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RESEARCH ARTICLE

Urinary mutagenicity and other biomarkers of occupational smoke exposure of wildland firefighters and oxidative stress

Anna M. Adetona[a], William Kyle Martin[b], Sarah H. Warren[c], Nancy M. Hanley[c], Olorunfemi Adetona[d], Junfeng (Jim) Zhang[e], Christopher Simpson[f], Mike Paulsen[f], Stephen Rathbun[g], Jia-Sheng Wang[a], David DeMarini[c] and Luke P. Naeher[a]

[a]Department of Environmental Health Sciences, College of Public Health, University of Georgia, Athens, GA, USA; [b]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA; [c]Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA; [d]Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH, USA; [e]Nicholas School of the Environment and Duke Global Health Institute, Duke University, Durham, NC, USA; [f]Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA, USA; [g]Department of Epidemiology and Biostatistics, College of Public Health, University of Georgia, Athens, GA, USA

ABSTRACT

Background: Wildland firefighters conducting prescribed burns are exposed to a complex mixture of pollutants, requiring an integrated measure of exposure.

Objective: We used urinary mutagenicity to assess if systemic exposure to mutagens is higher in firefighters after working at prescribed burns versus after non-burn work days. Other biomarkers of exposure and oxidative stress markers were also measured.

Methods: Using a repeated measures study design, we collected urine before, immediately after, and the morning after a work shift on prescribed burn and non-burn work days from 12 healthy subjects, and analyzed for malondialdehyde (MDA), 8-isoprostane, 1-hydroxypyrene (OH-pyrene), and mutagenicity in *Salmonella* YG1041 +S9. Particulate matter ($PM_{2.5}$) and carbon monoxide (CO) were measured by personal monitoring. Light-absorbing carbon (LAC) of $PM_{2.5}$ was measured as a surrogate for black carbon exposure. Linear mixed-effect models were used to assess cross-work shift changes in urinary biomarkers.

Results: No significant differences occurred in creatinine-adjusted urinary mutagenicity across the work shift between burn days and non-burn days. Firefighters lighting fires had a non-significant, 1.6-fold increase in urinary mutagenicity for burn versus non-burn day exposures. Positive associations were found between cross-work shift changes in creatinine-adjusted urinary mutagenicity and MDA ($p = 0.0010$), OH-pyrene ($p = 0.0001$), and mass absorption efficiency which is the LAC/$PM_{2.5}$ ratio ($p = 0.2245$), respectively. No significant effect of day type or work task on cross-work shift changes in MDA or 8-isoprostane was observed.

Conclusion: Urinary mutagenicity may serve as a suitable measure of occupational smoke exposures among wildland firefighters, especially among those lighting fires for prescribed burns.

Abbreviations list.: ACCEL: Composite variable computed from triaxial accelerometer measurements; BC: Black carbon; CO: Carbon monoxide; HPLC: High-performance liquid chromatography; IARC: International Agency for Research on Cancer; LAC: Light absorbing carbon; MDA: Malondialdehyde; OH-pyrene: 1-hydroxypyrene; OH-PAH: Hydroxylated polycyclic aromatic hydrocarbons; PM2.5: Particulate matter with median aerodynamic diameter $\leq$ 2.5 microns; USFS-SRS: United States Forest Service at Savannah River Site

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Introduction

Extensive studies show that smoke from wildland fires is associated with increased respiratory and cardiovascular disease among the general public (Adetona et al. 2016; Reid et al. 2016; Black et al. 2017; Cascio 2018). Wood smoke alone is composed of hundreds of constituents, many of which are mutagenic and carcinogenic (Naeher et al. 2007). There has been only one study of the mutagenicity of smoke from a wildland fire, and organic extracts of particulate matter <10 μm in diameter (PM_{10}) in which such fires in the Brazilian Amazon were found to be highly mutagenic in a *Salmonella* mutagenicity assay (de Oliveira Galvão et al. 2018). In addition, the International Agency for Research on Cancer (IARC 2010) has classified the household combustion of biomass (primarily wood) as a probable (Group 2A) human carcinogen.

Prescribed burning is a special category of wildland firefighting that involves the preplanned lighting of fires under the direction of certified management, usually for the

CONTACT Luke P. Naeher LNaeher@uga.edu Department of Environmental Health Science, College of Public Health, University of Georgia, 206 Environmental Health Science Building, Athens, GA 30602-2102, USA

prevention of wildland fires or to inhibit the advance of such fires, and these fires also can produce environmental and health impacts (Haikerwal et al. 2015; Adetona et al. 2016). Individuals initiating prescribed burns may experience exposures in addition to wood smoke, such as emissions from drip-torches fueled by a mixture of diesel and gasoline (Adetona et al. 2016). Diesel and gasoline emissions are also mutagenic and are classified, respectively, as known (Group 1) or possible (Group 2B) human carcinogens (IARC 2014). Although protective clothing is worn, wildland firefighters are not protected from inhalation exposures and typically do not wear protective masks, unlike municipal firefighters who use self-contained breathing apparatuses with supplied fresh air.

Many studies document the elevated exposure of wildland firefighters to potential mutagens based on concentrations of hydroxylated urinary metabolites of polycyclic aromatic hydrocarbons (OH-PAHs) (Adetona et al. 2016; Keir et al. 2017). Studies involving charcoal workers or people using wood-fired steam baths (temazcales) have also found that exposure to such wood smoke results in mutagenic urine, indicative of a systemic exposure to mutagenic and potentially carcinogenic compounds (Kato et al. 2004; Long et al. 2014). Although there is a recent report showing elevated urinary mutagenicity in municipal firefighters (Keir et al. 2017), no such study is reported for wildland firefighters. The purpose of this pilot study is to evaluate systemic genotoxicity in wildland firefighters exposed to smoke from wood and/or diesel/gasoline fuel exhaust before and after a prescribed burn through the assessment of urinary mutagenicity in the *Salmonella* (Ames) mutagenicity assay.

Urinary mutagenicity provides an integrated measure of exposure to complex mixtures of genotoxic pollutants (Černá et al. 1997). Previous studies have found that urinary mutagenicity correlates with other biomarkers in subjects exposed to wood smoke as well as a variety of other pollutants, including benzidine dyes, cigarette smoke, heterocyclic amines, and nitrotoluenes (Černá et al. 1997; DeMarini et al. 1997; Kato et al. 2004; Sabbioni et al. 2006; Shaughnessy et al. 2011; Long et al. 2014). Therefore, we explored the relationship between urinary mutagenicity, a variety of personal occupational exposure measurements, and urinary OH-PAHs among wildland firefighters at prescribed burns. Because air pollution exposure may lead to increased body burden of oxidative stress (IARC 2016), we also measured urinary concentrations of two common biomarkers of oxidative stress, malondialdehyde (MDA) and 8-isoprostane, and we explored the relationships between each of these biomarkers and urinary mutagenicity.

We hypothesized that urinary mutagenicity would be significantly higher among 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 would impact urinary mutagenicity, with firefighters lighting fires with diesel-gasoline-fueled drip-torches having higher urinary mutagenic potency compared to those performing other work tasks.

Methods

Study population and design

Twelve healthy subjects (10 wildland firefighters and 2 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 their voluntary participation.

Baseline questionnaires and daily work activity questionnaires were administered to subjects 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 firelines (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 work days, 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' because subjects reported experiencing occupational exposures to vehicle exhaust, diesel, dust, or possible exposures to smoke from smoldering when patrolling areas where recent burns were conducted. Non-burn work days also included subjects performing office work. An assigned primary work task was determined by reported time spent during a specific work task, that is, 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 (MA) a work shift. Samples were frozen at −5 °C 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 to room temperature to prepare randomized aliquots into sterile, polypropylene, conical 50-ml tubes and again

stored at −80 °C until further analyses. All urine samples were analyzed in a blinded fashion.

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. The subjects did not use respiratory protection during the study. A detailed description of the sample collection methods and gravimetric analyses were reported previously (Adetona 2016). In brief, all exposure monitoring instruments were calibrated prior to use, and gravimetric analyses followed the United States Environmental Protection Agency (U.S. EPA 2006) specifications. $PM_{2.5}$ samples were collected using MicroPEM™ (RTI International, Research Triangle Park, NC) loaded with 25-mm polytetrafluoroethylene membrane filters (porosity: 3.0 µm) (Pall Life Sciences, Ann Arbor, MI). Filters were weighed using the Cahn C-35 microbalance (sensitivity of ±1.0 µg; Thermo Electron, Waltham, MA). All filter weights were blank-adjusted as described previously (Adetona 2016). After gravimetric analyses, PM on the filters was analyzed for light-absorbing carbon by reflectance analysis using the Evans Electroselenium Limited smoke stain reflectometer (Model 43 D, Diffusion Systems Ltd, London, UK) as described (Adetona 2016), and used as a surrogate for black carbon (BC). 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). Time-weighted averages were calculated for both $PM_{2.5}$ (µg/m^3) and CO (ppm).

MDA analysis

Concentrations of MDA were measured using a High-Performance Liquid Chromatography (HPLC) system coupled with a fluorescent detector (Lärstad et al. 2002). A 150-µl sample was added into a mixture of 750 µl phosphoric acid (440 mM) and 150 µl thiobarbituric acid (42 mM). After 1-h incubation at 80 °C, 20 µl of this final solution was injected into the HPLC system with the 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) 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 from eight replicate injections), respectively. MDA was presented in nmol/L. Creatinine-adjusted concentrations, expressed as µmol MDA per mol creatinine, were calculated to correct for urine dilution. Urinary creatinine (mg/dl) was measured by a Beckman Coulter AU Analyzer for Creatinine (Beckman Coulter, Inc., Brea, CA).

8-Isoprostane analysis

Concentrations of 8-isoprostane in urine aliquots were measured using an enzyme-linked immunosorbent assay kit [15-F2t-isoprostane (8-iso-PGF2α), ELISA kits, #NWK-ISO02; Northwest Life Specialties, LLC, Vancouver, WA]. Urine samples were run in duplicate, standards were run in duplicate or triplicate, and creatinine-adjusted concentrations (µmol 8-isoprostane per mol creatinine) were calculated.

1-Hydroxypyrene analysis

Urine aliquots underwent hydrolysis and solid phase extractions before analysis for OH-pyrene using an HPLC-fluorescence technique. A full description of the method has been reported (Birch 2017). The concentrations were expressed in pg/mL urine metabolite concentration and creatinine-adjusted concentrations (µmol OH-pyrene/mol creatinine).

Sample extraction and preparation for mutagenicity assay

Organic extracts from the urine samples were prepared as described previously (Kato et al. 2004). In brief, urine aliquots were thawed and filtered to remove urothelial cells. 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) containing β-glucuronidase (6 units/ml urine; Sigma, St. Louis, MO) and sulphatase (2 units/ml urine, Sigma, St. Louis, MO) for 16 h at 37 °C. The de-conjugated urinary metabolites were then extracted and concentrated by pouring the urine through two C-18 silica-gel columns stacked in tandem (Waters Sep-Pak WAT04305, Milford, MA). The eluted urine was discarded, and a new tube was placed under the column to collect the organics, which were eluted by pouring 10 ml of methanol through the column. The methanol extract was filtered through a 0.22-µm filter and then solvent-exchanged with dimethyl sulfoxide (DMSO) to produce an organic concentrate at 150X. These extracts were stored at 4 °C until mutagenicity assays were conducted.

Mutagenicity assay

The *Salmonella* (Ames) mutagenicity assay was performed using the plate-incorporation method (Maron and Ames 1983) to evaluate the organic extracts of the urines in a blinded fashion. A detailed description of the assay method has been reported (Kato et al. 2004; Mutlu et al. 2015). Briefly, the urine concentrates were evaluated in seven batch experiments at 0.0 and 10.0, 0.0 and 12.0, or 0.0 and 15.0 ml-equivalents of urine/plate (ml-eq/plate) with 4–7 doses per sample depending on the volume of urine sample available (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, or 10.0 ml-equivalents of urine/plate; 0.0, 2.25, 4.0, 6.75, 9.0, 12 or 15 ml-eq/plate) in the presence of metabolic activation (S9 mix) made from Aroclor-induced, Sprague-Dawley rat-liver S9 (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 medium, and plates were incubated at 37 °C for 3 days (72 h), after which the colonies were counted using an automatic colony counter (ProtoCOL 3, Synbiosis, Frederick, MD). Data were stored automatically in an Excel spreadsheet on the ProtoCOL3. Ten percent of the aliquots had replicate samples (identical urines that were divided into two sample tubes) and were analyzed according to the methods described herein. Method blanks were included per batch of experiments with an average ± standard deviation of 0.9 ± 0.3 ml-equivalents of urine/plate.

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 (Mutlu et al. 2015). We used YG1041, a derivative of TA98 that overexpresses nitroreductase and acetyltransferase, permitting it to detect frameshift mutagens that are nitroarenes or aromatic amines. A previous study (Kato et al. 2004) of wood smoke-associated urinary mutagenicity in charcoal workers in Brazil showed that strain YG1041 + S9 was the most sensitive detector of such mutagenicity. Likewise, this strain was the most sensitive among the strains used to evaluate the mutagenicity of PM_{10} from wildland fires in Brazil (de Oliveira Galvão et al. 2018). Thus, we used the same strain with S9 for this study.

A negative or solvent control (DMSO at 100 µl/plate) and two positive controls (2-nitrofluorene at 3 µg/plate and 2-aminoanthracene at 0.5 µg/plate) were included with each experiment (note: the mean of seven batches of experiments that were conducted for the DMSO control for YG1041 +S9 was 60 revertants (rev)/plate, while the mean of the seven batches of experiments for 2-nitrofluorene for YG1041 −S9 and 2-aminoanthracene for YG1041 +S9 were 1715 and 1634 rev/plate, respectively). Three plates were used for each type of control for each experiment, and extracts were tested between 0.0 and 10.0, 0.0 and 12.0, or 0.0 and 15.0 ml-eq/plate with 4–7 doses per sample depending on the volume of urine sample available. 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 dose-related response with a twofold or greater increase in revertants (rev)/plate relative to the DMSO control plates. Urinary values were also creatinine-adjusted and reported as rev/µmole creatinine.

Statistical analysis

Linear regressions over the linear portions of the dose–response curves were performed for all samples to determine the mutagenic potencies (expressed as rev/ml-eq). This included samples that did not approach a twofold increase over the solvent control and would otherwise be considered as a negative mutagenic response so that we could determine cross-work shift changes in mutagenicity that included these samples. However, a few urine samples showed clear cytotoxicity ($n = 9$ of the 150 analyzed), as evidenced by negative slopes of the linear regressions. These sample values were disregarded in determining cross-work shift changes and in the final statistical analyses because they were indicative of clear cytotoxicity and therefore mutagenicity was unmeasurable.

Heteroscedasticity and normality of the data were assessed for all variables. Log transformations were applied to normalize the variables, including time-weighted averages of $PM_{2.5}$ concentration, CO concentration, absorption coefficient (surrogate for BC), MDA concentration, 8-isoprostane concentration, OH-pyrene concentration, and urinary mutagenicity data. Note that to achieve normality, mass absorption efficiencies (surrogate for BC/$PM_{2.5}$ ratio) were arcsine-square root transformed. The results of the statistical analysis were back-transformed.

We assessed cross-work shift (pre- to post-; and pre- to MA) changes in urinary mutagenicity by day type (burn and non-burn day) and by work task (holding, lighting, non-burn day exposure, and non-burn day office), while also evaluating possible associations with the exposure measures including $PM_{2.5}$, CO, absorption coefficient, mass absorption efficiency, and inhaled dose of $PM_{2.5}$.

The approach used to estimate inhaled dose of $PM_{2.5}$ has been reported (Adetona 2016) previously. Briefly, accelerometer activity measurements recorded by the MicroPEM were used to estimate minute ventilation rate (L/min) using the linear regression equation published by Rodes et al. (2012) (i.e. $V = m \times \text{ACCEL} + b$ 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). Next, inhaled mass of $PM_{2.5}$ (µg) was calculated by multiplying $PM_{2.5}$ exposure concentrations by the total volume of inhaled air which was estimated by multiplying the ventilation rate by the length of the monitoring period (Adetona 2016). Inhaled $PM_{2.5}$ dose (µg/kg body weight) was then calculated by dividing the inhaled mass of $PM_{2.5}$ by the subjects' body weight (Adetona 2016).

Cross-work shift changes in other outcomes, MDA, 8-isoprostane, and OH-pyrene, according to day type and work task were also individually evaluated in a similar manner. Associations between urinary mutagenicity and the other outcomes, MDA, 8-isoprostane, and OH-pyrene, were explored using the same statistical approach. To account for longitudinal within-subject correlations of the data, we included subject and date as random effect variables in the model. Using the forward-stepwise procedure, we also tested for the effects of possible confounding factors such as smokeless tobacco used (chew), grilled-foods, age, length of firefighter career, work-shift duration, and days since last

prescribed burn. Only significant covariates were included in the final model.

Cross-work shift changes are reported as post-work-shift/pre-work shift ratios or MA 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. Descriptive statistics and linear mixed-effect models were conducted using SAS v.9.4 (SAS Institute, Cary, NC). Statistical significance was set at $p < 0.05$, and the Bonferroni method was used for multiple comparisons.

Results

A total of 201 spot urine samples were collected throughout the study period. One sample was spilled during analytical procedures and therefore could not be analyzed. Thus, 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 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 give urine samples from time to time, there were a few occasions (six in total) in which the MA in the sample set (pre, post, MA) was not completed, and these were not included in the person-day samples noted above.

Study population characteristics were described previously (Adetona 2016). In brief, the average age of the 12 subjects (9 men and 3 women) who participated in the study was 33 ± 5.4 years with subjects having less than 1 year to 22 years of experience as wildland firefighters. All subjects were nonsmokers, although three subjects reported occasionally chewing smokeless tobacco. On average, 280 acres (range: 38–1000 acres) were burned per day, and the duration of work-shifts averaged 4.5 h (range: 1.9–9.4 h) on burn days and 6.2 h (range: 3.9–7.8 h) on non-burn work days. The average $PM_{2.5}$ and CO concentrations were 35.1 (95% CL: 15.9, 77.3) μg/m^3 and 0.005 (95% CL: 0.002, 0.016) ppm, respectively, on non-burn work days, and 259.4 (95% CL: 156.1, 431.1) μg/m^3 and 0.8 (95% CL: 0.4, 1.8) ppm, respectively, on burn days (Adetona 2016). Unadjusted arithmetic and geometric means of crude and creatinine-corrected urinary mutagenicity, MDA, and 8-iso-prostane, and OH-pyrene concentrations according to day type and time of sample collection are presented in Table 1. The number of days between the last prescribed burn and sample collection was from 3 to 30 days and from 1 to over 30 days for non-burn and burn day assessments respectively. Urinary creatinine values were between 10 and 382 mg/dl with 92% of the samples above the lower end of the normal range (30–300 mg/dl).

Cross-work shift changes in urinary mutagenicity

Percent positive results for mutagenicity and corresponding unadjusted arithmetic and geometric means by day type and time of sample collection are presented in Table 2. Twenty-eight percent of the post-work shift samples (14 of 50) on burn days were positive for mutagenicity, compared to 20% positive post-work shift samples (4 of 20) on non-burn work days (Table 2). Unadjusted means (95% CL) for crude and creatinine-corrected post-work shift urinary mutagenicity levels on burn days were 7.7 (95% CL: 4.2, 11.2) (arithmetic) rev/mL-eq and 0.83 (95% CL: 0.19, 1.48) (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 2). On non-burn work days, unadjusted mean crude and creatinine-corrected post-work shift urinary mutagenicity levels were 4.8 (95% CL: 3.6, 5.9) (arithmetic) rev/mL-eq and 0.33 (95% CL: 0.19, 0.46) (arithmetic) rev/μmol creatinine, and ranged from 4.2 to 5.8 rev/mL-eq and 0.22 to 0.41 rev/μmol creatinine, respectively (Table 2).

Adjusted cross-work shift (pre- to post-, and pre- to MA) changes in crude and creatinine-adjusted urinary mutagenicity according to day type are presented in Figure 1(a–d). No statistically significant effect of day type on cross-work shift (pre- to post- or pre- to MA) changes in crude or creatinine-adjusted mutagenicity was observed ($p > 0.05$). However, data trended in the expected direction, with slightly higher (though not significant) cross-work shift creatinine-adjusted change observed on burn days compared to non-burn work days [1.0 (95% CL: 0.6, 1.6) and 0.6 (95% CL: 0.5, 1.3), respectively] (Figure 1(b)). Likewise, pre- to MA cross-work shift changes in creatinine-adjusted mutagenicity also trended in similar directions, with burn days having 1.4 times higher cross-work shift (pre- to MA) increases compared to non-burn work days [1.1 (95% CL: 0.5, 2.2) and 0.8 (95% CL: 0.3, 1.9), respectively] (Figure 1(d)).

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 Figure 2(a–d). Although lighters appeared to have the highest cross-work shift (pre- to post-) increase in urinary mutagenicity and were 1.6-fold higher compared to holding, no significant difference was observed between the two ($p = 0.1487$) [1.6, (95% CL: 1.0, 2.7) and 1.0 (95% CL: 0.5, 1.9), respectively] (Figure 2(a)). Likewise, no significant difference was seen between lighting and non-burn day exposures ($p = 0.0901$) [1.6, (95% CL: 1.0, 2.7) and 0.8 (95% CL: 0.4, 1.8), respectively] (Figure 2(a)). No significant overall effect of work task was seen among crude or creatinine-adjusted cross-work shift [pre- to post- (Figure 2(a,b)) and pre- to MA (Figure 2(c,d))] changes.

Cross-work shift changes 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

Table 1. Unadjusted arithmetic and geometric means of urinary mutagenicity, malondialdehyde, 8-isoprostane, and OH-pyrene 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 MDA (nmol/l)	Creatinine adjusted MDA (μmol MDA/mole creatinine)	Crude 8-isoprostane (ng/ml)	Creatinine adjusted 8-isoprostane (μg/g creatinine)	Crude OH-pyrene (pg/ml)	Creatinine adjusted OH-pyrene (ng/g creatinine)
Burn day									
	Pre-work shift	Sample size $n = 50$[a]		Sample size $n = 50$		Sample size $n = 41$		Sample size $n = 50$	
	Arithmetic mean (95% CL)	3.5 (2.4, 4.5)	0.41 (0.29, 0.52)	980.4 (789.3, 1171.5)	105.6 (82.3, 128.8)	1.5 (1.1, 1.8)	0.5 (0.3, 0.7)	333.5 (151.3, 515.6)	235.7 (151.5, 320.0)
	Geometric mean (95% CL)	2.4 (1.9, 3.2)	0.29 (0.23, 0.37)	758.9 (607.5, 947.9)	89.2 (76.4, 104.0)	1.0 (0.7, 1.4)	0.4 (0.3, 0.5)	152.0 (108.6, 212.5)	157.8 (124.4, 200.2)
	Ranges	0, 23.6	0, 1.98	134.4, 3172.1	35.8, 551.4	0.1, 4.5	0.1, 3.5	17.5, 4237.9	32.9, 1793.8
	Post-work shift	Sample size $n = 50$		Sample size $n = 50$		Sample size $n = 41$		Sample size $n = 41$	
	Arithmetic mean (95% CL)	4.0 (2.9, 5.2)	0.47 (0.27, 0.68)	1074.1 (879.4, 1268.9)	92.0 (80.5, 103.5)	1.5 (1.1, 1.8)	0.4 (0.3, 0.4)	550.0 (299.0, 801.2)	402.9 (254.1, 551.6)
	Geometric mean (95% CL)	2.9 (2.3, 3.8)	0.28 (0.20, 0.37)	894.6 (748.5, 1069.0)	84.3 (74.9, 95.0)	1.1 (0.9, 1.4)	0.3 (0.2, 0.4)	272.6 (197.3, 376.8)	227.2 (168.7, 306.0)
	Ranges	0.1, 28	0.01, 4.46	175.3, 3415.5	37.1, 212.5	0.2, 4.7	0.0, 0.9	18.1, 5245.3	40.7, 2343.0
	Morning-after work shift	Sample size $n = 43$[a]		Sample size $n = 44$		Sample size $n = 32$		Sample size $n = 50$	
	Arithmetic mean (95% CL)	3.8 (2.9, 4.7)	0.44 (0.26, 061)	969.2 (798.0, 1140.3)	82.6 (70.0, 95.2)	1.6 (1.2, 2.0)	0.3 (0.3, 0.4)	397.2 (279.4, 515.0)	257.4 (198.4, 316.5)
	Geometric mean (95% CL)	2.9 (2.3, 3.7)	0.26 (0.19, 0.37)	829.9 (694.1, 992.2)	74.4 (65.0, 85.3)	1.1 (0.7, 1.7)	0.3 (0.2, 0.4)	247.6 (181.1, 338.6)	196.4 (155.8, 247.4)
	Ranges	0, 13.6	0, 3.46	159.5, 3255.0	34.0, 204.3	0.0, 4.9	0.0, 0.8	40.2, 1527.2	32.0, 753.3
Non-burn day									
	Pre-work shift	Sample size $n = 20$[a]		Sample size $n = 20$		Sample size $n = 19$		Sample size $n = 20$	
	Arithmetic mean (95% CL)	3.3 (2.5, 4.1)	0.77 (0.38, 1.15)	611.1 (430.8, 791.4)	90.8 (68.7, 112.8)	1.0 (0.5, 1.4)	0.3 (0.2, 0.4)	165.0 (104.5, 225.4)	204.5 (152.1, 256.9)
	Geometric mean (95% CL)	2.8 (2.1, 3.8)	0.45 (0.27, 0.76)	512.0 (383.4, 683.7)	81.6 (65.8, 101.1)	0.5 (0.3, 1.0)	0.2 (0.2, 0.3)	126.3 (88.5, 180.3)	177.9 (137.7, 229.9)
	Ranges	0, 7.3	0, 3.24	157.3, 1776.2	35.8, 551.4	0.1, 2.7	0.0, 0.6	23.5, 548.6	70.0, 466.6
	Post-work shift	Sample size $n = 20$[a]		Sample size $n = 20$		Sample size $n = 17$		Sample size $n = 20$	
	Arithmetic mean (95% CL)	2.7 (1.9, 3.5)	0.35 (0.21. 0.49)	1089.3 (615.1, 1563.4)	114.6 (77.1, 152.1)	1.4 (0.8, 2.0)	0.4 (0.3, 0.5)	198.2 (126.6, 270.0)	174.8 (130.0, 219.7)
	Geometric mean (95% CL)	2.3 (1.6, 3.2)	0.27 (0.19, 0.38)	828.6 (592.5, 1158.9)	98.6 (77.5, 125.5)	1.0 (0.7, 1.6)	0.4 (0.3, 0.5)	146.2 (99.2, 215.6)	153.8 (121.0, 195.6)
	Ranges	0, 7.3	0, 1.33	225.4, 4533.4	37.1, 212.5	0.3, 4.3	0.1, 0.8	33.1, 529.3	60.1, 460.5
	Morning-after work shift	Sample size $n = 18$[a]		Sample size $n = 18$		Sample size $n = 14$[b]		Sample size $n = 18$	
	Arithmetic mean (95% CL)	3.6 (2.3, 4.9)	0.33 (0.20, 0.46)	944.2 (783.0, 1105.3)	93.6 (56.2, 131.1)	1.4 (0.8, 1.9)	0.4 (0.2, 0.5)	247.2 (147.7, 346.6)	178.6 (116.0, 241.2)
	Geometric mean (95% CL)	2.9 (1.9, 4.4)	0.25 (0.16, 0.40)	892.6 (750.2, 1062.1)	78.8 (60.1, 103.2)	1.1 (0.8, 1.6)	0.3 (0.2, 0.4)	172.2 (108.6, 272.8)	134.3 (88.5, 204.0)
	Ranges	0, 8	0, 0.89	489.0, 1778.8	34.0, 204.3	0.6, 3.6	0.1, 0.7	35.9, 647.6	29.4, 465.4

Note: Revertants/ml-equivalent of urine (rev/ml-eq), Malondialdehyde (MDA), n=person-day samples, 95% Confidence Limits (95% CL).
[a]For geometric mean, zero values were replaced with the LOD/$\sqrt{2}$.
[b]$n = 13$ for creatinine adjusted 8-isoprostane morning-after work shift.

Table 2. Percent positive results for mutagenicity and corresponding unadjusted arithmetic and geometric means by day type and time of sample collection.

	Sample collection time		
Day type	Pre-work shift	Post-work shift	Morning-after work shift
Burn day			
Number and percent positive for mutagenicity, *n* of total, %	9 of 50 18%	14 of 50 28%	10 of 43 23%
Unadjusted arithmetic means (95% CL)			
Crude urine mutagenicity (rev/ml-eq)	8.4 (3.3, 13.5)	7.7 (4.2, 11.2)	7.8 (5.5, 10.0)
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.44 (0.20, 0.68)	0.83 (0.19, 1.48)	0.63 (0.39, 0.88)
Unadjusted geometric means (95% CL)			
Crude urine mutagenicity (rev/ml-eq)	6.8 (4.1, 11.2)	6.7 (5.1, 8.8)	7.3 (5.5, 9.6)
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.37 (0.24, 0.58)	0.55 (0.34, 0.88)	0.56 (0.39, 0.81)
Ranges (Min, Max)			
Crude urine mutagenicity (rev/ml-eq)	3.7, 23.6	4.4, 28.0	4.6, 13.6
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.20, 1.16	0.24, 4.46	0.32, 1.41
Non-burn day			
Number and percent positive for mutagenicity, *n* of total, %	3 of 20 15%	4 of 20 20%	4 of 18 22%
Unadjusted Arithmetic Means (95% CL)			
Crude urine mutagenicity (rev/ml-eq)	5.9 (2.5, 9.4)	4.8 (3.6, 5.9)	7.4 (6.1, 8.6)
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.61 (−0.29, 1.50)	0.33 (0.19, 0.46)	0.55 (0.30, 0.79)
Unadjusted geometric means (95% CL)			
Crude urine mutagenicity (rev/ml-eq)	5.8 (3.2, 10.7)	4.7 (3.7, 6.0)	7.3 (6.1, 8.7)
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.54 (0.11, 2.57)	0.32 (0.20, 0.49)	0.53 (0.34, 0.84)
Ranges (Min, Max)			
Crude urine mutagenicity (rev/ml-eq)	4.5, 7.3	4.2, 5.8	6.3, 8.0
Creatinine-corrected urine mutagenicity (rev/μmole creatinine)	0.28, 1.00	0.22, 0.41	0.37, 0.74

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

creatinine, respectively (Table 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 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 Figure 3(a–d). No significant difference was observed between cross-work shift changes on burn day and non-burn days (Figure 3(a,b,d)). However, a marginally significant difference in pre- to MA work shift crude MDA changes was observed between day types ($p = 0.0502$) (Figure 3(c)), 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 Figure 4(a–d). No significant effect of work tasks was observed across all cross-work shift changes (Figure 4(a–d)); however, non-burn work day exposures showed marginally significant higher crude MDA cross-work shift (pre- to MA) changes 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 4(c)).

Association between urinary mutagenicity, oxidative stress, and exposure

We found positive correlations between cross-work shift (pre- to post) changes in urinary mutagenicity and MDA ($p = 0.0010$) (Figure 5), and between cross-work shift (pre- to post-) changes in urinary mutagenicity and OH-pyrene ($p = 0.0001$) (Figure 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) ($p = 0.2245$) (Figure 7).

No significant correlation was 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 exposure measures ($PM_{2.5}$, inhaled dose of $PM_{2.5}$, CO, absorption coefficient, and mass absorption efficiency (results not shown). Lastly, we found no significant effects for age, wildland firefighter career length, work-shift duration, days since last prescribed burn, use of smokeless tobacco (chew), or grilled foods on cross-work shift changes in MDA, 8-iso-prostane, or urinary mutagenicity (results not shown).

Discussion

Constituents of wood smoke and non-wood smoke occupational exposures such as diesel exhaust and wood smoke during wildland firefighting are mutagenic and carcinogenic (IARC 2010, 2014, 2016; Adetona et al. 2015, 2016). A prior study of wildland firefighters found measurably higher urinary metabolites of OH-PAHs after occupational exposure, indicating that subjects received an internal dose of exposure to possible mutagenic and carcinogenic PAHs (Adetona et al. 2015). 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 in this population. Therefore, we explored the use of urinary

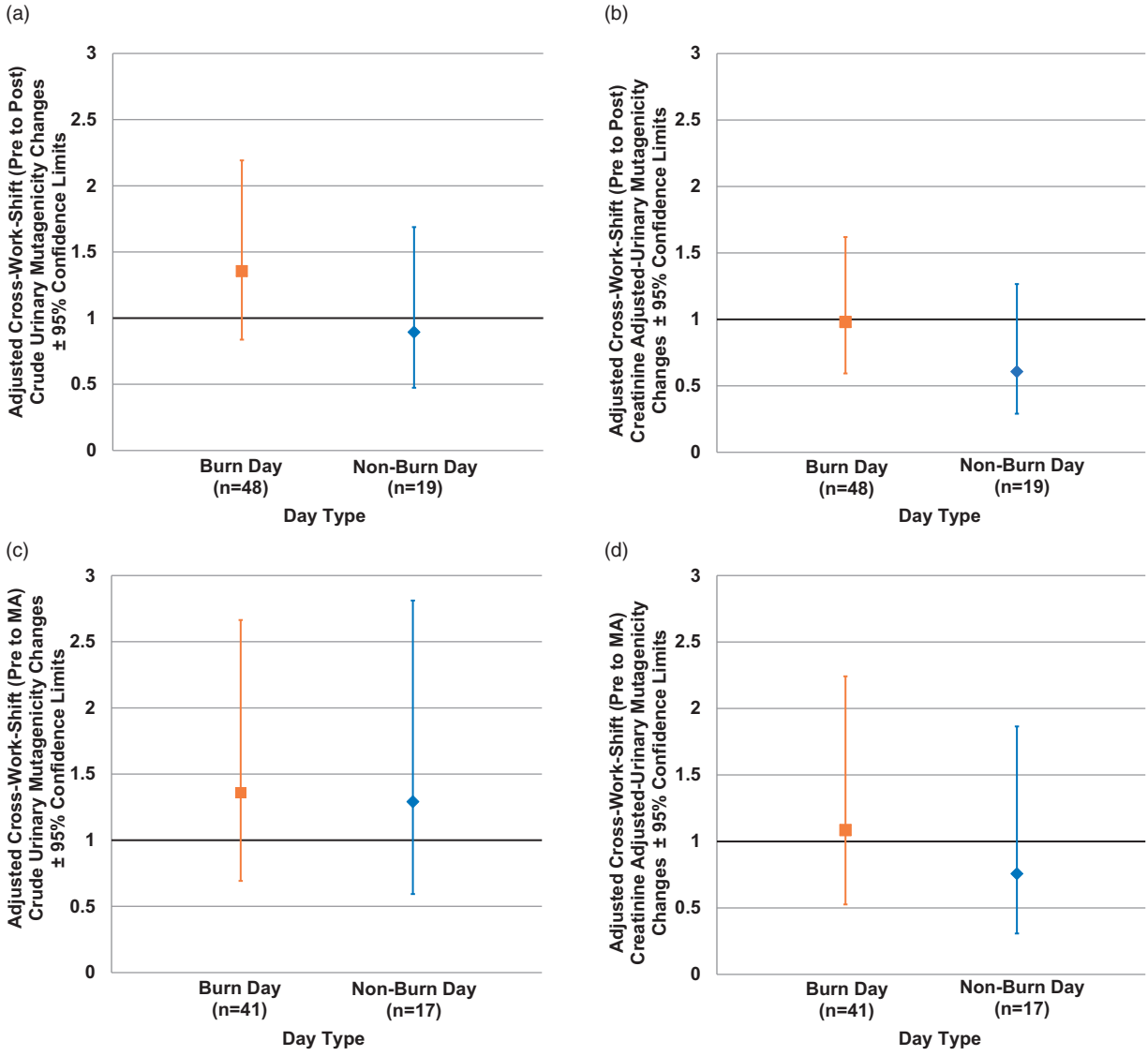

Figure 1. Adjusted cross-work shift changes in crude and creatinine-adjusted urinary mutagenicity concentrations according to day type. Pre- to post- work shift changes are depicted in (a) and (b). Pre to morning-after (MA) work shift changes are depicted in (c) and (d). No significant difference was observed between burn day and non-burn day samples 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 MA 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).

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 (Kato et al. 2004; Long et al. 2014). 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 nonsmoking charcoal workers exposed to wood smoke in Brazil. Our burn day pre-work shift creatinine-adjusted urinary mutagenicity levels were 2.8X less than levels observed among the no-wood-smoke exposure group reported in Kato et al. (2004); and our burn day post-work shift and MA work shift levels were 4.6X and 4.1X less, respectively, compared to the low-wood-smoke exposure group reported by Kato et al. (2004). Personal or ambient exposure monitoring, that is, $PM_{2.5}$ or CO levels, was not measured in the charcoal study; rather, subjects were grouped based on task-related exposure levels (Kato et al. 2004). However, it is possible that exposures in Kato et al. may have been significantly higher compared to our study. Exposures in our current study were among the lower levels measured among firefighters. This could have been due to the changes in prescribed burn operations that reduced both the size and period of burns conducted compared to historical data (Adetona 2011; Adetona, Simpson et al. 2013; Hejl et al. 2013).

We found a non-significant 1.6-fold higher 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 prevalence odds ratios of 2.33 (95% CL: 0.83, 6.57) for urinary mutagenicity at low-wood-smoke exposure levels and 5.31 (95% CL: 1.85, 15.27) at high-wood-smoke

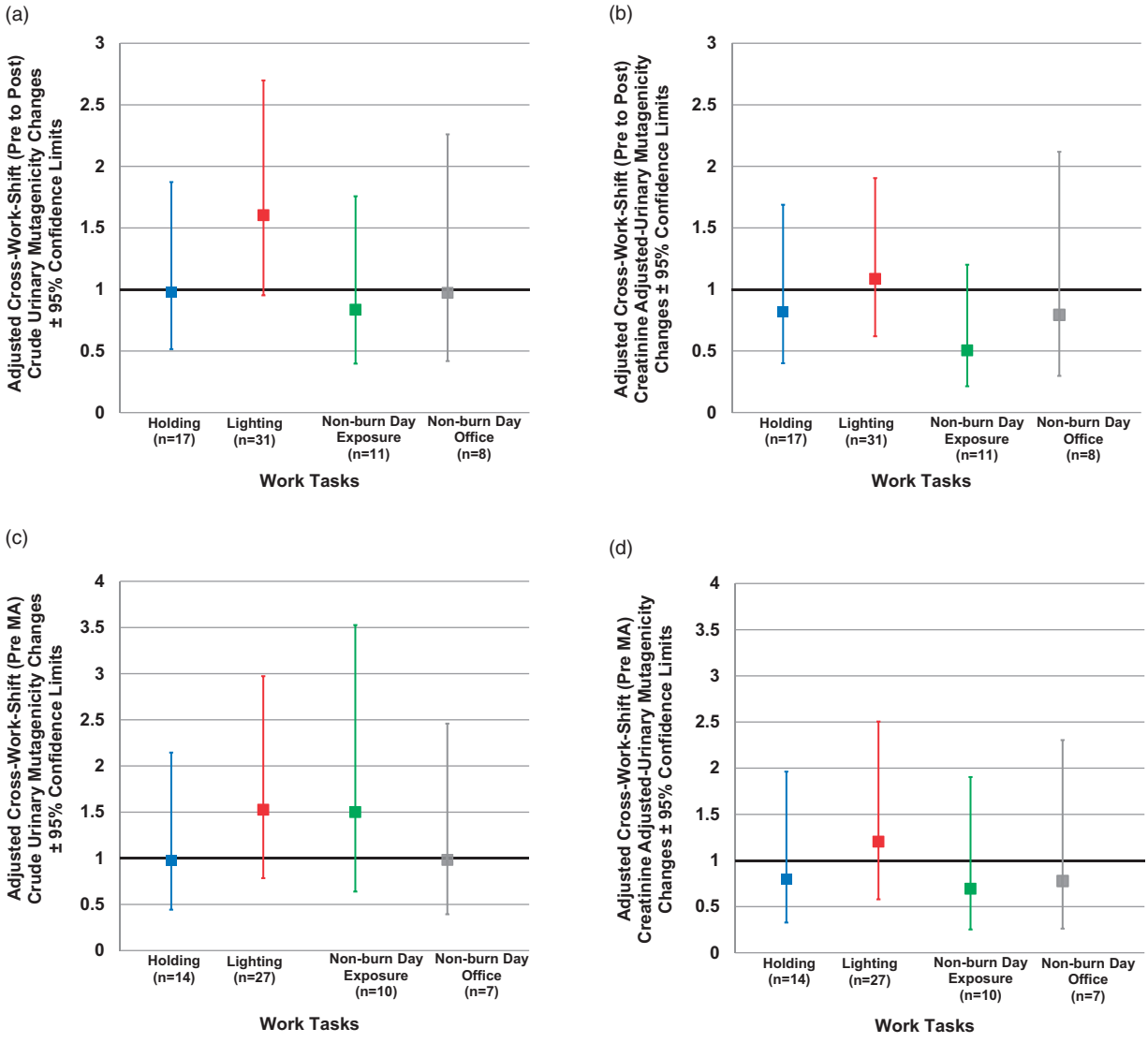

Figure 2. Adjusted cross-work shift changes in crude and creatinine-adjusted urinary mutagenicity concentrations according to work tasks. Pre- to post-work shift changes are depicted in (a) and (b). Pre to morning-after (MA) work shift changes are depicted in (c) and (d). No significant difference was 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 MA work-shift/pre-work shift ratios, r espectively. Where 95% confidence limits do not cross the x-axis, cross-work shift changes are statistically different from 1 (p-values < 0.05).

exposure levels compared to the non-exposed group of workers (Kato et al. 2004). 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 mutagenic potency compared to pre-exposure samples and also compared to control samples ($n = 9$, unexposed individuals from the same population) (Long et al. 2014).

In a study of occupational exposures to combustion emissions among structural firefighters, mutagenicity was assessed in urine collected before and after a fire event (Keir et al. 2017). Event-related increases in urinary mutagenicity were 4.3-fold higher in post-urine samples (Keir et al. 2017). One reason why our results may have differed compared to this study may be due to different levels of exposures to mutagenic compounds. Moreover, the $PM_{2.5}$ exposure levels reported in our present study were at least two times lower compared to studies conducted at the same site and elsewhere (Reinhardt and Ottmar 2004; Adetona, Simpson, et al. 2013; Hejl et al. 2013).

Our data suggest that lighters may have higher cross-work shift increases in crude urinary mutagenicity compared to other work tasks (Figure 2(a)). These results are understandable in that firefighters who light fires may be exposed to diesel and wood smoke particulate matter and, therefore, may have an additive or perhaps synergistic systemic dose to mutagenic compounds (Mutlu et al. 2015). A recent experimental study found that extractable organic material from diesel exhaust particles had significantly 50–85% higher mutagenic potency compared to extracts from soy-biodiesel emission particles (Mutlu et al. 2015).

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

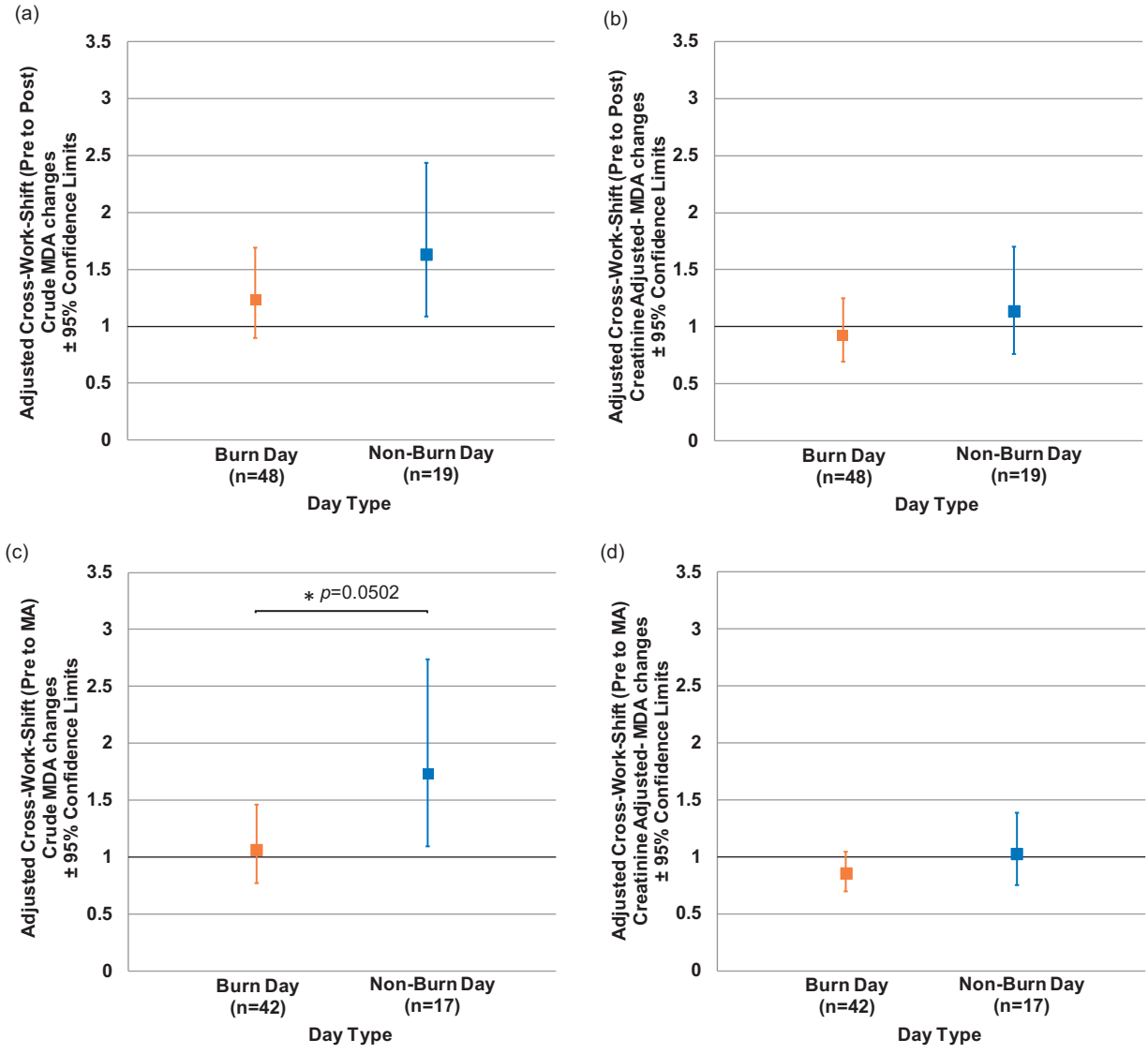

Figure 3. Adjusted cross-work shift changes in crude and creatinine-adjusted urinary malondialdehyde concentrations according to day type. Pre- to post-work shift changes are depicted in (a) and (b). Pre to morning-after (MA) work shift changes are depicted in (c) and (d). No significant difference was observed between burn day and non-burn day samples for (a), (b), or (d), however marginal significant differences were observed in (c) (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 MA 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).

in crude MDA on non-burn days compared to burn days (Figure 3(c)), and a marginally significant higher cross-work shift (pre- to MA) increase in crude MDA for non-burn work day tasks that may have had other associated work exposures (Figure 4(c)). These results may be due to non-reported exposures on non-burn days. For instance, MDA levels in the body may be impacted by diet, such as lipid-rich foods (Richelle et al. 1999; Bloomer et al. 2010). For example, a human cross-over study among young healthy men ($n = 9$) found increased MDA blood levels after eating heavy whipping cream (Bloomer et al. 2010).

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 1); Adetona, Zhang, 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]. 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) µg/m^3 and 1.0 ($n = 78$, 95% CL: 0.07, 13) ppm, respectively (Adetona, Zhang, et al. 2013). In addition, similar to our study's findings, no significant cross-work shift changes were observed for MDA in the urine samples of firefighters in the study of Adetona, Zhang, et al. (2013). In contrast, results from a human chamber study found measurable increases in exhaled breath MDA levels after subjects were exposed to wood smoke (Barregard

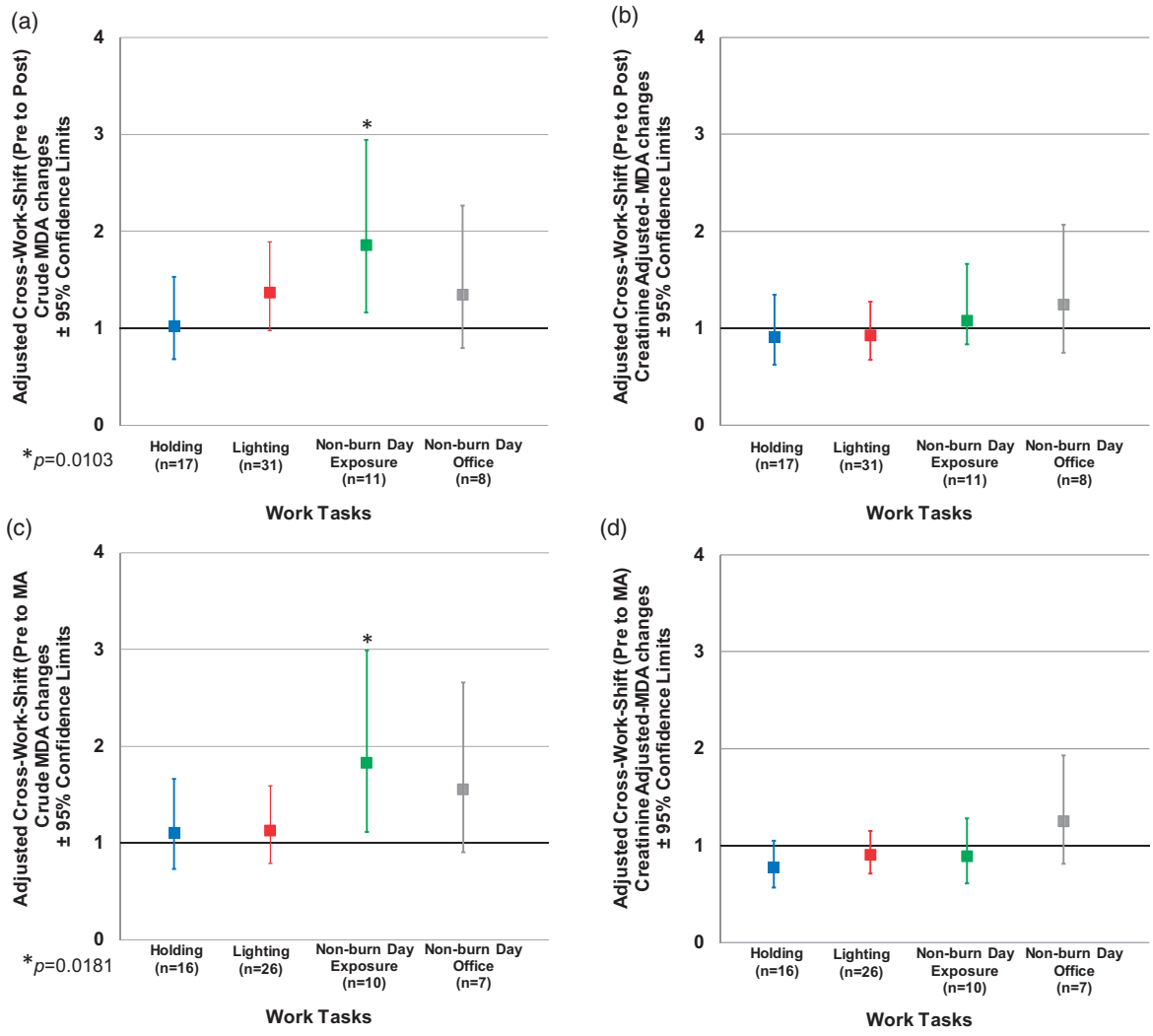

Figure 4. Adjusted cross-work shift changes in crude and creatinine-adjusted urinary malondialdehyde concentrations according to work tasks. Pre- to post-work shift changes are depicted in (a) and (b). Pre to morning-after (MA) work shift changes are depicted in (c) and (d). No significant difference 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 MA 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).

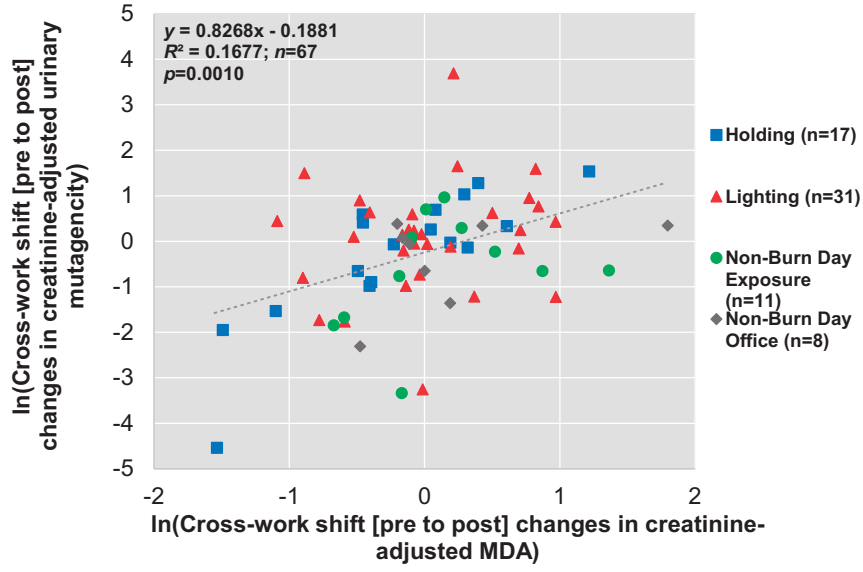

Figure 5. Correlation between log-transformed cross-work shift (pre- to post-) changes in creatinine-adjusted urinary mutagenicity and log-transformed creatinine-adjusted MDA. Person days are indicated as n. Linear mixed effect model results showed a positive significant correlation ($p = 0.0010$).

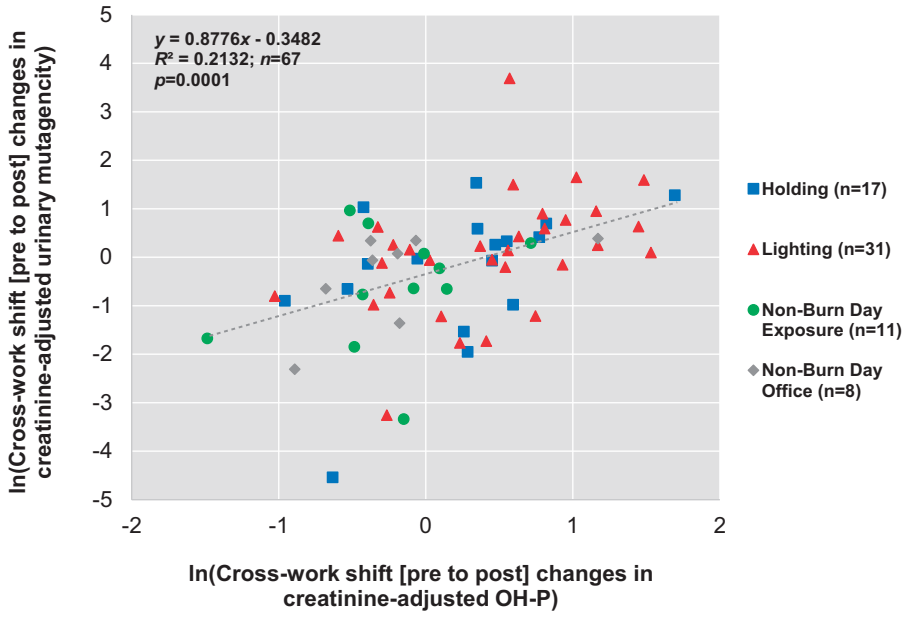

Figure 6. Correlation between log-transformed cross-work shift (pre- to post-) changes in creatinine-adjusted urinary mutagenicity and log-transformed creatinine-adjusted OH-pyrene. Person days are indicated as *n*. Linear mixed effect model results showed a positive significant correlation ($p = 0.0001$).

Figure 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 cluster of data by work task and a positive (though not significant) correlation ($p = 0.2245$).

et al. 2008). However, it is known that urinary MDA and exhaled breath MDA are not necessarily correlated (Gong et al. 2013). We did not observe any significant cross-work shift changes in urinary 8-isoprostane. Although Keir et al. (2017) observed increases in urinary mutagenicity after structural fire events, they did not find significant cross-work shift changes in urinary 8-isoprostane levels.

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). This association is plausible because MDA is potentially mutagenic (Hartman 1983; Niedernhofer et al. 2003). MDA may act as an endogenous genotoxic product of lipid peroxidation of polyunsaturated fatty acids in the body (Niedernhofer et al. 2003). 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 (Niedernhofer et al. 2003). MDA induced multiple types of mutations, including large insertions and deletions (most frequently), base pair substitutions, and inter-strand cross-links (Niedernhofer et al. 2003).

Although urinary mutagenicity was not associated with $PM_{2.5}$ exposure or inhaled dose of $PM_{2.5}$, it was correlated with exposure to components of particulate matter. A significant correlation was observed between adjusted cross-work shift (pre- to post-) changes in creatinine-adjusted urinary mutagenicity and OH-pyrene exposure (Figure 6). Various PAHs, found in wood smoke and diesel exhaust, are potentially mutagenic and carcinogenic (Kato et al. 2004). Results from Kato et al. (2004) 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)]. Furthermore, the study reported significantly higher levels of 2-naphthol and OH-pyrene among the highly exposed group compared to the non-exposed (Kato et al. 2004). Higher PAH exposures were also observed in structural firefighters with higher urinary mutagenicity levels (Keir et al. 2017).

Increased cross-work shift mutagenicity on non-burn days observed for some subjects could have been due to their exposures to mutagenic compounds. Some of the subjects reported performing tasks at which they could have experienced mutagenic exposures such as exhaust from engines, smoke from smoldering when patrolling areas where recent burns were conducted or dust during field prep work (Adetona 2016). In addition, exposures of a few subjects during the work-shift on non-burn days to OH-PAHs were apparent as indicated by their large increase in OH-pyrene excretion across the work-shift (Figure 6).

Cross-work shift (pre- to post-) changes in urinary mutagenicity concentrations were not associated with absorption coefficient, which is rather a direct measure of the amount of light absorption by the particle deposited on the filter. Since this measure is also dependent on the mass of particles deposited on the filter, it was essential to normalize it against $PM_{2.5}$ concentration (mass absorption efficiency: surrogate for $BC/PM_{2.5}$ ratio) in order to obtain a surrogate measure of the BC content of $PM_{2.5}$ and an indirect measure of BC exposure. We observed a positive (though not significant) correlation between cross-work shift changes in creatinine-adjusted urinary mutagenicity and the mass absorption efficiency (surrogate for $BC/PM_{2.5}$ ratio) (Figure 7). This relationship suggests that urinary mutagenicity is dependent on the type of particles and composition of PM, that is, BC content. Firefighters lighting with diesel-gasoline fueled drip-torches appear to have higher urinary mutagenicity as depicted in Figure 7 and suggested in Figure 2(a). A previous study using extracted particles from diesel engine exhaust, which has a higher BC proportion compared to wood smoke, (Naeher et al. 2007; Ris 2007) had a higher mutagenic potency compared to soy-biodiesel particles (Mutlu et al. 2015).

Limitations

This study was a pilot study exploring the use of urinary mutagenicity among wildland firefighters, and the study sample size was powered initially using an effect size for a different health endpoint estimated from a previous piloted study (Hejl et al. 2013). Increasing the sample size in follow up studies will be important to determine small effects and ability to dissociate the effect of confounders. MDA could be influenced by dietary intake of high-lipid content foods. Biomarkers of lipid peroxidation that are not affected by lipid intake, such as urinary isoprostanes (Richelle et al. 1999), may be used as an alternative in future studies. Additionally, occupational exposures observed in this study were relatively low. Future studies should investigate mutagenicity at higher exposure levels because there was an association with particulate matter components in this study, and wood smoke is mutagenic (Kato et al. 2004; Kim et al. 2018). 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 would be valuable because 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, nonsmoking 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 when exposure levels are likely higher than what was observed in this study. No statistically significant cross-work shift increases were observed in creatinine-adjusted urinary mutagenicity between burn and non-burn days. However, our results suggest that firefighters using drip-torches to light fires have potentially higher urinary mutagenicity during prescribed burns than during other work tasks. Findings from this study suggest that occupational smoke exposure, especially related to tasks involving lighting fires on prescribed burns, may contribute to systemic exposure to mutagens.

Disclaimer

This article was reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication. Approval does not signify that the contents reflect the views of the agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Disclosure statement

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