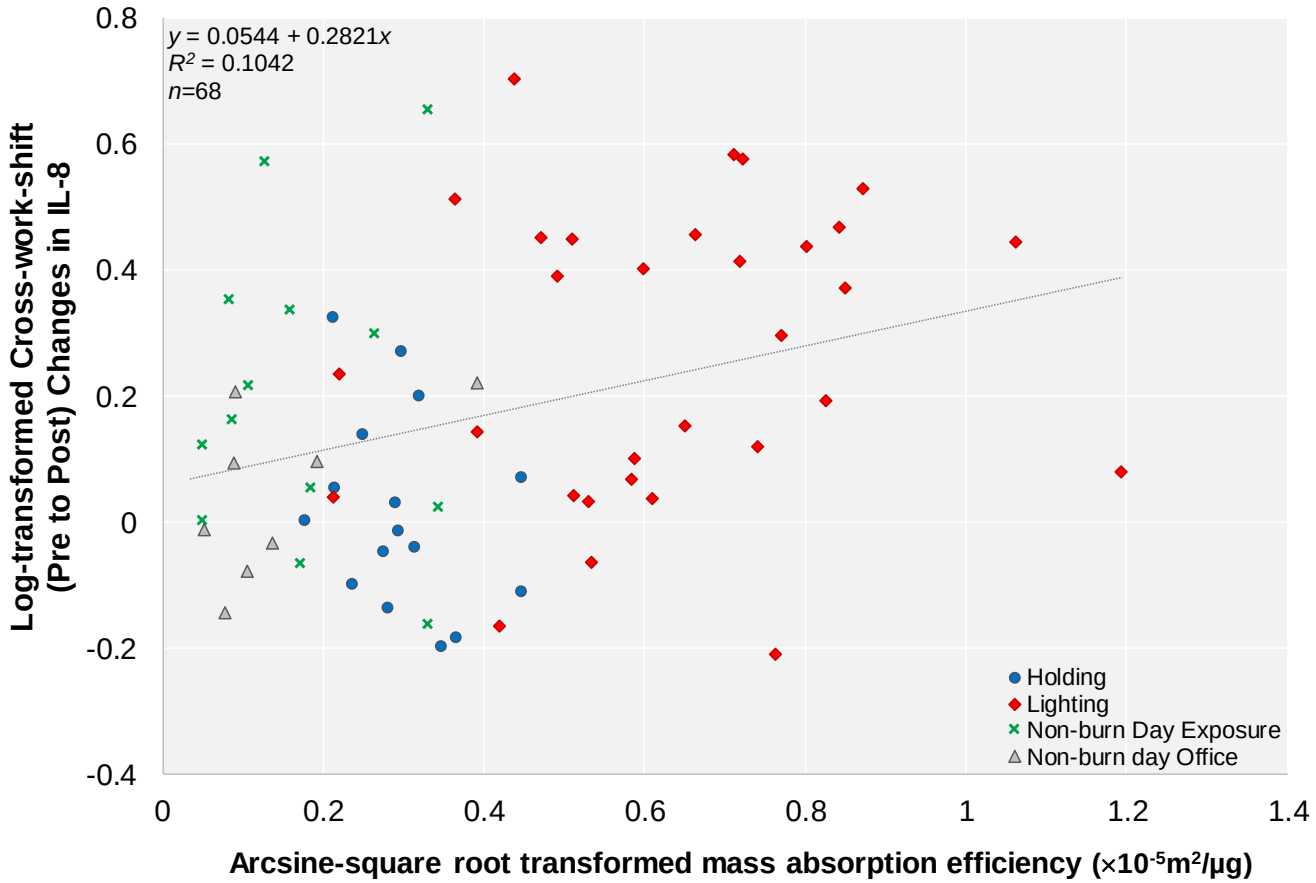

Figure 4.5. Correlation Between Log-transformed Cross-work-shift (Pre to Post) Changes in IL-8 and Arcsine-square root Transformed Mass Absorption Efficiency. Person days are indicated as *n*. Linear mixed effect model results showed statistical significant positive correlation (p=0.0080). Note: Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios.

CHAPTER 5

URINARY MUTAGENICITY AS A BIOMARKER OF OCCUPATIONAL SMOKE EXPOSURES OF WILDLAND FIREFIGHTERS AT PRESCRIBED BURNS [1]

[1] Adetona AM, DeMarini DM, Warren SH, Martin K, Hanley NM, Zhang J, Simpson C, Paulsen M, Rathbun SL, Adetona O, Wang JS, & Naeher LP. To be submitted to *Inhalation Toxicology*.

ABSTRACT

Background: Wildland firefighters conducting prescribed burns are exposed to a mixture of pollutants which include exposure to a wide array of constituents found in wood smoke, diesel and gasoline. An integrated measure of exposure to a complex mixture of genotoxic pollutants is needed to better characterize occupational exposures among wildland firefighters.

Objective: The purpose of the study was to evaluate systemic genotoxicity of wildland firefighters before and after a prescribed burn through the assessment of urinary mutagenicity in *Salmonella* YG1041 +S9 (Ames assay). We hypothesized that urinary mutagenicity will be significantly higher from firefighters after working at prescribed burns compared to non-burn work days.

Methods: Using a repeated measures study design, urine samples were collected before, immediately after, and the morning-after a work shift on prescribed burn and non-burn work days from twelve healthy subjects with the United States Forest Service, Aiken, SC. Urine samples were analyzed for malondialdehyde and mutagenic potency. Personal particulate matter ($PM_{2.5}$) and carbon monoxide (CO) were collected from each participant. Light absorbing carbon of $PM_{2.5}$ was measured as a surrogate for black carbon. Linear-mixed effect models were used to assess cross-work shift (pre to post work shift) changes in urinary biomarkers.

Results: 48 pre-post paired work shift urine samples were collected on burn days and 21 on non-burn work days. A two-fold (marginally significant) higher creatinine-adjusted urinary mutagenicity cross-work shift increase on burn days compared to non-burn day samples (p=0.0906). Though not significant, lighters had a two-fold higher cross-work shift increase in crude urinary mutagenicity compared to holding and non-burn day exposures. Positive associations were found between cross-work shift (pre to post) changes in creatinine-adjusted urinary mutagenicity and creatinine-adjusted MDA (p=0.0905), CO (p=0.0459), and mass

absorption efficiency (surrogate for black carbon/$PM_{2.5}$ ratio) (p=0.1333), respectively. No significant effect of day type or work task on cross-work shift (pre to post) changes in MDA was observed.

Conclusion: Urinary mutagenicity may serve as a suitable measure of occupational smoke exposures among wildland firefighters. Findings from this study suggest that diesel exposure may also contribute to increases in urinary mutagenic potency.

INTRODUCTION

Wildland firefighters are exposed to a mixture of pollutants.[1] Wood smoke alone is composed of hundreds of constituents, some of which are carcinogenic in nature.[2] Specific to prescribed burning (preplanned ignited fires under the direction of certified management), additional exposures may be experienced by wildland firefighters igniting fires by drip-torches fueled by a mixture of diesel and gasoline. The purpose of the study was to evaluate systemic genotoxicity of wildland firefighters before and after a prescribed burn through the assessment of urinary mutagenicity in *Salmonella* (Ames assay). While traditional exposure monitoring techniques were used to characterize occupational exposures, we proposed to use urine collected from firefighters exposed to smoke from burns and/or diesel/gasoline fuel exhaust, to more fully characterize their exposures. Urinary mutagenicity provides an integrated measure of exposure to a complex mixture of genotoxic pollutants.[3] Previous studies show that urinary mutagenicity correlates with other biomarkers in subjects exposed to wood smoke as well as a variety of other pollutants.[3-7] Therefore, we explored the relationship between urinary mutagenicity and personal occupational exposure measurements among wildland firefighters at prescribed burns. In additional, we report urinary concentrations for a biomarker of oxidative, malondialdehyde (MDA), and explore the relationship between it and urinary mutagenicity. We hypothesized that urinary mutagenicity will be significantly higher from firefighters after working at prescribed burns (post-work shift levels) compared to pre-work shift levels, and compared to non-burn work days. We also hypothesized that work task will have an effect on urinary mutagenicity with firefighters lighting fires with diesel-gasoline fueled drip-torches having higher urinary mutagenic potency compared to other work tasks.

METHODS

Study Population and Design

Twelve healthy subjects (ten wildland firefighters and two work-certified volunteers) with the United States Department of Agriculture Forest Service at Savannah River Site (USFS-Savannah River), South Carolina were monitored for occupational exposures during their work shifts at prescribed burns and on working days when prescribed burns were not conducted during January-July of 2015. Before and after-work-shift spot urine samples were collected each sampling day. This study was approved by the University of Georgia Institutional Review Board and written informed consent was obtained from each subject before participation. Participation in the study was voluntary. Baseline questionnaires and daily work activity questionnaires were administered to subjects in order to gain information on personal work history, length of firefighter career, health habits (i.e. exercise frequency, tobacco use), disease history, medication, diet (i.e. grilled-foods), daily work tasks, and other factors that could be considered influences on exposure and/or on mutagenic responses. Subjects' self-reported work tasks were categorized into four major categories, two of which were on burn days ("Holding" and "Lighting"), and two on non-burn work days ("Non-burn day—Exposures" and "Non-burn day—Office"). On burn days, work tasks included holding prescribed fire-lines (subjects were referred to as "Holders") where firefighters used fire engine vehicles to patrol and contain fires from escaping established boundaries, and lighting (herein subjects were referred to as "Lighters") frequently by hand using a drip-torch or less often by aerial methods (i.e. helicopter). Subjects also conducted various tasks on non-burn workdays which included patrolling of areas where recent burns were conducted, field prep work, engine maintenance, etc. These tasks were all classified as "Non-burn day—Exposures" since subjects reported experiencing occupational exposures to vehicle exhaust, diesel, dust, or others.

Non-burn workdays also involved subjects performing office work (herein classified as "Non-burn day—Office"). An assigned primary work task was determined by reported time spent during a particular work tasks (i.e. the subject had spent more than 50% of the duration of a work-shift conducting the task).

Urine Sample Collection

Subjects were instructed on how to properly collect their urine samples in sterile polypropylene 4.5 ounce (~133 ml) cups. Spot urine samples were collected immediately before a work shift, immediately after a work shift, and the morning-after a work shift. Samples were frozen in a -5°C storage freezer immediately after collection and transported on dry ice to a -80°C freezer at the University of Georgia, Department of Environmental Health Science. The samples were thawed once at room temperature to prepare randomized aliquots into sterile, polypropylene, conical 50-ml tubes and again stored in -80°C freezer until further analyses.

Occupational Exposure Assessment

Gravimetric particulate matter with an aerodynamic diameter of ≤ 2.5 μm ($PM_{2.5}$) and carbon monoxide (CO) were measured in the breathing zone of the subjects on burn and non-burn work days. No respiratory protection of the subjects was used during the study. A detailed description of the sample collection methods and gravimetric analyses are previously reported in a companion paper.[8] In brief, all exposure monitoring instruments were calibrated prior to use and gravimetric analyses followed the United States Environmental Protection Agency (U.S. EPA) specifications.[9] $PM_{2.5}$ samples were collected using MicroPEM™ (RTI International, Research Triangle Park, NC, USA) which were loaded with 25 mm polytetrafluoroethylene membrane filters (porosity: 3.0 μm) (Pall Life Sciences, Ann Arbor, MI, USA). Filters were weighed using the Cahn C-35 microbalance (sensitivity of ±1.0 μg; Thermo Electron, Waltham, MA, USA). All

filter weights were blank-adjusted accordingly (previously described).[8] After gravimetric analyses, PM on the filters was analyzed for light absorbing carbon (LAC) and used as a surrogate for black carbon (BC) by reflectance analysis using the Evans Electroselenium Limited smoke stain reflectometer (Model 43D, Diffusion Systems Ltd, London, United Kingdom) (methods previously described).[8] Absorption coefficients ($\times 10^{-5} m^{-1}$) were calculated according to ISO 9835 and $PM_{2.5}$ mass absorption efficiencies ($\times 10^{-5} m^2/\mu g$) were determined by dividing the absorption coefficients by gravimetric $PM_{2.5}$ concentrations. Real-time CO was measured using the Dräger Pac III (DrägerSafety Inc., Pittsburgh, PA, USA). Time-weighted averages were calculated for both $PM_{2.5}$ ($\mu g/m^3$) and CO (ppm).

Malondialdehyde Analysis

In a blinded fashion, concentrations of free MDA were measured using a High-Performance Liquid Chromatography (HPLC) system with fluorescent detection.[10] A 150 µl aliquot sample was added into a mixture of 750 µl phosphoric acid (440 mM) and 150 µl thiobarbituric acid (TBA, 42 mM). After 1-hour incubation at 80°C, a 20 µl aliquot of this final solution was injected into the HPLC system with fluorescence detector set at 532 nm for the excitation wavelength and 553 nm for the emission wavelength. A Nova-Pak C_{18} column (Waters, Milford, MA, USA) was used with a mobile phase that was composed of 40% methanol and 60% water containing 50 mM KH_2PO_4 (pH=6.8) at a flow rate of 0.8 ml/min. The detection limit, extraction recovery and analytical precision of this method were 1.8 nM, 75.9%, and 2.2% (measured as relative standard deviation [RSD] from 8 replicate injections), respectively. Free MDA was presented in nmol/L. Creatinine-adjusted concentrations (the weight of free MDA per unit weight of creatinine in urine: µmol MDA/mol creatinine) were calculated in order to correct

for urine dilution. Urinary creatinine (mg/dL) was measured by a Beckman Coulter AU Analyzer for Creatinine (Beckman Coulter, Inc., Brea, CA, USA).

Sample Extraction and Concentration

Organic extracts from the urine samples was done by a method described previously.[4] In brief, urine aliquots were thawed and filtered to remove urothelial cells. Technicians were blinded to the sample codes. The volume of each sample was recorded and the urine was enzymatically de-conjugated in 0.2-M (10% v/v) sodium acetate buffer (pH 5.0) (Sigma, St. Louis, MO, USA), containing β-glucuronidase (6 units/ml urine; Sigma, St. Louis, MO, USA) and sulphatase (2 units/ml urine, Sigma, St. Louis, MO) for 16 h at 37ºC. The de-conjugated urinary metabolites were then extracted and concentrated by pouring the urine through two C-18 silica-gel columns stacked in tandem (Avantor Performance Materials, Center Valley, PA, USA). The eluted urines were discarded, and a new tube was placed under the column to collect the organics, which was eluted by pouring 10 ml of methanol through the column. The methanol was solvent-exchanged with dimethyl sulfoxide (DMSO) to produce an organic concentrate at 150X. These extracts were stored at 4ºC until mutagenicity assays were conducted.

Mutagenicity Assay

The *Salmonella* (Ames) mutagenicity assay, using the plate-incorporation method[11] was used to evaluate the organic extracts of the urines in a blinded fashion. A detailed description of the assay method is previously reported.[12] Briefly, the urine concentrates were evaluated in five batch experiments at 0.0, 0.3, 0.6, 1.0, 3.0, 6.0, and 10.0 ml-equivalents of urine/plate (Note: Dosing sometimes varied based on total urine volume available. i.e. 0.0, 0.5, 1.5, 3.0, 7.5, 12.0 or 15 ml-equivalents of urine/plate; 0.0, 4.0, 6.0, 10.0 ml-equivalents of urine/plate; 0.0, 2.25, 4.0, 6.75, 9.0, 12 or 15 ml-equivalents of urine/plate in some instances were used) in the presence of

metabolic activation (S9 mix) made from Aroclor-induced, Sprague-Dawley rat-liver S9 from Moltox, Boone, NC. The concentrate (not exceeding 100 µl of DMSO/plate) was added to 2.5-ml of top agar, along with 100 µl of overnight cell suspension, with 500 µl of S9 mix. The contents of the tube were vortexed and poured onto bottom-agar plates containing Vogel-Bonner Minimal E (VBME) medium, and incubated at 37ºC for 3 days (72 h), after which the colonies were counted using an automatic colony counter (AccuCount 1000, Manassas, VA). Data was recorded manually from the reading of the colony counter onto standard score sheets. 10% of the aliquots had replicate samples (identical urines that were divided into two sample tubes) and were analyzed according to the methods described herein.

As described in Mutlu et al. (2015), TA98 [*hisD3052 chl-1008 (bio uvrB gal) rfa-1004* pKM101$^+$, Fels-1$^+$, Fels-2$^+$ Gifsy-1$^+$ Gifsy-2$^+$] detects frameshift mutagens.[12] We used YG1041, a derivative of TA98 that over-expresses nitroreductase and acetyltransferase, permitting it to detect frameshift mutagens that are nitroarenes or aromatic amines. A previous study[4] of wood smoke-associated urinary mutagenicity in charcoal workers in Brazil showed that strain YG1041 + S9 was the most sensitive detector of such mutagenicity; thus, we used the same strain with S9 for this study.

A negative control (DMSO at 100 µl/plate) and two positive controls (2-nitrofluorene at 0.5 µg/plate and 2-aminoanthracene at 0.5 µg/plate) was included with each experiment (Note: The mean of five batches of experiments thus far conducted for the DMSO control value for YG1041 +S9 was 63 revertants (rev)/plate, while the mean of the five batches of experiments thus far conducted for 2-nitrofluorene for YG1041 −S9 and 2-aminoanthracene for YG1041 +S9 were 1741 and 1687 rev/plate, respectively). Three plates were used for each type of control for each experiment and extract were tested at 0.3, 0.6, 1.0, 3.0, 6.0, and 10.0 ml-equivalents/plate (ml-

eq/plate) (or at dosing specific to each experiment). Due to limited urine volumes, only one independent experiment for each extract per one plate/dose was conducted. A positive mutagenic response was defined as a reproducible, dose-related response with a twofold or greater increase in revertants (mutant colonies) relative to the DMSO control plates. Urinary values were also creatinine-adjusted and reported in revertants per µmole creatinine.

Statistical Analysis

Linear regressions over the linear portions of the dose-response curves were performed to determine the mutagenic potencies (expressed as rev/ml-eq). Several urine samples were extracted with compromised methanol and showed clear cytotoxicity (n=9 of the 150 analyzed), as evidence by negative slopes of the linear regressions. These sample values were disregarded in the final statistical analyses.

Primary analyses consisted of using linear mixed-effects models to assess cross-work-shift (pre/before work-shift to post/after work-shift) urinary mutagenicity changes in firefighters. To determine cross-work shift changes in urinary mutagenicity, we regressed all the data (with the exception of the 9 compromised samples); this included the samples that did not approach a twofold increase over the zero control and would otherwise be considered as a negative mutagenic response. The linear regressions were computed using Microsoft Excel 2016. Urinary creatinine and the mutagenic potencies were expressed relative to creatinine concentration as rev/µmole urinary creatinine. Descriptive statistics and linear mixed effect models were conducted using SAS v.9.4 (SAS Institute, Cary, NC, USA). Statistical significance was set at $p < 0.05$ and adjusted *p*-value (i.e. Bonferroni Method) was used when doing multiple comparisons.

Heteroscedasticity and normality of the data was assessed. Log-transformations were applied to normalize the variables including TWA gravimetric $PM_{2.5}$, CO, absorption coefficient

(surrogate for black carbon), MDA, and urinary mutagenicity data. Note that to achieve normality, mass absorption efficiencies (surrogate for black carbon/$PM_{2.5}$ ratio) were arcsine-square root transformed. The appropriate back transformations were applied to transformed variables before reporting final results. We assessed cross-work-shift (pre to post; and pre to morning-after) changes in urinary mutagenicity by day type (burn and non-burn day), while also evaluating possible associations with other outcome variables which included TWA $PM_{2.5}$, CO, absorption coefficient, mass absorption efficiency, inhaled dose of $PM_{2.5}$, and MDA. To account for longitudinal within subject correlation of the data, we included subject and date as random effect variables in the model. Using the forward elimination procedure, we also tested for the effects of possible confounding factors such as smokeless tobacco used (chew), grilled-foods, age and length of firefighter career. Only significant covariates were included in the final model.

Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios or morning-after-work-shift/pre-work-shift ratios, respectively. Therefore, cross-work-shift ratios were statistically different if the mean ratios' corresponding 95% confidence limits (95% CL) did not include the value of 1.

RESULTS

A total of 201 spot urine samples were collected throughout the study period. Therefore, 48 person-day paired (pre-post) samples were collected from 12 subjects on 7 prescribed burn days. We also collected 19 person-day paired (pre-post) samples from 8 subjects on 3 non-burn working days during the season. In addition, we collected morning after (ma) work day samples, resulting in 40 person-day paired (pre-post-ma) samples on burn days and 16 person-day paired (pre-post-ma) samples on non-burn days. Because some study participants were unable to donate

urine from time to time, a few occasions in which only pre- or pre-ma or post-ma samples were available (6 in total) and were not included in the person-day samples noted above.

To date, 150 urine samples have been analyzed for mutagenic potency. One sample was spilled during analytical procedures and therefore could not be analyzed. Analyses on the remaining 50 urine samples are expected to be complete at a later time. We understand results reported herein may change given the inclusion of the remaining data, but the results thus far are in the expected direction. The following mutagenicity results are based on matched pre-post work-shift paired samples consisting of 44% of the data on burn days (n=20 of 45 total) and 56% of the data on non-burn days (n=9 of 16 total). All samples have been analyzed for creatinine and MDA concentrations.

Study population characteristics are described previously.[8] In brief, the average age of the twelve subjects who participated in the study was 33 ± 5.4 years with subjects having less than 1 year to 22 years of experience as a wildland firefighter. All subjects were non-smokers although three subjects reported chewing smokeless tobacco on occasions. On average, 280 acres (Range: 38-1000 acres) were burned per day and the duration of work-shifts averaged 4.5 hr (Range: 1.9-9.4 hr) on burn days and 6.2 hr (Range: 3.9-7.8 hr) on non-burn work days. The average $PM_{2.5}$ and CO concentrations on non-burn work days and burn days were 35.1 [95% CL: 15.9, 77.3] $\mu g/m^3$ and 0.005 [95% CL: 0.002, 0.016] ppm, and 259.4 [95% CL: 156.1, 431.1] $\mu g/m^3$ and 0.8 [95% CL: 0.4, 1.8] ppm, respectively.[8] Unadjusted means of crude and creatinine-adjusted urinary mutagenicity and MDA concentrations according to day type and time of sample collection are presented in Table 5.1.

Urinary Mutagenicity

Percent positive results for mutagenicity and corresponding unadjusted means by day type and time of sample collection are presented in Table 5.2. Thirty-eight percent of the post-work shift samples (12 of 32 thus far analyzed) on burn days were positive for mutagenicity, compared to only 14% positive post-work shift samples (2 of 14 thus far analyzed) on non-burn work days (Table 5.2). Unadjusted mean ± standard deviation (SD) crude and creatinine-adjusted post-work shift urinary mutagenicity levels on burn days were 8.1 ± 6.5 (arithmetic) rev/mL-eq and 0.90 ± 1.21 (arithmetic) rev/µmol creatinine, and ranged from 4.4 to 28.0 rev/mL-eq and 0.24 to 4.46 rev/µmol creatinine, respectively (Table 5.2). On non-burn work days, unadjusted mean crude and creatinine-adjusted post-work shift urinary mutagenicity levels were 5.3 ± 0.7 (arithmetic) rev/mL-eq and 0.40 ± 0.03 (arithmetic) rev/µmol creatinine, and ranged from 4.8 to 5.8 rev/mL-eq and 0.38 to 0.41 rev/µmol creatinine, respectively (Table 5.2).

Adjusted cross-work shift (pre to post, and pre to morning after [MA]) changes in crude and creatinine-adjusted urinary mutagenicity according to day type are presented in Figures 5.1 (a-d). A marginally significant effect of day type on cross-work shift (pre to post) changes in creatinine-adjusted mutagenicity was observed (p=0.0906), with a two-fold times higher cross-work shift change observed on burn days compared to non-burn work days (1.4 [95% CL: 0.9, 2.3, and 0.7 [95% CL: 0.3, 1.4], respectively) (Figure 5.1b). Though not significant (p= 0.4847), pre to MA cross-work shift changes in creatinine-adjusted mutagenicity appear to be trending in similar directions, with burn days having 1.5 times higher cross-work shift (pre to MA) increases compared to non-burn work days (1.2 [95% CL: 0.6, 2.8], and 0.9 [95% CL: 0.4, 2.0], respectively) (Figure 5.1d).

Adjusted cross-work shift (pre to post, and pre to MA) changes in crude and creatinine-adjusted urinary mutagenicity according to work tasks are presented in Figures 5.2 (a-d). Though lighters appeared to have the highest cross-work shift (pre to post) increase in urinary mutagenicity and were 2-folds higher compared to holding, no significant difference was observed between the two (p=0.1945) (2.0, [95% CL: 1.0, 3.6], and 1.1 [95% CL: 0.5, 2.4], respectively) (Figure 5.2a). Likewise, no significant difference was seen between lighting and non-burn day exposures (p=0.1454) (2.0 [95% CL: 1.0, 3.6], and 1.0 [95% CL: 0.4, 2.4], respectively) (Figure 5.2a). No significant overall effect of work task was seen among crude or creatinine-adjusted cross-work shift (pre to post [Figure 5.2a] and pre to MA [Figures 5.2c and 5.2d]) changes. However, a marginally significant (p=0.0971) effect of work task was observed for creatinine-adjusted cross-work shift (pre to post) changes [Figure 5.2b], with lighting having significantly higher cross-work shift changes in mutagenicity compared to non-burn day exposures (p=0.0205) (1.5 [95% CL: 0.8, 2.8], and 0.4 [95% CL: 0.2, 1.0], respectively).

Urinary Concentrations of MDA

Unadjusted geometric mean crude and creatinine-adjusted post-work shift changes in MDA on burn days were 894.6 (95% CL: 748.5, 1069.0) nmole/l and 84.3 (95% CL: 74.9, 95.0) µmol MDA/mole creatinine, and ranged from 175.3 and 3415.5 nmole/l and 37.1 and 212.5 µmol MDA/mole creatinine, respectively (Table 5.1). On non-burn work days, unadjusted geometric mean crude and creatinine-adjusted post-work shift changes in MDA were 828.6 (95% CL: 592.5, 1158.9) nmole/l and 98.6 (95% CL: 77.5, 125.5) µmol MDA/mole creatinine, and ranged from 4225.4 and 4533.4 nmole/l and 37.1 and 212.5 µmol MDA/mole creatinine, respectively (Table 5.1).

Adjusted cross-work shift (pre to post, and pre to MA) changes in crude and creatinine-adjusted MDA according to day type are presented in Figures 5.3 (a-d). No significant difference was observed between cross-work shift changes on burn day and non-burn days (Figures 5.3a, 5.3b, and 5.3d). However, a marginal significant difference in pre to MA work shift crude MDA changes was observed between day types (p=0.0502) (Figure 5.3c), with non-burn days having a higher crude MDA cross-work shift (pre to MA) increase compared to burn days (1.7 [95% CL: 1.1, 2.7], and 1.1 [95% CL: 0.8, 1.5], respectively).

Adjusted cross-work shift (pre to post, and pre to MA) changes in crude and creatinine-adjusted MDA according to work tasks are presented in Figures 5.4 (a-d). No significant effect of work tasks was observed across all cross-work shift changes (Figures 5.4a-d), however non-burn work day exposures showed marginally significant higher crude MDA cross-work shift (pre to MA) increase compared to lighting (p=*0*.0565) (1.8 [95% CL: 1.1, 3.0], and 1.1 [95% CL: 0.8, 1.6], respectively) (Figure 5.4c).

Correlations and Other Covariates

We found positive correlations between cross-work shift (pre to post) changes in urinary mutagenicity and MDA (marginally significant, p=0.0905) (Figure 5.5), and between cross-work shift (pre to post) changes in urinary mutagenicity and CO (p=0.0459) (Figure 5.6). Additionally, a positive relationship, though not significant, was observed between cross-work shift (pre to post) changes in urinary mutagenicity and mass absorption efficiency (surrogate for BC/$PM_{2.5}$ ratio) (p=0.1333) (Figure 5.7).

No significant correlations were found between the remaining outcomes variables ($PM_{2.5}$, inhaled dose of $PM_{2.5}$, absorption coefficient) and cross-work shift changes in urinary mutagenicity, nor between the remaining outcomes variables ($PM_{2.5}$, inhaled dose of $PM_{2.5}$, CO,

absorption coefficient, mass absorption efficiency) (results not shown). Lastly, we found no significant effects for age, wildland firefighter career length, use of smokeless tobacco (chew), or grilled foods on cross-work shift changes in MDA or urinary mutagenicity (results not shown).

DISCUSSION

Constituents of wood smoke and non-wood smoke occupational exposures such a diesel exhaust during wildland firefighting may be mutagenic and carcinogenic.[1, 13] A recent study on wildland firefighters found measurably higher urinary metabolites of hydroxy-polycyclic aromatic hydrocarbons (OH-PAHs) after exposure to wood smoke, indicating that subjects received an internal dose of exposure to possible mutagenic and carcinogenic PAHs.[13] Because firefighters are exposed to a mixture of pollutants throughout a given work day and receive cumulative exposures throughout the length of their career, it was necessary to find an integrated approach in assessing internal (systemic) dose to smoke constituents among this population. Therefore, we explored the use of urinary mutagenicity in Salmonella YG1041 +S9 among firefighters working at prescribed burns and during non-burn work days.

Our results are comparable to the values reported in the literature.[4, 5] However, we observed generally lower geometric means for pre-, post- and MA- work shift creatinine-adjusted urinary mutagenicity (positive results) among our study subjects compared to the geometric means reported among non-smoking charcoal workers exposed to wood smoke in Brazil (Our study: Burn day pre-work shift levels: 0.63 ± 1.91 rev/µmol creatinine; Burn day post-work shift levels: 0.57± 2.38 rev/µmol creatinine; Burn day morning after-work shift levels: 0.65 ± 1.71 rev/µmol creatinine [Table 5.2]; Kato et al. (2004): No wood smoke exposure group: n=34, 1.79 [95% CL: 1.26, 2.54] rev/µmol creatinine; Low wood smoke exposure group: n=49, 2.65 [95% CL: 2.01, 3.66] rev/µmol creatinine; High wood smoke exposure group: n=49, 4.22 [95% CL: 3.27, 5.45])

rev/µmol creatinine).[4] Personal or ambient exposure monitoring (i.e. $PM_{2.5}$ or CO levels) was not measured in the study; rather, subjects were grouped based on tasks-related exposure levels (i.e. No wood smoke exposure group consisted of lumberjacks, helpers, and log carriers; Low wood smoke exposure group consisted of tractor or truck drivers, and helpers who unloaded logs in the kiln area and bricklayers in the kiln area; High wood smoke exposure group consisted of kiln workers who loaded the kiln with wood).[4] However, it is possible that exposures in this study may have been significantly higher compared to our study.

We found a two-fold higher (marginally significant) creatinine-adjusted urinary mutagenicity cross-work shift change on burn days compared to non-burn day samples. These results are in the expected direction and are consistent with previous findings reported in the literature. For instance, Kato et al. 2004 found that charcoal workers exposed to wood smoke had a prevalence odds ratios of 2.33 (95% CL: 0.83, 6.57) at low wood smoke exposure levels and 5.31 (95% CL: 1.85, 15.27) at high wood smoke exposure levels compared to the non-exposed group of workers.[4] Likewise, another study on individuals from Mayan families from Guatemala (n=32) who regularly used woodfired temazcales (steam baths), found that post-exposure samples were on average 1.7 times higher in urinary mutagen potency compared to pre-exposure samples and also compared to control samples (n=9, unexposed individuals from the same population).[5]

Our data suggest that lighters may have higher cross-work shift increases in crude urinary mutagenicity compared to other work tasks (Figure 5.2a). These results are understandable in that firefighters who light fires may be exposed to diesel and wood smoke particulates and therefore may have an additive or perhaps synergistic systemic dose to mutagenic compounds.[12] A recent experimental study found that extractable organic material (EOM) from diesel engine produced

particles had significantly 50-85% higher mutagenic potency compared to soy-biodiesel emission particle extracts.[12]

We found no significant effect of day type or work task on cross-work shift (pre to post) changes in MDA. However, we found a marginally significant higher cross-work shift (pre to MA) increase in cross-work shift changes in crude MDA on non-burn days compared to burn days (Figure 5.3c), and a marginally significant higher cross-work shift (pre to MA) increase in crude MDA for non-burn work day tasks that may have associated exposures versus lighting (Figure 5.4c). These findings are difficult to explain and may be influenced by non-reported exposures on non-burn days or perhaps outside of the work place. For instance, MDA levels in the body may be impacted by diet, such as lipid rich foods.[14, 15] A human cross-over study among young healthy men (n=9) had increased MDA blood levels after eating lipid rich foods (i.e. heavy whipping cream).

Our unadjusted creatinine-MDA concentrations are comparable to a similar study on wildland firefighters exposed to wood smoke (Our study: Unadjusted pre- and post- work shift arithmetic mean creatinine-adjusted MDA [95% CL]: 105.6 [95% CL: 82.3, 128.8] and 92.0 [95% CL: 80.5, 103.5], respectively [Table 5.1]; Adetona et al. 2013: Unadjusted pre- and post- work shift arithmetic mean creatinine-adjusted MDA ± SD: n=104, 88.14 ± 48.59 µmol MDA/mole creatinine, and n=96, 107.35 ± 33.90 µmol MDA/mole creatinine, respectively).[16] Similarly, our unadjusted exposure concentrations are comparable to this study's reported corresponding unadjusted geometric mean $PM_{2.5}$ and CO exposure concentrations of 248 (n=82, 95% CL: 184, 333) $\mu g/m^3$ and 1.0 (n=78, 95% CL: 0.07, 13) ppm, respectively.[16] Likewise to our study's findings, no significant cross-work shift changes was observed for MDA in the urine samples of

firefighters in Adetona et al. 2013.[16] Contrastingly, results from a human chamber study found measurable increases in exhaled breath MDA levels after subjects were exposed to wood smoke.[17]

We found a marginally significant correlation between adjusted cross-work shift (pre to post) changes in creatinine-adjusted urinary mutagenicity and cross-work shift (pre to post) changes in creatinine-adjusted MDA (Figure 5.5). This association is plausible since MDA is thought to be potentially mutagenic.[18, 19] MDA may act as an endogenous genotoxic product of lipid peroxidation of polyunsaturated fatty acids in the body.[19] An *in vitro* study evaluated the mutagenic potential of MDA in human cells and found that MDA induced a 15-fold increase in mutation frequency in the *supF* reporter gene compared with untreated DNA, while sequence analysis showed that MDA-induced mutations occurred more often at GC base pairs.[19] MDA induced multiple types of mutations, including large insertions and deletions (most frequently), base pair substitutions, and interstrand cross-links.[19]

We found a significant correlation between adjusted cross-work shift (pre to post) changes in creatinine-adjusted urinary mutagenicity and CO exposure (Figure 5.6). CO is often used as a surrogate for wood smoke exposure and incomplete combustion.[20] Exposure to CO is suggested to be potentially mutagenic, in that urinary mutagenic potency has been found to be associated with other metrics of exposure such as exhaled CO.[5] Urinary mutagenicity was significantly positively correlated with exhaled CO level (p <0.0001, R^2=0.53) among human subjects exposed to wood smoke from steam baths.[5]

Although we did not find any significant association between urinary mutagenicity and $PM_{2.5}$ exposure or inhaled dose of $PM_{2.5}$, we observed a positive (though not significant) correlation between cross-work shift (pre to post) changes in creatinine-adjusted urinary mutagenicity and mass absorption efficiency (surrogate for BC/$PM_{2.5}$ ratio) (Figure 5.7). This

relationship suggests that particle type (i.e. BC content) could influence urinary mutagenicity. Firefighters lighting with diesel-gasoline fueled drip-torches appear to have higher urinary mutagenicity as depicted in Figure 5.7 and suggested in Figure 5.2a. A previous study using extracted particles from diesel engine exhaust, which has a higher BC proportion compared to wood smoke,[2, 21] had a higher mutagenic potency compared to soy-biodiesel associated particles.[12]

In the future, it would be interesting to compare our urinary mutagenicity data to other urinary metabolites of wood smoke exposure such as hydroxy-polycyclic aromatic hydrocarbon (OH-PAH) metabolites (1-hydroxypyrene [1-OHP]) or hydroxy-metabolites of napthalene (1-NAP and 2-NAP), as in Kato et al. (2004). Results from this study showed that urinary mutagenicity increased significantly after wood smoke exposure and was modified by smoking among 154 Brazilian charcoal workers (Prevalence odds ratio of highly exposed workers versus non-exposed: 5.31 [95% CL: 1.85, 15.27]).[4] Furthermore, the study reported significantly higher levels of 2-NAP and 1-OHP among the highly exposed group compared to the non-exposed.[4]

Limitations

This study was a pilot study exploring the use of urinary mutagenicity among wildland firefighter. Therefore, the study sample size was initially powered using an effect size for a different health endpoint estimated from a previous piloted study.[22] Increasing the sample size in follow up studies will be important to determine small effect sizes and ability to dissociate the effect of confounders. MDA could be influenced by dietary intake of high lipid content foods. It is possible that other biomarkers of lipid peroxidation not effected by lipid intake, such as urinary isoprostanes may be useful for comparison.[14] Moreover, other biomarkers of exposure such as urinary OH-PAH metabolites would be useful in comparison with urinary mutagenicity, as various PAHs, also found in wood smoke and diesel exhaust, are potentially mutagenic and carcinogenic.[4]

Although our study was a repeated measures study design where subjects' served as their own controls, inclusion of a non-exposed control group in the future will be valuable since wildland firefighters often perform various tasks on non-burn days that may result in inadvertent exposures to pollutants.

CONCLUSION

Results from this study suggest that healthy, non-smoking wildland firefighters are exposed to genotoxic compounds during prescribed burning. Urinary mutagenicity may serve as a suitable measure of occupational smoke exposures among this worker population. We found significant (marginally) higher cross-work shift (pre to post) changes in urinary mutagenicity on burn days compared to non-burn days. Moreover, results suggest that firefighters using drip-torches to light fires during prescribed burns potentially have higher urinary mutagenicity compared to other work tasks. Findings from this study suggest that diesel exposure may also contribute to increases in urinary mutagenic potency.

Disclaimer

These conclusions do not reflect the view or policies of the U.S. EPA.

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NC and performed there. Sincere gratitude goes to William Crolly, Chris Hobson, Paul Varnedoe, John Blake, and the USFS-Savannah River crew and subjects who participated in the study.

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Table 5.1. Unadjusted Means of Urinary Mutagenicity and Malondialdehyde Concentrations by Day Type and Time of Sample Collection.

Day Type	Sample Collection Time	Crude Urine Mutagenicity (rev/ml-eq)	Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	Crude Free MDA (nmol/l)	Creatinine Adjusted Free MDA (µmol MDA/mole creatinine)
Burn Day					
	Pre-Work Shift	Sample Size *n* =34[a]		Sample Size *n* =50	
	Arithmetic Mean (95%CL)	4.0 (2.5, 5.5)	0.46 (0.30, 0.62)	980.4 (789.3, 1171.5)	105.6 (82.3, 128.8)
	Geometric Mean (95%CL)	3.1 (2.2, 4.4)	0.36 (0.27, 0.49)	758.9 (607.5, 947.9)	89.2 (76.4, 104.0)
	Ranges	0, 23.6	0, 1.98	134.4, 3172.1	35.8, 551.4
	Post-Work Shift	Sample Size *n* =33		Sample Size *n* =50	
	Arithmetic Mean (95%CL)	5.1 (3.4, 6.8)	0.56 (0.28, 0.84)	1074.1 (879.4, 1268.9)	92.0 (80.5, 103.5)
	Geometric Mean (95%CL)	3.9 (3.0, 5.0)	0.37 (0.28, 0.50)	894.6 (748.5, 1069.0)	84.3 (74.9, 95.0)
	Ranges	0.7, 28	0.10, 4.46	175.3, 3415.5	37.1, 212.5
	Morning-after Work Shift	Sample Size *n* =28[b]		Sample Size *n* =44	
	Arithmetic Mean (95%CL)	4.7 (3.4, 5.9)	0.53 (0.28, 0.79)	969.2 (798.0, 1140.3)	82.6 (70.0, 95.2)
	Geometric Mean (95%CL)	4.3 (3.4, 5.4)	0.39 (0.27, 0.54)	829.9 (694.1, 992.2)	74.4 (65.0, 85.3)
	Ranges	0, 13.6	0, 3.46	159.5, 3255.0	34.0, 204.3
Non-Burn Day					
	Pre-Work Shift	Sample Size *n* =18		Sample Size *n* =20	
	Arithmetic Mean (95%CL)	4.3 (3.2, 5.5)	0.84 (0.42, 1.27)	611.1 (430.8, 791.4)	90.8 (68.7, 112.8)
	Geometric Mean (95%CL)	3.8 (3.0, 5.0)	0.54 (0.34, 0.88)	512.0 (383.4, 683.7)	81.6 (65.8, 101.1)
	Ranges	1.1, 11.0	0.14, 3.20	157.3, 1776.2	35.8, 551.4
	Post-Work Shift	Sample Size *n* =14[c]		Sample Size *n* =20	
	Arithmetic Mean (95%CL)	3.4 (2.0, 4.8)	0.45 (0.23, 0.67)	1089.3 (615.1, 1563.4)	114.6 (77.1, 152.1)
	Geometric Mean (95%CL)	3.9 (2.9, 5.4)	0.49 (0.33, 0.73)	828.6 (592.5, 1158.9)	98.6 (77.5, 125.5)
	Ranges	0, 7.3	0, 1.33	225.4, 4533.4	37.1, 212.5
	Morning-after Work Shift	Sample Size *n* =16[d]		Sample Size *n* =18	
	Arithmetic Mean (95%CL)	5.5 (4.0, 7.0)	0.53 (0.36, 0.69)	944.2 (783.0, 1105.3)	93.6 (56.2, 131.1)
	Geometric Mean (95%CL)	5.4 (4.3, 6.8)	0.50 (0.37, 0.66)	892.6 (750.2, 1062.1)	78.8 (60.1, 103.2)
	Ranges	0, 11.8	0, 1.11	489.0, 1778.8	34.0, 204.3

Note: Revertants/ml-equivalent of urine (rev/ml-eq), Malondialdehyde (MDA), *n* =person-day samples, 95% Confidence Limits (95% CL).

[a] Geometric mean *n* =31

[b] Geometric mean *n* =26

[c] Geometric mean *n* =11

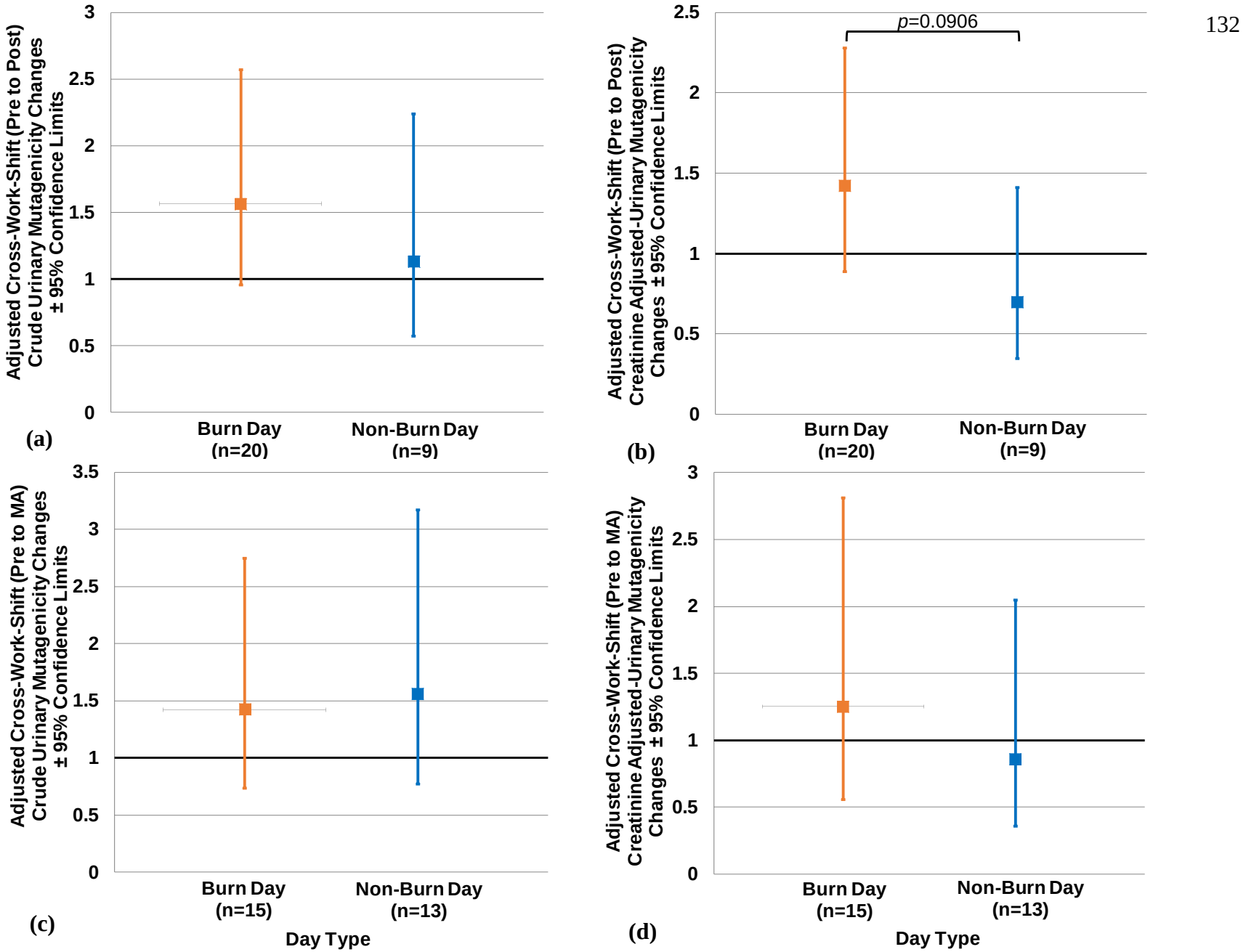
[d] Geometric mean *n* =15

Table 5.2. Percent Positive Results for Mutagenicity and Corresponding Unadjusted Means by Day Type and Time of Sample Collection

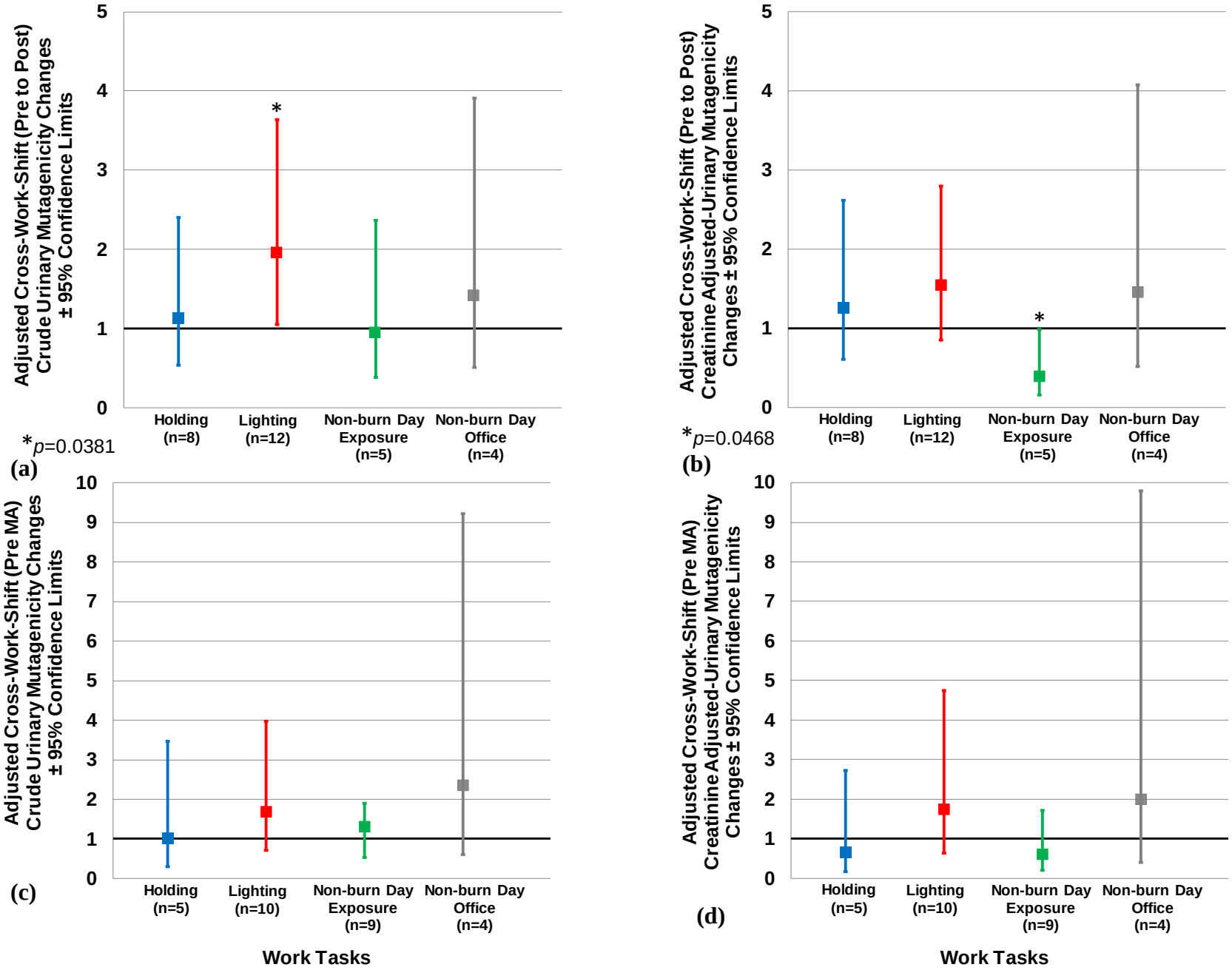
Day Type	Sample Collection Time		
	Pre-Work Shift	**Post-Work Shift**	**Morning-after Work Shift**
Burn Day			
Number and Percent Positive for Mutagenicity, *n* of total (%)	3 of 33 (9%)	12 of 32 (38%)	7 of 27 (26%)
Unadjusted Arithmetic Means ± SD			
Crude Urine Mutagenicity (rev/ml-eq)	14.6 ± 8.6	8.1 ± 6.5	8.5 ± 3.4
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.72 ± 0.42	0.90 ± 1.21	0.73 ± 0.37
Unadjusted Geometric Means ± SD			
Crude Urine Mutagenicity (rev/ml-eq)	12.8 ± 1.9	6.9 ± 1.7	8.0 ± 1.5
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.63 ± 1.91	0.57 ± 2.38	0.65 ± 1.71
Ranges (Min, Max)			
Crude Urine Mutagenicity (rev/ml-eq)	6.5, 23.6	4.4, 28.0	5.4, 13.6
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.32, 1.16	0.24, 4.46	0.32, 1.41
Non-Burn Day			
Number and Percent Positive for Mutagenicity, *n* of total (%)	2 of 18 (11%)	2 of 14 (14%)	2 of 16 (13%)
Unadjusted Arithmetic Means ± SD			
Crude Urine Mutagenicity (rev/ml-eq)	5.3 ± 1.1	5.3 ± 0.7	8.0 ± 0.1
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.64 ± 0.51	0.40 ± 0.03	0.66 ± 0.1
Unadjusted Geometric Means ± SD			
Crude Urine Mutagenicity (rev/ml-eq)	5.2 ± 1.2	5.3 ± 1.1	7.9 ± 1.0
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.53 ± 2.44	0.40 ± 1.07	0.66 ± 1.2
Ranges (Min, Max)			
Crude Urine Mutagenicity (rev/ml-eq)	4.5, 6.0	4.8, 5.8	7.9, 8.0
Creatinine Adjusted Urine Mutagenicity (rev/µmole creatinine)	0.28, 1.00	0.38, 0.41	0.58, 0.74

Note: *n* = person-day samples; Revertants/ml-equivalent of urine (rev/ml-eq); Standard deviation (SD).

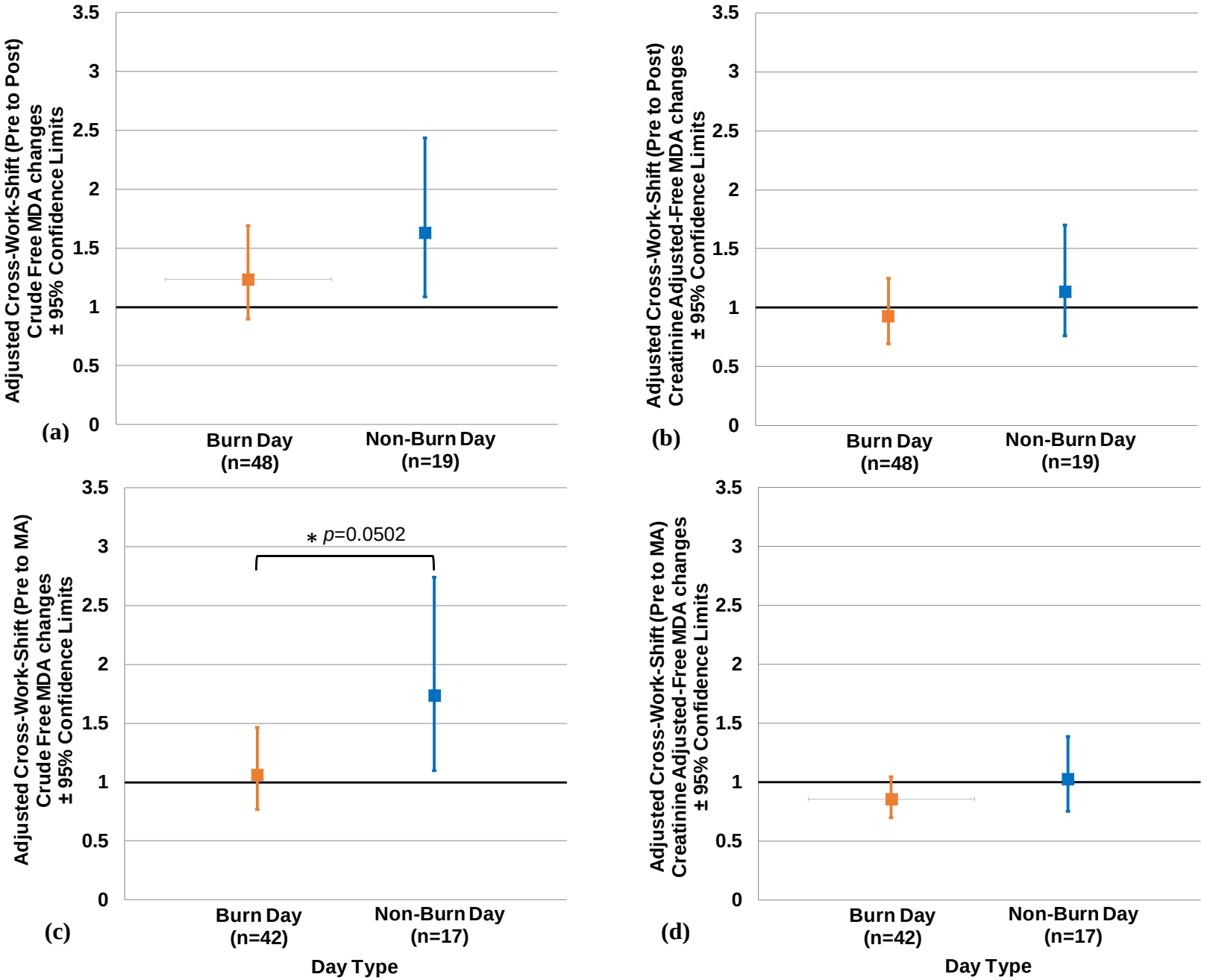
Additional Note: *n* of total = positive samples out of the total samples thus far analyzed.



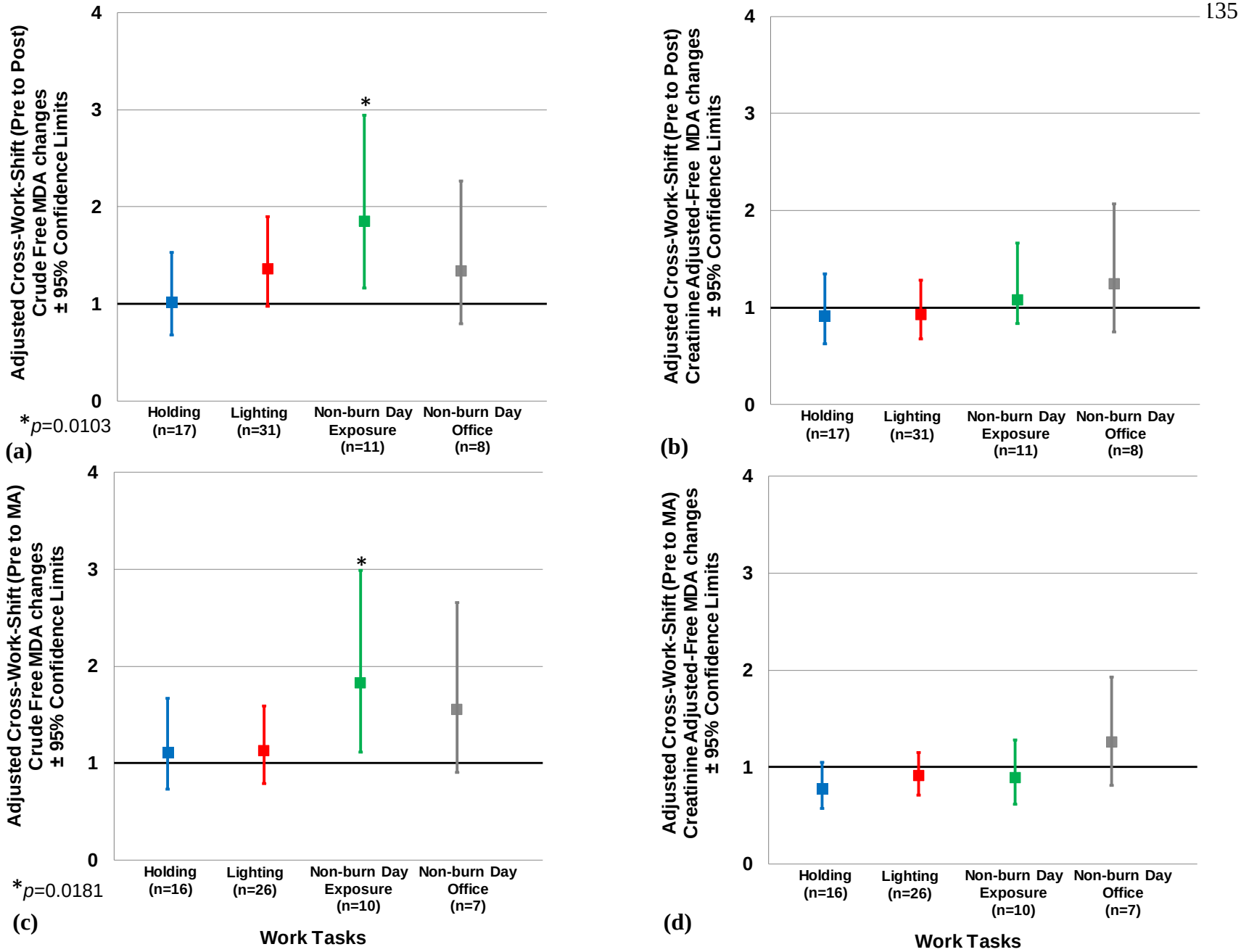
Figures 5.1(a-d). Adjusted Cross-Work Shift Changes in Crude and Creatinine-adjusted Urinary Mutagenicity Concentrations according to Day Type. Pre to post work shift changes are depicted in Figs. 5.1a and 5.1b. Pre to morning-after (MA) work shift changes are depicted in Figs. 5.1c and 5.2d. No significant difference was observed between burn day and non-burn day samples for Figs. 5. 2a, 5.2b, or 5.2d, however marginal significant differences were observed in Fig. 5.2c (p-value=0.0906). Note: n= person-day pre-post paired or pre-ma paired samples, respectively; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios or morning-after-work-shift/pre-work-shift ratios, respectively. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes are statistically different from 1 (p-values < 0.05).



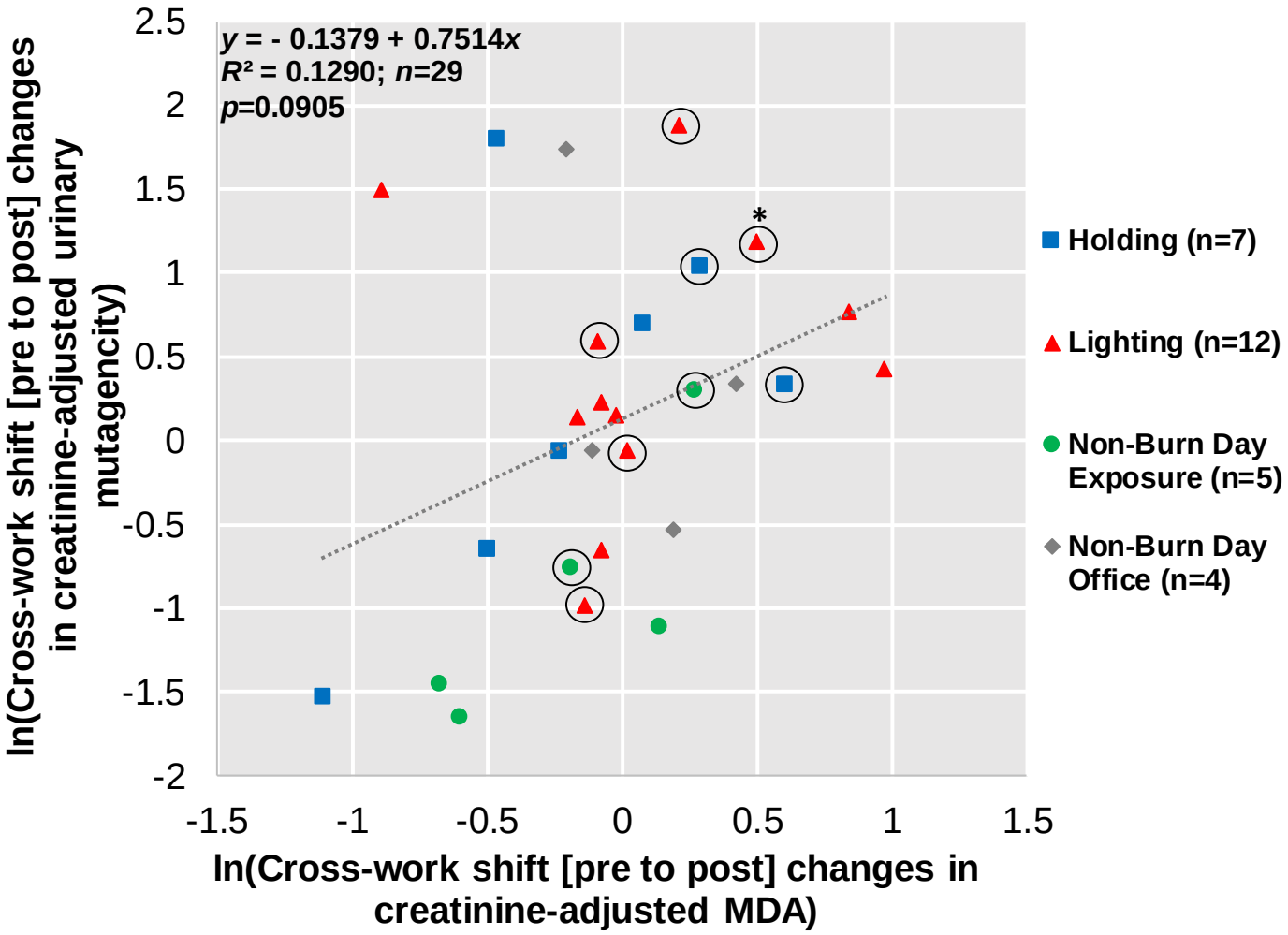
Figures 5.2(a-d). Adjusted Cross-Work Shift Changes in Crude and Creatinine-adjusted Urinary Mutagenicity Concentrations according to Work Tasks. Pre to post work shift changes are depicted in Figs. 5.2a and 5.2b. Pre to morning-after (MA) work shift changes are depicted in Figs. 5.2c and 5.2d. No significant difference was observed across work tasks for all figures. Note: *n*= person-day pre-post paired or pre-ma paired samples, respectively; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios or morning-after-work-shift/pre-work-shift ratios, respectively. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes are statistically different from 1 (*p*-values < 0.05).



Figures 5.3(a-d). Adjusted Cross-Work Shift Changes in Crude and Creatinine-adjusted Urinary Malondialdehyde Concentrations according to Day Type. Pre to post work shift changes are depicted in Figs. 5.3a and 5.3b. Pre to morning-after (MA) work shift changes are depicted in Figs. 5.3c and 5.3d. No significant difference was observed between burn day and non-burn day samples for Figs. 5.3a, 5.3b, or 5.3d, however marginal significant differences were observed in Fig. 5.3c (p-value <0.05 was considered significant). Note: n= person-day pre-post paired or pre-ma paired samples, respectively; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios or morning-after-work-shift/pre-work-shift ratios, respectively. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes are statistically different from 1 (p-values < 0.05).



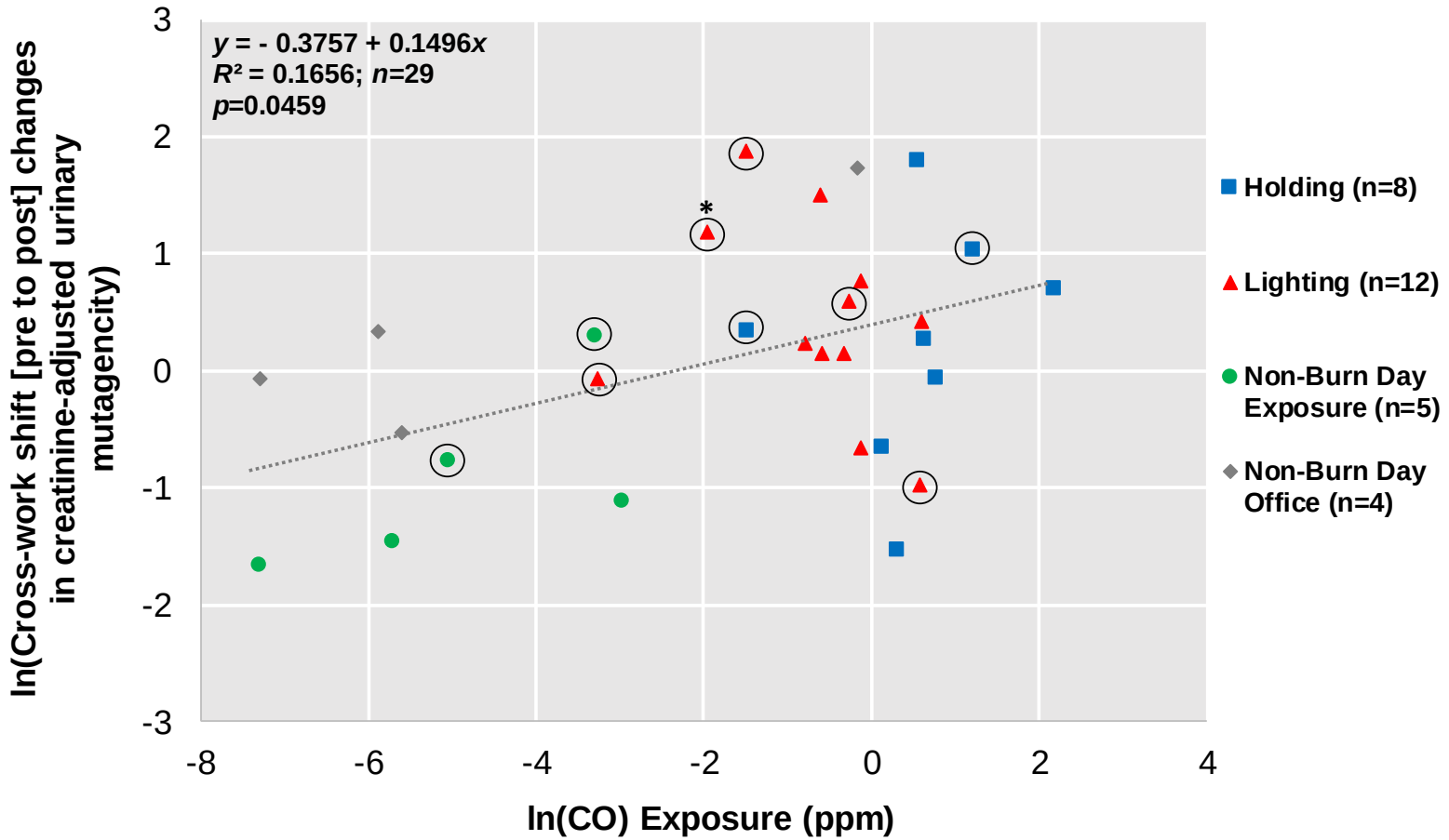
Figures 5.4(a-d). Adjusted Cross-Work Shift Changes in Crude and Creatinine-adjusted Urinary Malondialdehyde Concentrations according to Work Tasks. Pre to post work shift changes are depicted in Figs. 5.4a and 5.4b. Pre to morning-after (MA) work shift changes are depicted in Figs. 5.4c and 5.4d. No significant difference was observed across work tasks for all figures. Note: *n*= person-day pre-post paired or pre-ma paired samples, respectively; Cross-work-shift changes are reported as post-work-shift/pre-work-shift ratios or morning-after-work-shift/pre-work-shift ratios, respectively. Where 95% confidence limits do not cross the x-axis, cross-work-shift changes are statistically different from 1 (*p*-values < 0.05).



◯ Indicates a cross-work shift (pre to post) paired sample is positive for mutagencity

* Pre-work shift rather than the post-work shift sample was positive for mutagenicity

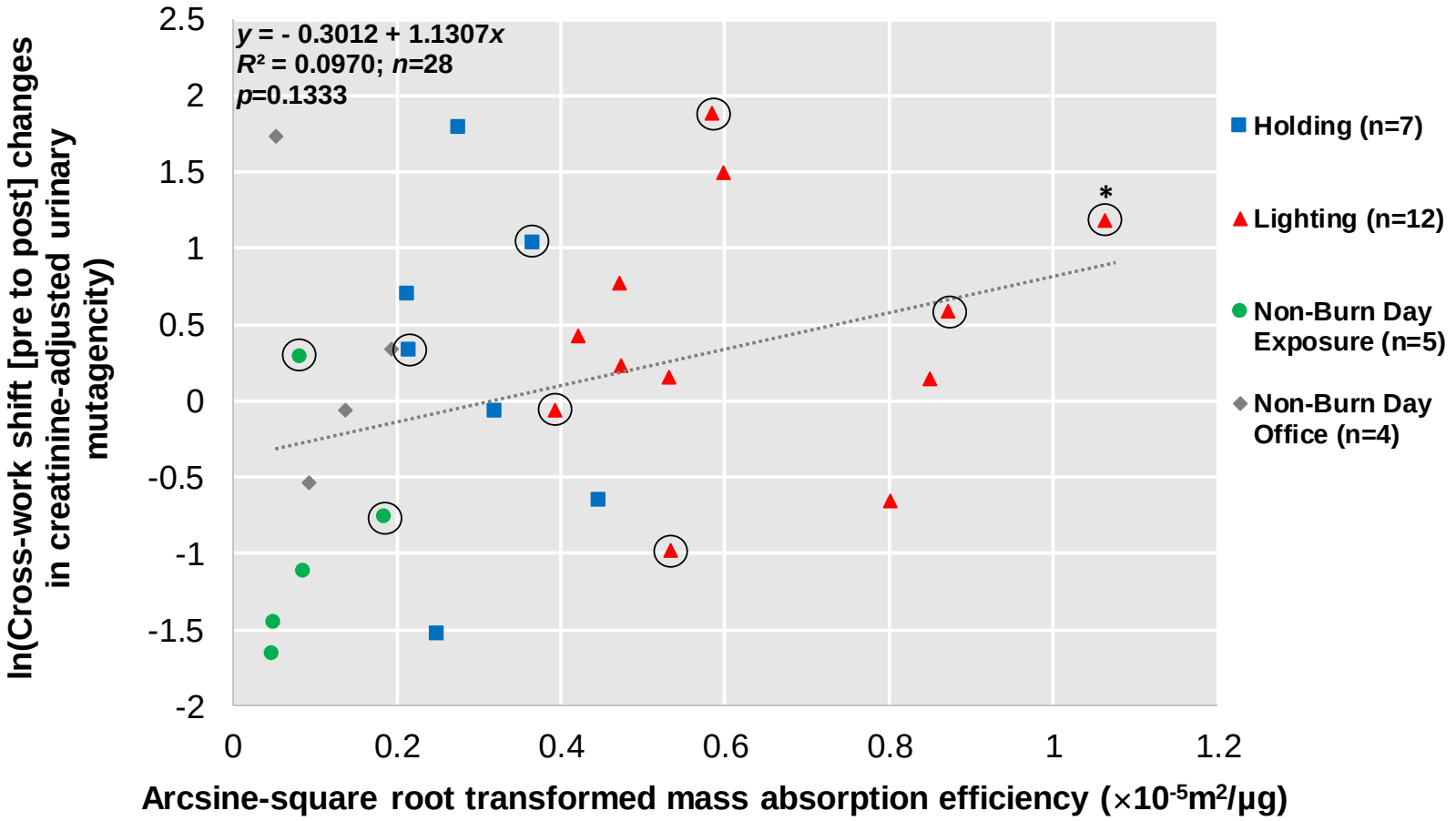
Figure 5.5. Correlation between Log-transformed Cross-Work Shift (Pre to Post) Changes in Creatinine-adjusted Urinary Mutagenicity and Log-transformed Creatinine-adjusted Free MDA. Person days are indicated as *n*. Linear mixed effect model results showed a positive marginally significant correlation (p=0.0905).



◯ Indicates a cross-work shift (pre to post) paired sample is positive for mutagencity

*Pre-work shift rather than the post-work shift sample was positive for mutagenicity

Figure 5.6. Correlation between Log-transformed Carbon Monoxide Exposure and Log-transformed Cross-Work Shift (Pre to Post) Changes in Creatinine-adjusted Urinary Mutagenicity. Person days are indicated as *n*. Linear mixed effect model results showed a positive significant correlation (p=0.0459).



◯ Indicates a cross-work shift (pre to post) paired sample is positive for mutagencity

*Pre-work shift rather than the post-work shift sample was positive for mutagenicity

Figure 5.7. Correlation between Arcsine-square root Transformed Mass Absorption Efficiency (Surrogate for Black Carbon/$PM_{2.5}$ Ratio) and Log-transformed Cross-Work Shift (Pre to Post) Changes in Creatinine-adjusted Urinary Mutagenicity. Person days are indicated as *n*. Linear mixed effect model results showed a positive (though not significant) correlation (*p*=0.1333).

CHAPTER 6

SUMMARY AND CONCLUSION

Standard grade respiratory protection is typically not worn among wildland firefighters.[1] Constituents of wood smoke and non-wood smoke occupational exposures such as diesel and gasoline exhaust among firefighters working at prescribed burns may be a concern for health; however, few studies on the topic have been conducted.[2] Wood smoke exposure is thought to induce oxidative stress in lung cells, lipid peroxidation, DNA damage, and systemic inflammation,[2-5] all of which are likely precursors and contributors to adverse health outcomes like cardio-respiratory diseases.[2, 6] Constituents of wood smoke such as particulate matter ($PM_{2.5}$), carbon monoxide (CO), acrolein, and formaldehyde have been shown to be elevated among wildland firefighters and orders of magnitude above recommended ceiling limits.[2] Health effect seen among wildland firefighters have been limited to respiratory symptoms, acute airway inflammation, acute systemic inflammation, oxidative stress, arterial stiffness (a factor involved in the pathogenesis of cardiovascular diseases such as heart attacks), and perhaps temporary lung function declines over burn seasons.[2, 4, 7-12] One of the many data gaps impeding the understanding of the underlying health risks of wood smoke exposure is the lack of health studies among firefighters and the characterization of their occupational exposures. Results from our previous study suggest that firefighting work tasks may influence particulate composition and toxicity of inhaled exposure.[11] Improved exposure assessment approaches capable of distinguishing among the sources and estimating internal systemic dose of exposures are needed for determining accurate

dose-response relationships in epidemiological studies. Therefore, this dissertation sought to characterize work task-related occupational smoke exposures and assess their association with select biomarkers of exposure and effect, with the intention of advancing the understanding of underlying mechanisms involved in acute health effects seen after wildland firefighting.

This dissertation presents biological and exposure data collected from a repeated measures study on a small cohort of wildland firefighters working at prescribed burns and non-burn days during January-July 2015 at Savannah River Site (SRS), SC, USA. SRS is a United States Department of Energy National Environmental Research Park with the majority of the park composed of a mixture of hardwood or pine species.[13] About 12% of the site has had or remains designated for the use of nuclear processing purposes or for designated personnel research and office buildings.[14]

Summary findings from the first manuscript included in this dissertation showed that $PM_{2.5}$ exposures were not significantly different between lighters and holders, which typically had been observed in previous studies.[14, 15] These observations may be due to shorter burn durations, fewer acres burned, and current use of enclosed fire engine vehicles rather than open-air mule utility vehicles during holding seen in our current study compared to previous years. Ventilation rates among firefighters lighting by foot with drip-torches had significantly higher ventilation rates compared to other work tasks. When ventilation rates were applied, lighters had on average an estimated 1.3 and 1.2 times higher, though not significant, inhaled amount of $PM_{2.5}$ (µg) and inhaled dose of $PM_{2.5}$ (µg/kg body weight), respectively, compared to holders. Light absorbing carbon (LAC) of $PM_{2.5}$, used as a surrogate for black carbon (BC), was significantly 3 times higher on personal air filter samples from lighters compared holders and other work tasks.

Summary findings from the second manuscript presented in this dissertation showed that significant cross-work shift increases in systemic inflammation was observed among firefighters who lit fires by drip-torch. Lighters had significantly higher cross-work shift increases in three proinflammatory mediators included interleukin-8, C-reactive protein, and serum amyloid A, compared to holders. These findings were consistent with our previous pilot study results, where a significant cross-work shift increase in interleukin-8 was also observed among lighters.[11] Moreover, we found positive significant associations between interleukin-8 and segmented-neutrophil (p=0.0179), and mass absorption efficiency (p=0.0080), respectively.

The third manuscript of this dissertation include the exploration of urinary mutagenicity as a biomarker of genotoxic exposures among the same cohort of wildland firefighters. Overall findings presented herein showed a two-fold (marginally significant) higher cross-work shift change in creatinine-adjusted urinary mutagenicity on burn days compared to non-burn day samples (p=0.0906). Moreover, lighters had a two-fold higher (though not significant) cross-work shift increase in crude urinary mutagenicity compared to holding and non-burn day exposures. We also observed positive associations between creatinine-adjusted urinary mutagenicity and MDA (p=0.0905), CO (p=0.0459), and mass absorption efficiency (p=0.1333), respectively.

CONCLUSION

In conclusion, results from this dissertation suggest that intermittent occupational diesel exposures along with wood smoke exposure contribute to systemic inflammation and perhaps increases in urinary mutagenic potency in healthy, seasonal wildland firefighters at prescribed burns. Also, data suggest that urinary mutagenicity may serve as a suitable measure of occupational smoke exposure. Work tasks-related exposures were observed and data suggest that wildland firefighters may also be inadvertently exposed to various pollutants on non-burn work

days. PM speciation will be important in the assessment of toxicity as it relates to its source and composition. Moreover, we were able to mechanistically determine significant cross-work shift changes in acute systemic inflammation as indicated by a positive significant correlation between elevated interleukin-8 levels and peripheral blood segmented-neutrophils. However, future studies are needed to evaluate the chronic effects of occupational exposures among wildland firefighters. Information gained from this dissertation's results can be used to inform the United States Forest Service whether occupational work tasks should be modified in order to reduce exposure, specifically during prescribed burning. Results presented herein may be used to inform the development of occupational exposure limits for wildland firefighters and ultimately toward the protection and improvement of health among firefighters. At large, findings from this dissertation may be relevant for other exposure situations, such as biomass and/or wood smoke-related household air pollution exposures in low-income countries or community wide exposures to wood smoke during wildland fire events.

FUTURE DIRECTIONS

Exhaled breath condensate (EBC) samples were also collected from the same study population reported in this dissertation. In the future (contingent on funding), data gained from the analyses of the EBC samples could be useful for the assessment pulmonary lung inflammation and oxidative stress. Several human and *in vivo* studies have identified several biomarkers of inflammation and oxidative stress in EBC.[16-21] Some inflammatory mediators that have been measurable in EBC include leukotriene B_4, cysteinyl-leukotrienes, prostaglandins, histamine, adenosine, interleukin-4, interleukin-6, interleukin-8, interferon-γ, and hydrogen ions (pH).[16] Collection of EBC is non-invasive and may be more amenable to subject participation compared to bronchoalveolar lavage or induced sputum collection procedures.

Lastly, field intervention studies to help identify effective methods to mitigate occupational smoke exposure and reduce its acute health effects observed in wildland firefighters will be a useful next step, building upon the information presented in this dissertation. It would be interesting to determine if effective exposure mitigation methods (i.e. masks or real-time sensor alarms)[22] would be associated with decreases in select biomarkers of exposure and effect, similar to those analyzed in this dissertation. To date, few intervention studies have been conducted among wildland firefighters and results so far have been limited to the assessment of lung function parameters.[23, 24]

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APPENDICES

APPENDIX A: STUDY CONSENT FORM

UNIVERSITY OF GEORGIA
CONSENT FORM #1
THE EFFECT OF WORK TASK AND SMOKE CONSTITUENTS ON INFLAMMATION IN FIREFIGHTERS WORKING AT PRESCRIBED BURNS AND WILDLAND FIRES

Researcher's Statement
We are asking you to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. This form is designed to give you the information about the study so you can decide whether to be in the study or not. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent." A copy of this form will be given to you.

Principal Investigator: *Dr. Luke Naeher*
Department of Environmental Health Science
University of Georgia, USA
lneaher@uga.edu
706.542.4104

Purpose of the Study
The reason for this study is to obtain information on firefighter exposure to air pollution from prescribed forest burns and wild fires in forested locations within the US in order to establish information used for future invention purpose to protect firefighter health. We are recruiting participants in this study who are in general good health (i.e. Non-Asthmatic and No Respiratory Diseases), currently non-smokers, not pregnant, and 18 years old and older.

Study Procedures
If you agree to participate, you will be asked to:

1. *Answer one baseline questionnaire before the study begins. However, you as a potential participant may be withdrawn from further participation based on your baseline questionnaire responses*
2. *Answer 7 short questionnaires throughout the duration of the study to better characterize your daily exposure*
3. *Wear personal air sampling equipment for 8-12 hours for 4 work days at which you are working at a prescribed burn/wild fire, and for 3 work days at which you are or not working at a prescribed burn/wildfire*
4. *Provide one before (morning) work-shift, one after (afternoon/evening) work-shift, and one immediate next-day morning after work-shift finger stick blood sample for the 7 sampling days (estimated time commitment: 5 minutes for each collection visit)*
5. *Provided one before (morning) work-shift, one after (afternoon/evening) work-shift, and one immediate next-day morning after work-shift exhaled breath condensate sample for 7 sampling days (Estimated time commitment: 10 minutes for each collection visit)*
6. *Provided one before (morning) work-shift, one after (afternoon/evening), and one immediate next-day morning after urine sample for 7 sampling days*

In summary: The estimated total time commitment for you as a participant in the study is approximately 5-6 hours over the course of the entire study

Risks and discomforts

- *The risks of drawing blood from a finger stick include discomfort at the site of the stick; possible redness; rarely an infection; and, uncommonly faintness from the procedure. These risks will be minimized by the use of standard sterile techniques. The amount of blood taken should have no negative effects.*
- *The questionnaires will seek self-reported health-related information. However, information provided will be kept confidential and you can skip any questions that you do not feel comfortable responding to. Furthermore, to maintain your confidentiality, your employer or supervisor must not look at or review your answers. Your completed questionnaires should be immediately handed directly to the research professional who will review it. All partially or completed questionnaires will be locked in a secure location in a locked cabinet and locked room outside of your employer's workplace.*
- *There are no foreseeable risks for urine or breath collection. They are safe and non-invasive procedures. You are encouraged to wash your hands before and after sampling collection to maintain sanitation.*
- *The personal air monitors will be placed within your breathing zone for 8-12 hours each sampling day. A low humming noise can be heard when the personal air monitor are running. The personal air monitors are relatively compact and lightweight (less than 0.5 pounds) and easily fit into a shirt pocket or clip on a belt.*

Benefits

- *The benefits for your workplace and community are that the air pollution and corresponding health response to woodsmoke may help leaders in occupational and public health agencies reduce elevated occupational and environmental exposures to you and others in your workplace and community.*
- *The potential benefits to society or humankind is that this study will provide understanding of the health risk and information on exposure to air pollution from prescribed forest burns/wild fires in order to establish information used for future exposure mitigation invention purposes to protect firefighter health. Data from this research can be used to inform the development of occupational woodsmoke exposure standards for wildland firefighters, as well as protecting and improving the health and well-being of wildland firefighters around the United States.*

Incentives for participation

You will receive a $10 Walmart gift certificate for each sampling day completed (i.e. if sampled for 2 days, a $20 Walmart gift certificate will be issued to you; 7 days equates to a $70 Walmart gift certificate) as compensation for being in this study. If you decided to withdraw from the study, you will be compensated based on the activities you completed.

Privacy/Confidentiality

The blood will not be tested for HIV-AIDS. Your finger stick blood, breath, and urine samples will not be used for diagnostic purposes and will not be tested for drug screening.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. You will be assigned an identifying number and this number will be used on all air monitoring and questionnaires you fill out. All completed questionnaire forms and personal information will be kept confidential and locked in a file cabinet in a locked room. Information that links your personal name to your assigned number will be properly shredded and disposed after data collection is completed.

In order to process the payment for your participation, the researchers need to collect your name and mailing address on a separate payment form. This completed form will be sent to the Department of Environmental Health Science business office and then to the University of Georgia business office. The researcher has been informed that these offices will keep your information private, but may have to release your name and the amount of compensation paid to you to the IRS, if ever asked. The researchers connected with this study have gone to great lengths to protect your survey information and will keep this

confidential in locked files. However, the researcher is not responsible once your name and mailing address leave his office for processing of your payment.

Taking part is voluntary
Your involvement in the study is voluntary. You should understand that you do not have to take part in the study if you do not want to. You can refuse to participate or stop taking part without giving any reason, and without penalty or loss of benefits to which you are otherwise entitled. If you decide to withdraw from the study, the information that can be identified as yours will be kept as part of the study and may continue to be analyzed, unless you make a written request to remove, return, or destroy the information.

Consent to bank blood, exhaled breath and urine
This requests you to allow us to store a portion of your blood, exhaled breath, and urine sample in the University of Georgia, Department of Environmental Health Science Laboratory in Athens, Georgia, USA so that it could be used for possible future studies. Blood samples may be used to test for changes in the cell's gene material due to woodsmoke exposure. If you agree to have the blood, exhaled breath, and urine stored, you can ask that the stored blood, exhaled breath, or urine be destroyed at any time by contacting Dr. Luke Naeher at 706-542-2454. Refusal to agree to this consent to bank would in no way prevent you from participating in the study.

Please Check One and Initial:

( ) Initial:_____ 1. **I agree** to allow a portion of my blood, exhaled breath, and urine sample from this study to be stored for possible future testing as explained above.

OR

( ) Initial:_____ 2. **I do not agree** to allow a portion of my blood, exhaled breath, and urine sample from this study to be stored for future testing. These samples should not be used for anything but this study, and the samples should be destroyed one year after this study is finished.

If you have questions
The main researcher conducting this study is *Dr. Luke Naeher*, a professor at the University of Georgia. Please ask any questions you have now. If you have questions later, you may contact *Dr. Luke Naeher* at *lneaher@uga.edu* or at *706.542.4104*. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at 706.542.3199 or irb@uga.edu.

Research Subject's Consent to Participate in Research:
To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form, and have had all of your questions answered.

_____	_____	_____
Name of Researcher (Print)	Signature	Date
_____	_____	_____
Name of Participant (Print)	Signature	Date

Please sign both copies, keep one and return one to the researcher.

APPENDIX B: BASELINE QUESTIONNAIRE

Office Use Only – Technician: _____

SRS Fire Crew Baseline Questionnaire, page 1

Please Note: To maintain your confidentiality, your employer or supervisor must not look at or review your answers. Your completed questionnaires should be immediately handed directly to the research professional who will review it.

Subject ID #: _____ **Date:** _____

1. What is your Job Title?

2. What is your Age?

3. Weight? lbs.

4. Height? Feet Inches

5. How many years have you been a firefighter? (If less than 1 year, please indicate # of months)

6. How many months/years have you been working at prescribed burns? Years Months

7. Approximately how many PRESCRIBED BURNS have you worked in your career? (Circle One)

Less than 50 | 50-100 | 101-200 | 201-450 | 451-800 | 801 or more

8. Approximately how many WILD FIRES have you worked in your career? (Circle One)

Less than 50 | 50-100 | 101-200 | 201-450 | 451-800 | 801 or more

9. How many work days a year would you estimate that you are exposed to smoke from a fire at work? This estimation includes smoke from prescribed burns and wild fires. (Circle One)

0-10 | 11-30 | 31-50 | 51-100 | 101-150 | 151-200 | 201 or more

10. Do you wear any form of respiratory protection when fighting fires? (Circle Answer)

Yes No

If YES, what type of respiratory protection do you use and under what conditions do you use it? _____

11. How many times a week do you do cardiovascular exercise for greater than 15 min? (Circle One)

0 | 1-2 | 3-4 | 5-7

12. Before working at this job have you had any respiratory or cardiovascular problems? (Circle Answer) Yes No

If YES, please explain: _____

13. Currently, do you have any respiratory or cardiovascular problems? (Circle Answer) Yes No

If YES, please explain: _____

14. Have you ever used a bronchodilator or a puffer? (Circle Answer) Yes No

If YES, do you currently use one?: _____

Office Use Only – Technician: _____

SRS Fire Crew Baseline Questionnaire, page 2

15. Do you usually cough first thing in the morning? (Circle Answer)	Yes	No
16. Do you usually cough at other times during the day or night? (Circle Answer)	Yes	No
17. Has your chest <u>ever</u> sounded wheezy or whistling when you have had a cold? (Circle Answer)	Yes	No
18. Has your chest <u>ever</u> sounded wheezy or whistling when you <u>have not</u> had a cold? (Circle Answer)	Yes	No
19. Have you <u>ever</u> had episodes of shortness of breath? (Circle Answer)	Yes	No
20. Do you have elevated blood pressure? (Circle Answer)	Yes	No
21. Do you have asthma? (Circle Answer)	Yes	No
22. Do you have allergies? (Circle Answer)	Yes	No

If <u>YES</u>, please describe type of allergy <u>and</u> symptoms _____

_____

23. Have you ever done a spirometry (lung function) test before? (Circle Answer)	Yes	No

If <u>YES</u>, did you have any problems?: _____

If problems, please explain: _____

24. Does your biological father <u>or</u> mother have any of the following health problems?: (Circle Answer)		
Chronic Bronchitis	Yes	No
Emphysema	Yes	No
Chronic Obstructive Pulmonary Disease (Lung Disease)	Yes	No
Asthma	Yes	No
Lung Cancer	Yes	No
Cardiovascular Diseases (Heart Diseases/Heart Attack)	Yes	No
25. Have you ever had thoracic surgery? (Circle Answer)	Yes	No
26. Are you pregnant? (Circle Answer)	Yes	No

Office Use Only – Technician: _____

SRS Fire Crew Baseline Questionnaire, page 3

27. Have you <u>ever</u> smoked any tobacco products? (Circle Answer)	Yes	No

If <u>YES</u>, how recent ago? _____

And how many months/years did you smoke? _____

28. Do you <u>currently</u> smoke cigarettes? (Circle Answer)	Yes	No

If <u>YES</u>, how many cigarettes do you smoke a day? _____

29. Do you <u>currently</u> smoke pipes or cigars? (Circle Answer)	Yes	No

If <u>YES</u>, how many pipes or cigars do you smoke a day? _____

30. Do you <u>currently</u> smoke pipes or cigars? (Circle Answer)	Yes	No

If <u>YES</u>, how many pipes or cigars do you smoke a day? _____

31. Do you <u>currently</u> use smokeless tobacco products (i.e. chew/dip tobacco)? (Circle Answer)	Yes	No

If <u>YES</u>, how many times a day? _____

32. Does anyone smoke cigarettes within your home on a regular basis? (Circle Answer)	Yes	No

If <u>YES</u>, how many cigarettes does that person smoke a day? _____

33. What kind of heating do you use in your home during the colder months out of the year?: (Check all that apply)

- ☐ **District Heating**
- ☐ **Central Heating (inside your building)**
- ☐ **Single Stoves/ Heaters**
  - ☐ **with electricity**
  - ☐ **with gas**
  - ☐ **with wood**
  - ☐ **with kerosene/paraffin**
  - ☐ **with fuel/heating oil**
- ☐ **Fireplace**
- ☐ **There is no heating**
- ☐ **Other:** _____

APPENDIX C: DAILY EXPOSURE-WORK TASK QUESTIONNAIRE

Office Use Only – Technician: _____ Time of Questionnaire: _____

Daily Smoke Exposure -Task Questionnaire

Please Note: To maintain your confidentiality, your employer or supervisor must not look at or review your answers. Your completed questionnaires should be immediately handed directly to the research professional who will review it.

ID Number: _____ **Date:** _____

Time of **start** of shift: _____ Time of **end** of shift: _____

1. Did you assist in the prescribed burn today? (Circle Answer)	Yes	No

If YES, approximately how many acres were burned? _____

2. How many days has it been since your last work at a prescribed burn?

_____Days

3. What was your primary job task during the work day today? (Check One)

☐ Mostly (more than half a day) **Lighting** ☐ Mostly (more than half a day) **Holding**

☐ **Lighting & Holding** (Approx. equal time spent) ☐ **Other** (please specify) _____

_____

4. Did you use any sort of respiratory protection today? (Circle Answer)	Yes	No

If YES, what type? _____

5. Did you smoke any cigarettes/cigars during the work day? (Circle Answer)	Yes	No

If YES, how many? _____

6. Were you around any people who were smoking tobacco today? (Circle Answer)	Yes	No

If YES, how long? _____

7. Did you chew/dip any smokeless tobacco products today? (Circle Answer)	Yes	No

If YES, how much? _____

8. How much wood smoke would you feel that you encountered today? (Circle One)

None to very little	low level	medium level	medium to high level	high level

Office Use Only – Technician: _____ Time of Questionnaire:_____

9. Did you experience exposure to <u>any</u> of the following today? (Circle all that apply)		
Chain Saw	Dust	Vehicle exhaust
10. Did you notice or have any problems with the sampling equipment today? (Circle Answer)	Yes	No

If <u>YES</u>, please explain (hose disconnection, early shut off, cyclone fell, etc.) _____

_____

11. Have you been exposed to <u>woodsmoke</u> *OUTSIDE* of work <u>within the last 24 hours</u>? (Circle Answer)	Yes	No

If <u>YES</u>, how long? _____

12. Have you been exposed to <u>cigarette smoke</u> *OUTSIDE* of work <u>within the last 24 hours</u>? (Circle Answer)	Yes	No

If <u>YES</u>, how long? _____

13. Did you have any form of upper/lower respiratory (virus/ bacterial) infection known to you <u>within the last 2 weeks</u>?(Circle One)	Yes	No

If <u>YES</u>, are you <u>currently</u> still feeling ill today? _____

What are your symptoms? _____

14. Did you take any medication (prescribed or over-the counter medicines) <u>within the last 1 week</u>? (Circle Answer)	Yes	No

When? _____

Please specify (i.e. Ibuprofen, Aspirin, etc.):_____

15. Did you take any <u>vitamin</u> supplements <u>within the last 1 week</u>? (Circle Answer)	Yes	No

When? _____

Please specify (type i.e. multivitamin; brand):_____

16. Did you take any other dietary supplements (i.e. minerals, herbs, energy bars, etc.) <u>within the last 1 week</u>? (Circle Answer)	Yes	No

When?_____

Please specify (type; brand):_____

17. Did you take any <u>energy drinks</u> (i.e. Monster, Red Bull, 5-Star Energy, etc.) <u>within the last 1 week</u>? (Circle Answer)	Yes	No

When?_____ **Please specify:** _____

Office Use Only – Technician: _____ Time of Questionnaire: _____

---

18. Did you consume fruits or vegetables today? (Circle Answer)	Yes	No

Please list them: _____

19. Please circle the following beverages if you had any within the last 24 hours:

Coffee	Green Tea	Other types of Tea	Red wine	Fruit Juice

20. Did you consume food that has been grilled/charbroiled within the last 24 hours? (Circle Answer)	Yes	No

21. Have you experienced any of the following symptoms today?: (Check all that apply)

Please also rank severity of symptoms: (Circle One)
1 – Little
2 – Low
3 – Medium
4 – High
5 – Very High

☐ Watery Eyes	1	2	3	4	5
☐ Red Eyes	1	2	3	4	5
☐ Coughing	1	2	3	4	5
☐ Loss/Shortness of Breath	1	2	3	4	5
☐ Runny Nose	1	2	3	4	5
☐ Chest Pain	1	2	3	4	5
☐ Phlegm	1	2	3	4	5
☐ Chest Tightness	1	2	3	4	5
☐ Chest Congestion	1	2	3	4	5
☐ Sore Throat	1	2	3	4	5
☐ Other (Please specify): _____	1	2	3	4	5

APPENDIX D: MORNING-AFTER QUESTIONNAIRE

Office Use Only – Technician: _____ Time of Questionnaire:_____

Daily Smoke Exposure -Task Questionnaire

Please Note: To maintain your confidentiality, your employer or supervisor must not look at or review your answers. Your completed questionnaires should be immediately handed directly to the research professional who will review it.

ID Number: _____ **Date:**_____

1. Have you been exposed to woodsmoke *OUTSIDE* of work since you finished your work-shift yesterday?	Yes	No
2. Have you been exposed to cigarette smoke *OUTSIDE* of work since you finished your work-shift yesterday?	Yes	No
3. Do you have any form of upper/lower respiratory (virus/ bacterial) infection (i.e. cold, flu, allegeries) known to you this morning?	Yes	No
4. Did you take any medication (prescribed or over-the counter medicines) since you finished your work-shift yesterday?	Yes	No

Please specify (i.e. Ibuprofen, Aspirin, etc.):_____

5. Did you take any vitamin supplements since you finished your work-shift yesterday?	Yes	No

Please specify (type i.e. multivitamin; brand):_____

6. Did you take any energy drinks (i.e. Monster, Red Bull, 5-Star Energy, etc.) this morning?	Yes	No

Please specify: _____

7. Did you consume fruits or vegetables since you finished your work-shift yesterday?	Yes	No
8. Did you consume food that has been grilled/charbroiled within the last 24 hours?	Yes	No
9. Did you do cardio-based excersize (running, jogging, cycling, swiming, etc.) since you finished your work-shift yesterday ?	Yes	No

10. Please circle the following beverages if you had any since you finished your work-shift yesterday:

Coffee	Green Tea	Other types of Tea	Red wine	Fruit Juice

APPENDIX E:

REVIEW OF THE HEALTH EFFECTS OF WILDLAND FIRE SMOKE ON WILDLAND FIREFIGHTERS AND THE PUBLIC[1]

[1] Adetona O, Reinhardt TE, Domitrovich J, Broyles G, Adetona AM, Kleinman M, Ottmar RD, & Naeher LP. 2016. *Inhalation Toxicology*, *28*(3), 95-139, http://dx.doi.org/10.3109/08958378.2016.1145771.

ABSTRACT

Each year, the general public and wildland firefighters in the US are exposed to smoke from wildland fires. As part of an effort to characterize health risks of breathing this smoke, a review of the literature was conducted using five major databases, including PubMed and MEDLINE Web of Knowledge, to identify smoke components that present the highest hazard potential, the mechanisms of toxicity, review epidemiological studies for health effects and identify the current gap in knowledge on the health impacts of wildland fire smoke exposure. Respiratory events measured in time series studies as incidences of disease-caused mortality, hospital admissions, emergency room visits and symptoms in asthma and chronic obstructive pulmonary disease patients are the health effects that are most commonly associated with community level exposure to wildland fire smoke. A few recent studies have also determined associations between acute wildland fire smoke exposure and cardiovascular health end-points. These cardiopulmonary effects were mostly observed in association with ambient air concentrations of fine particulate matter ($PM_{2.5}$). However, research on the health effects of this mixture is currently limited. The health effects of acute exposures beyond susceptible populations and the effects of chronic exposures experienced by the wildland firefighter are largely unknown. Longitudinal studies of wildland firefighters during and/or after the firefighting career could help elucidate some of the unknown health impacts of cumulative exposure to wildland fire smoke, establish occupational exposure limits and help determine the types of exposure controls that may be applicable to the occupation.