

ORIGINAL RESEARCH

Urinary mutagenicity and oxidative status of wildland firefighters working at prescribed burns in a Midwestern US forest

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► Supplemental material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/oemed-2020-106612>).

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Received 14 April 2020

Revised 10 October 2020

Accepted 13 October 2020

ABSTRACT

Objective Wildland firefighters (WLFFs) experience repeated exposures to wildland fire smoke (WFS). However, studies about WLFFs remain regionally limited. The objective of this study was to assess the effect of WFS exposure on urinary mutagenicity and cell oxidation among WLFFs who work at prescribed burns in the Midwestern USA.

Methods A total of 120 spot urine samples was collected from 19 firefighters right before (pre-shift), immediately after (post-shift), and the morning (next-morning) following work shifts on prescribed burn days (burn days) and regular workdays (non-burn days). The levels of urinary mutagenicity, 8-isoprostane, malondialdehyde and oxidised guanine species (Ox-GS) were measured. Linear mixed-effect models were used to determine the difference of cross-shift changes in the concentrations of urinary biomarkers.

Results Post-shift levels of creatinine-corrected urinary mutagenicity and 8-isoprostane were non-significantly higher than pre-shift levels (1.16× and 1.64×; $p=0.09$ and 0.07) on burn days. Creatinine-corrected Ox-GS levels increased significantly in next-morning samples following WFS exposure (1.62×, $p=0.03$). A significant difference in cross-shift changes between burn and non-burn days was observed in 8-isoprostane (2.64×, $p=0.03$) and Ox-GS (3.00×, $p=0.02$). WLFFs who contained the fire (performed holding tasks) had a higher pre-morning to next-morning change in urinary mutagenicity compared with those who were lighting fires during the prescribed burns (1.56×, $p=0.03$).

Conclusions Compared with the other regions, WLFFs who worked in Midwestern forests had an elevated urinary mutagenicity and systemic oxidative changes associated with WFS exposure at prescribed burns.

INTRODUCTION

Health impacts of wildland fire emissions have become a major public health concern. Wildland fire smoke (WFS) is a heterogeneous mixture of air pollutants in the gaseous and particle phases, including carbon monoxide, volatile organic compounds, particulate matter (PM), black carbon (BC) and polycyclic aromatic hydrocarbons (PAHs).^{1–4} Wildland firefighters (WLFFs), who are the first defence against wildland fires, are exposed directly and consistently to WFS. Their occupational exposures are exacerbated by extended work

Key messages

What is already known about this subject?

- Exposure to biomass smoke emissions due to incomplete combustion has been associated with systemic health effects among exposed individuals, including wildland firefighters.
- However, limitations exist regarding geographical location and the assessment of the potential impact of occupational exposure to wildland fire smoke on wildland firefighters' health.

What are the new findings?

- Our study is the first to evaluate the effect of fire smoke exposure on systemic health status in wildland firefighters who worked at prescribed burns in the Midwest.
- Compared with the other regions, wildland firefighters in this study had a higher urinary mutagenicity and oxidative stress presumably due to repeated exposure to elevated levels of fire smoke emissions during prescribed burns compared with a previous study done in the Southeast.

How might this impact on policy or clinical practice in the foreseeable future?

- The results of this study suggest that wildland firefighters might need a suitable respiratory protection against peak smoke exposure situations as they consistently work under high physical exertion and breathing rate.
- This is especially the case given that firefighters typically approach a smouldering fire more than they do a flaming fire (for safety reasons), but the mutagenicity emission factor of smouldering biomass is ~10 times greater than that of flaming biomass.

hours without appropriate respiratory protection while performing physically demanding activities.²

Systemic exposure to the complex mixture of mutagens in biomass smoke can be assessed by measuring urinary mutagenicity.^{5–13} Although specific mutagens cannot be identified, urinary mutagenicity has the advantage of non-invasively measuring an integrated level of mutagenic activity without prior knowledge about the mutagens.⁷



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To cite: Wu C-M, Warren SH, DeMarini DM, et al. *Occup Environ Med* Epub ahead of print: [please include Day Month Year]. doi:10.1136/oemed-2020-106612

Previous epidemiological studies have found increased urinary mutagenicity levels among charcoal workers and wood-fired steam bath users.^{7,8} A significant positive association was observed between structural fire smoke exposure and increased urinary mutagenicity in municipal firefighters.⁶ Increased urinary mutagenicity was also observed after WFS exposure among WLFFs in Southeastern USA.⁵ However, the mutagenic potencies and mutagenicity emission factors vary among different types of biomass emissions.¹⁴ Thus, urinary mutagenicity and other systemic effects of WFS may vary due to the different types of biomass fuel in different regions.

PM from biomass emissions is composed of PAHs, other mutagenic organics and reactive oxygen species (ROS) that can induce oxidative stress.^{2,3,15} Acute redox activities can be assessed by changes in the levels of urinary oxidative biomarkers including 8-iso-prostaglandin F_{2α} (8-isoprostane), malondialdehyde (MDA) and oxidised guanine species (Ox-GS), that is, 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG) and 8-hydroxyguanine (8-OHGua). 8-Isoprostane and MDA are generated by cellular lipid peroxidation, whereas Ox-GS are formed during repair of oxidised DNA.^{6,16,17} These biomarkers have been used to assess systemic oxidative status resulting from exposure to biomass smoke.^{18–22}

To characterise occupational WFS exposure and assess potential health effects on WLFFs working in Midwestern USA, we examined cross-shift changes in the levels of mutagenicity and oxidative biomarkers in spot urine samples collected from WLFFs in Ohio.

MATERIALS AND METHODS

Study location and WLFFs

Between 2015 and 2018, a convenience sample of 19 healthy firefighters (17 men and 2 women) were recruited from the US Forest Service–Wayne National Forest (USFS-WNF). A baseline questionnaire was provided to each firefighter to obtain information about demography and work history, and two questionnaires were administered to collect self-evaluated WFS exposure and information about daily work activities and potentially confounding exposures immediately after and the morning following each prescribed burn or regular work shift.

Prescribed (or controlled) burn is a land management tool used to reduce vegetative fuel load on the forest floor. WLFFs working at prescribed burn are generally involved in two tasks: lighting and/or holding. Firefighters assigned to lighting use a drip torch to ignite fires in predesignated area, whereas those holding, patrol and quench fires at the boundaries to maintain fires within the preplanned burn areas. Prescribed burns are conducted at the USFS-WNF in early spring and late fall. WLFFs on burn days were involved in arduous tasks including lighting and/or holding, whereas they worked at the forest office on non-burn with few exceptions doing fire response training, field investigation and timber management that require less physical exertion compared with the burn day tasks.

Sample collection and exposure assessment

Spot (single) urine samples were collected from each firefighter right before (pre-shift, ~20 min before), immediately after (post-shift, ~20 min after), and the morning following (next morning, 12–15 hours after) prescribed burn (burn day) and regular (non-burn day) work shifts using 90 mL polypropylene containers. The volume of sample collected from the firefighters was 60–70 mL. An aliquot of 25–30 mL was used for analysis of urinary mutagenicity and the remaining (30–35 mL) raw urine

was used for the analyses of oxidative stress biomarkers. The samples were stored immediately after collection in light-proof containers with dry ice and subsequently transported to The Ohio State University. The samples were aliquoted, labelled to ensure confidentiality and stored at –80°C until analysis.

Personal exposure to PM_{2.5} during prescribed burns was measured by gravimetry in the breathing zone of the firefighters using the MicroPEM aerosol sensor (RTI International, Research Triangle Park, North Carolina, USA). The concentration of BC in WFS particulates was determined using a SootScan Model OT21 Optical Transmissometer (Magee Scientific, Berkeley, California, USA). To estimate the organic carbon (OC) content in WFS particulates, a linear relationship ($OC = 0.567 \times (PM - BC) - 0.119$, $r^2 = 0.885$) derived from a previous study investigating BC emissions from wood-fueled cookstoves was used.²³

Urinary mutagenicity and oxidative biomarkers

Urinary mutagenicity was assessed as described previously.^{5,8} Briefly, ~25 mL of urine was filtered and enzymatically de-conjugated in 0.2 M (10% v/v) sodium acetate buffer (pH 5.0) (Sigma, St. Louis, Missouri, USA), containing β-glucuronidase (6 U/mL urine; Cat. No. G-7017, Sigma, St. Louis, Missouri, USA) and sulfatase (2 U/mL urine, Cat. No. S-9751, Sigma, St. Louis, Missouri, USA) for 16 hours at 37°C. The urine was then poured through a C-18 silica-gel column (Waters Corp, Milford, Massachusetts, USA), and the organics were eluted with 10 mL of methanol, which was then solvent-exchanged into dimethyl sulfoxide (DMSO) to produce an organic concentrate at 150X; concentrates were stored at 4°C until used for bioassay. Methanol/C-18 blanks were also prepared and tested for mutagenicity.

We used the *Salmonella* (Ames) mutagenicity assay with strain YG1041 in the plate-incorporation method to evaluate the organic concentrates of the urine samples as described previously.⁸ Briefly, the concentrates were evaluated at 0.3, 0.6, 1.2, 3, 6 and 12 ml-equivalents (ml-eq) of urine at one plate per dose. All experiments were performed with metabolic activation (S9 mix) made from Aroclor-induced, Sprague-Dawley rat-liver S9 (Moltox, Boone, North Carolina, USA). Plates were incubated at 37°C for 3 days, after which the colonies were counted on an automatic colony counter (ProtoCOL 3, Synbiosis, Frederick, Maryland, USA). We used DMSO at 100 μL/plate as the negative control. The positive controls were 2-nitrofluorene at 3 μg/plate in the absence of S9 and 2-aminoanthracene at 0.5 μg/plate in the presence of S9. The coefficient of variation (CV) for urinary mutagenicity was not calculated because enough sample was available to test only once.

Oxidative stress biomarkers were measured in raw urine aliquots. Concentrations of 8-isoprostane and Ox-GS were measured using commercially available ELISA kits (Cayman, Ann Arbor, Michigan, USA). MDA level was determined using a colorimetric assay kit (Cayman, Ann Arbor, Michigan, USA). Standards and samples were measured in duplicate or triplicate according to the manufacturer's instructions. The CV for 8-isoprostane, MDA and Ox-GS are 14.90, 3.10, 9.92%, respectively. Urinary creatinine level was measured using a colorimetric kit (Cayman, Ann Arbor, Michigan, USA). Both non-creatinine-corrected (crude) and creatinine-corrected urinary biomarker concentrations were presented. The samples were banked and run as a group for the analyses.

Statistical analyses

The slope of the linear regressions over the linear portion of the dose–response curves, expressed as revertants/ml-eq, was

used to determine the mutagenic potency. The linear portion of the curve was defined by the initial doses that gave the highest r^2 value that also had a $p \leq 0.05$ based on a trend test. Samples that did not achieve both requirements were given mutagenic potency of zero.

Cross-shift changes in biomarkers and whether these changes were different between burn and non-burn days were analysed using linear mixed-effect models (LMMs) with the subject and date included as random effect variables. Because urinary biomarkers were not distributed normally, the concentrations were log-transformed to achieve normality before being included in the models. To estimate cross-shift changes, an intercept-only model was used in which the log-differences were the dependent variables. The difference in cross-shift changes on burn day between tasks (holding or lighting) was also analysed using LMMs while controlling for potential confounders including career length, smoking status, chewing of tobacco, eating grilled foods, shift duration and acreage of burn. Only duration and acres, which were uncorrelated, were significant for some of the biomarkers and, thus, were included in the final model. LMMs were also used to analyse the associations between the concentrations of air pollutants in WFS and the cross-shift changes in the biomarkers. $PM_{2.5}$ and BC data were also log-transformed to achieve normality. The association between urinary biomarkers was analysed using Pearson correlation. All statistical analyses were performed using SAS (V.9.4), and $p < 0.05$ was considered statistically significant.

RESULTS

Analyses were done for a total of 120 spot urine samples from 19 firefighters (age— 35.0 ± 7.2 years; career length— 9.2 ± 6.8 years). Of these, 81 and 39 urine samples were collected on 7 burn days and 3 non-burn days, respectively. All samples collected on burn days were completely paired. There were 15 urine sample sets collected on non-burn days, and of these 1 and 5 sample sets did not have post-shift and next-morning samples, respectively. The average length of time spent at prescribed burn shifts was 4.98 ± 1.34 hours, and the average area burned was 301.19 ± 157.86 acres. At prescribed burns, geometric mean concentrations of personal exposure to $PM_{2.5}$ and BC were 1.36 ± 0.16 mg/m³ and 59.39 ± 8.68 µg/m³, respectively.

The percentages of zero urinary mutagenicity were 33% in pre-shifts, 11% in post-shifts and 37% in next-morning of burn days, and the corresponding percentages on non-burn days were 47%, 31% and 45%. Crude and creatinine-corrected urinary biomarker concentrations on prescribed burn and regular workdays are presented in table 1, and cross-shift changes in the biomarkers examined using LMMs are shown in table 2. Crude values of urinary mutagenicity, 8-isoprostane and MDA increased significantly from pre-shift to post-shift on burn days ($2.56\times$, $2.45\times$ and $1.56\times$, $p < 0.01$) and returned to a level similar to that of the pre-shift the next morning.

Except for pre-shift to next-morning changes in crude urinary 8-isoprostane and MDA ($1.77\times$ and $1.34\times$, $p < 0.05$), no other significant temporal change in the levels of urinary biomarkers was observed for non-burn days. Post-exposure levels of creatinine-corrected mutagenicity and 8-isoprostane were non-significantly higher than pre-exposure levels on burn days ($1.16\times$ and $1.64\times$; $p = 0.09$ and 0.07), whereas creatinine-corrected 8-isoprostane decreased non-significantly in post-shift on non-burn days ($0.62\times$, $p = 0.08$). Creatinine-corrected Ox-GS levels increased significantly in the next-morning compared with pre-shift on burn days ($1.62\times$, $p = 0.03$). No significant temporal

change in creatinine-corrected urinary biomarkers was observed on non-burn days.

Differences in cross-shift changes in crude and creatinine-corrected values of urinary biomarkers between burn and non-burn days are shown in table 3. Pre-shift to post-shift changes in crude values of urinary mutagenicity, 8-isoprostane and MDA were 2.79-fold, 3.72-fold and 1.72-fold higher on burn days than on non-burn days ($p = 0.02$, 0.01 and 0.03). Following creatinine correction, pre-shift to post-shift change in urinary 8-isoprostane and pre-morning to next morning change in Ox-GS on burn days were 2.64 ($p = 0.03$) and 3.00 ($p = 0.02$) times greater than the changes on non-burn days.

The effects of work tasks on cross-shift changes in urinary biomarker levels are presented in table 4. No significant effect on pre-shift to post-shift changes was observed. However, WLFFs who performed holding had 1.56 times higher pre-morning to next-morning changes in the levels of creatinine-corrected urinary mutagenicity compared with those who lighted during prescribed burns ($p = 0.03$). Cross-shift change in urinary mutagenic potency was also associated significantly with the length of smoke exposure ($p = 0.01$).

Results of the evaluation of the association between exposure to air pollutants in WFS during prescribed burns and cross-shift changes in urinary biomarker levels are presented in table 5. BC exposure concentration was correlated positively with pre-shift to post-shift change in urinary MDA level ($\beta = 0.36$, $p = 0.01$) but correlated negatively with pre-morning to next-morning change in urinary mutagenic potency ($\beta = -0.27$, $p = 0.04$). $PM_{2.5}$ and OC exposure concentrations were not associated with the urinary biomarkers (online supplemental table S1).

Pre-morning to next-morning changes in creatinine-corrected 8-isoprostane and MDA were significantly correlated ($r = 0.59$, $p < 0.01$).

DISCUSSION

The assessment of related health responses of WFS exposure among WLFFs has been geographically limited to Southeastern and Western USA, where vegetative fuels might be different from the Midwest. In Ohio, the most dominant forest type is oak (~60%),²⁴ whereas pine (~40%) and fir groups (~40%) are the major forest types in the Southeast and West, respectively.²⁵ Furthermore, prescribed burn is mostly conducted in the Southeast (64%) followed by the West (33%) and the Northeast (3%).²⁶ Therefore, distinct urinary mutagenicity and oxidative injuries might be observed among WLFFs in the Midwest due to WFS exposure from combustion of different forest fuels and loads.

Urinary mutagenicity

Investigation of urinary mutagenic activity offers a quantitative assessment of integrated exposure to mutagens in the biomass/wood smoke. Additionally, elevated urinary mutagenicity has been reported to be associated with increased cancer risk.^{5 7 8 27}

In this study, the levels of creatinine-corrected urinary mutagenicity in WLFFs before, after and the morning following prescribed burns were approximately 5.3-fold, 6.4-fold and 6.4-fold higher, respectively, compared with those from a similar study conducted in the Southeastern USA.⁵ Creatinine-corrected mutagenic potencies in pre-shift, post-shift and next-morning on non-burn days were also 3.4-fold, 5.6-fold and 5.4-fold higher in the current study, respectively.⁵ The study design used in both studies are comparable. Both report urinary mutagenicity and 8-isoprostane levels in pre-shift, post-shift and next morning

Table 1 Geometric mean (GM), geometric SD (GSD), and range for crude and creatinine-corrected values of urinary biomarkers in WLFFs by types of workday (prescribed burn or regular work) and time of sample collection (pre-shift, post-shift or next-morning)

	Prescribed burn (N=81)		Regular work (N=39)	
	GM±GSD	Range	GM±GSD	Range
Crude values				
Urinary mutagenicity (rev/ml-eq)				
Pre-shift	4.31±0.97	0.00–27.54	3.92±1.36	0.00–18.95
Post-shift	11.03±2.54	0.00–72.86	4.44±1.37	0.00–15.52
Next-morning	4.54±1.16	0.00–40.00	3.65±1.44	0.00–23.81
8-isoprostane (ng/mL)				
Pre-shift	1.30±0.31	0.09–15.15	0.75±0.15	0.17–2.40
Post-shift	3.18±0.80	0.15–17.33	0.51±0.18	0.07–3.02
Next-morning	1.62±0.39	0.12–9.07	1.66±0.39	0.36–5.48
MDA (µM)				
Pre-shift	4.07±0.53	1.16–12.05	4.53±0.66	1.47–9.19
Post-shift	6.36±1.01	0.58–19.32	4.26±0.64	2.04–10.47
Next-morning	3.91±0.51	0.76–9.64	6.62±0.87	3.01–11.58
Ox-GS (ng/mL)				
Pre-shift	98.27±14.03	18.91–607.57	114.55±26.60	21.66–1147.40
Post-shift	116.99±16.48	24.93–509.38	129.66±39.24	30.96–1069.46
Next-morning	126.53±13.09	49.71–373.05	76.67±14.56	24.15–220.10
Creatinine-adjusted values				
Urinary mutagenicity (rev/µmol creatinine)				
Pre-shift	1.55±0.14	0.00–3.85	1.53±0.19	0.00–3.08
Post-shift	1.80±0.13	0.00–2.83	1.52±0.13	0.00–1.36
Next-morning	1.67±0.19	0.00–6.96	1.34±0.13	0.00–1.38
8-isoprostane (ng/mg creatinine)				
Pre-shift	1.05±0.21	0.13–15.01	0.82±0.13	0.17–2.18
Post-shift	1.72±0.36	0.37–125.09	0.50±0.12	0.13–1.64
Next-morning	1.72±0.47	0.08–66.90	0.96±0.20	0.20–2.53
MDA (µmol/g creatinine)				
Pre-shift	3.28±0.29	1.26–7.63	4.94±0.61	2.74–14.44
Post-shift	3.43±0.23	1.69–7.29	4.15±0.45	2.42–8.74
Next-morning	4.17±0.45	1.49–17.17	3.81±0.59	2.08–10.78
Ox-GS (ng/mg creatinine)				
Pre-shift	83.04±16.06	12.63–901.98	101.99±32.67	8.67–501.50
Post-shift	67.54±15.55	10.23–1142.56	91.46±38.25	8.35–1180.23
Next-morning	134.77±31.34	15.80–2644.84	55.21±17.11	10.18–239.31

MDA, malondialdehyde; Ox-GS, oxidised guanine species; WLFFs, wildland firefighters.

of burn and non-burn days. However, personal PM_{2.5} exposure concentration in this study was ~5-fold higher than in the Southeastern study. These results suggest that WLFFs working in the Midwest had three to five times higher mutagenic exposures than those in the other study.^{5 16 28–30}

Similarly, pre-exposure and post-exposure levels of creatinine-corrected mutagenic potencies on burn days in our study were 3.2-fold and 2.1-fold higher than the corresponding potencies reported in wood-fire steam bath users.⁷ WLFFs in our study who performed the holding task had higher levels of urinary mutagenicity than did those who performed lighting. The holding task was associated with exposure to smouldering emissions, which have much higher mutagenicity emission factors than do flaming emissions.¹⁴

Nonetheless, the firefighters in our study had pre-exposure and post-exposure levels of urinary mutagenicity that were 1.2-fold to 2.3-fold less than those reported for charcoal workers in South America.⁸ Although WLFFs are also exposed dermally to biomass smoke, the charcoal workers probably experienced more dermal exposure because firefighters have a relatively

higher dermal protection against occupational smoke exposure. The higher urinary mutagenicity of the charcoal workers could also be due to their routine exposure to biomass smoke, unlike the intermittent exposures of the WLFFs.

Both crude and creatinine-corrected urinary mutagenicity increased among firefighters following WFS exposure. WLFFs working in the southeast had a 1.6-fold higher creatinine-corrected cross-shift change in urinary mutagenicity on burn days compared with non-burn days.⁵ Following combustion smoke exposure, urinary mutagenicity increased by 1.9-fold and 1.7-fold among Ottawa municipal firefighters and wood-fire steam bath users, respectively.^{6 7} These results combined with our findings demonstrate that combustion emissions are capable of causing systemic mutagenicity among exposed individuals. However, the smaller cross-shift changes observed in this study might be due to a higher baseline level (pre-shift and non-burn days) of urinary mutagenicity in the WLFFs. Such high baseline might be due to repeated exposure of WLFFs in this study to more elevated WFS. Also, higher pre-existing elevated levels of urinary mutagenicity could attenuate the cross-shift changes on

Table 2 Cross-shift changes (post-shift or next-morning vs pre-shift) in crude and creatinine-corrected values of urinary biomarkers in WLFFs on prescribed burn days or regular work days using linear mixed-effect models

	Prescribed burn		Regular work	
	Ratio (95% CI)	P value	Ratio (95% CI)	P value
Crude values				
Pre-shift to post-shift*				
Mutagenicity	2.56 (1.49–4.40)	<0.01	0.92 (0.49–1.73)	0.77
8-isoprostane	2.45 (1.35–4.41)	<0.01	0.66 (0.32–1.36)	0.23
MDA	1.56 (1.16–2.11)	<0.01	0.91 (0.60–1.37)	0.62
Ox-GS	1.19 (0.74–1.91)	0.46	1.17 (0.49–2.80)	0.71
Pre-morning to next-morning*				
Mutagenicity	1.05 (0.60–1.85)	0.85	0.84 (0.26–2.73)	0.75
8-isoprostane	1.24 (0.69–2.25)	0.46	1.77 (1.07–2.95)	0.03
MDA	0.96 (0.73–1.27)	0.77	1.34 (1.01–1.78)	0.04
Ox-GS	1.29 (0.86–1.93)	0.21	0.69 (0.36–1.33)	0.23
Creatinine-corrected values				
Pre-shift to post-shift*				
Mutagenicity	1.16 (0.98–1.39)	0.09	0.93 (0.74–1.18)	0.52
8-isoprostane	1.64 (0.95–2.81)	0.07	0.62 (0.36–1.08)	0.08
MDA	1.04 (0.84–1.30)	0.69	0.86 (0.65–1.14)	0.27
Ox-GS	0.81 (0.47–1.40)	0.44	0.87 (0.37–2.03)	0.72
Pre-morning to next-morning*				
Mutagenicity	1.08 (0.88–1.33)	0.45	0.87 (0.65–1.16)	0.32
8-isoprostane	1.64 (0.77–3.49)	0.19	1.12 (0.76–1.65)	0.54
MDA	1.27 (0.94–1.72)	0.12	0.84 (0.66–1.08)	0.15
Ox-GS	1.62 (1.04–2.53)	0.03	0.54 (0.18–1.60)	0.24

*Cross-shift changes were defined as log (post-shift or next-morning) versus log (pre-shift) and results were backlog-transformed.

MDA, malondialdehyde; Ox-GS, oxidised guanine species; WLFFs, wildland firefighters.

burn days and differences in the changes between burn and non-burn days in this study.

Unlike the findings in the Southeastern study,⁵ WLFFs in this study who performed holding had a higher increase in creatinine-corrected urinary mutagenicity from pre-morning to next-morning compared with those who performed lighting (table 4). Meanwhile, exposure concentration of PM_{2.5} was 1.5-fold higher in holding compared with lighting firefighters (data not shown). Although the Southeastern study indicated that exposure to both diesel and woodsmoke might induce an additive or synergistic effect on urinary mutagenicity,⁵ the results in this study suggest that increased urinary mutagenicity was due primarily to exposure to particulate-phase and gas-phase mutagens in WFS.

Nonetheless, cross-shift change in creatinine-corrected urinary mutagenicity was not associated with personal PM_{2.5} concentration. Instead, pre-morning to next-morning change in the mutagenicity was correlated negatively with BC exposure and BC to PM_{2.5} ratio, which were higher among lighters (data not shown). These findings suggest that personal exposure concentration might not necessarily represent internal dose among the exposed firefighters.

Urinary oxidative status

Oxidative stress is a series of imbalances between ROS production and the capacity of antioxidant defence in cells. Exposure

Table 3 Comparison of cross-shift changes (post-shift or next-morning vs pre-shift) in crude and creatinine-corrected values of urinary biomarkers in WLFFs on prescribed burn days to regular workdays using linear mixed-effect models

	Pre-shift to post-shift*		Pre-shift to next-morning*	
	Ratio (95% CI)	P value	Ratio (95% CI)	P value
Crude values				
Mutagenicity	2.79 (1.16–6.69)	0.02	1.25 (0.41–3.76)	0.69
8-isoprostane	3.72 (1.42–9.75)	0.01	0.70 (0.27–1.85)	0.46
MDA	1.72 (1.04–2.84)	0.03	0.72 (0.45–1.14)	0.16
Ox-GS	1.02 (0.43–2.45)	0.96	1.87 (0.90–3.88)	0.09
Creatinine-adjusted values				
Mutagenicity	1.25 (0.93–1.67)	0.13	1.24 (0.86–1.79)	0.24
8-isoprostane	2.64 (1.13–6.16)	0.03	1.47 (0.44–4.85)	0.52
MDA	1.21 (0.84–1.75)	0.29	1.51 (0.92–2.47)	0.10
Ox-GS	0.94 (0.37–2.41)	0.89	3.00 (1.19–7.57)	0.02

*Cross-shift changes were defined as log (post-shift or next-morning) versus log (pre-shift) and results were backlog-transformed.

MDA, malondialdehyde; Ox-GS, oxidised guanine species; WLFFs, wildland firefighters.

Table 4 Difference of cross-shift changes in creatinine-corrected values of urinary biomarkers in WLFFs due to different work tasks (ie, holding or lighting) during prescribed burns

	Work task†	
	Ratio (95% CI)	P value
Pre-shift to post-shift*		
Mutagenicity	1.09 (0.73–1.64)	0.65
8-isoprostane	0.61 (0.18–2.06)	0.41
MDA	0.70 (0.43–1.13)	0.14
Ox-GS	1.00 (0.29–3.46)	1.00
Pre-morning to next-morning*		
Mutagenicity	1.56 (1.05–2.31)	0.03
8-isoprostane	2.56 (0.53–12.39)	0.23
MDA	1.15 (0.61–2.16)	0.66
Ox-GS	1.13 (0.40–3.18)	0.81

*Cross-shift changes were defined as log (post-shift or next-morning) versus log (pre-shift) and results were backlog-transformed.

†WLFFs worked holding task versus lighting task during prescribed burns. MDA, malondialdehyde; Ox-GS, oxidised guanine species; WLFFs, wildland firefighters.

to biomass-burning smoke is capable of inducing free radical-related oxidation in cells,¹ and urinary biomarkers are often used to study redox balance among exposed individuals.^{5 8 16 17} Oxidative stress is an important pathogenic process that is associated with many diseases such as cardiovascular disease and cancer.³¹

Pre-shift, post-shift and next-morning creatinine-corrected 8-isoprostane levels on burn and non-burn days were 2.6–5.7 and 1.3–4.1 times higher in the current study than those measured at corresponding work shift days in Southeastern USA.⁵ Although creatinine-corrected 8-isoprostane was not reported in a study conducted among western WLFFs,¹⁷ the average crude value of urinary 8-isoprostane on non-burn days observed in this study was 1.4-fold higher. Unlike WLFFs in the Southeastern US study, we observed a significant difference of pre-shift to post-shift change in creatinine-corrected 8-isoprostane between burn and non-burn days (table 3). Again, the higher WFS exposure concentration of prescribed burns in this study could be the possible explanation.

We also observed a non-significant 1.64-fold increase in creatinine-corrected 8-isoprostane in the morning following prescribed burns (table 2). Similar observations were made in

a controlled human exposure study in which 8-iso-PGFα was 1.45-fold and 1.20-fold greater among healthy adults in the post-morning and next morning following woodsmoke exposure, respectively.²¹ In addition, WLFFs in our study had a 1.6–3.6 times higher level of creatinine-corrected 8-isoprostane compared with cigarette smokers in previous cross-sectional studies.^{32–34} It is worth noting that the concentration of urinary 8-isoprostane determined by ELISA is ~40% greater than the concentration obtained from analysis by liquid chromatography–mass spectrometry.³⁵ Considering the different sensitivities of these analytical methods, WLFFs in this study still may have a higher level of urinary 8-isoprostane compared with the general population, including smokers.

Likewise, creatinine-corrected MDA levels observed before, after and the morning following burn and non-burn days in our study were 4.2–6.3 and 4.8–6.8 times higher than the corresponding levels reported in the Southeastern study (μmol/g creatinine=113 μmol MDA/mole creatinine).⁵ Pre-exposure and post-exposure levels of creatinine-corrected MDA in the present study were ~4-fold higher compared with those from another WLFF study in the Southeast.¹⁶ The urinary MDA concentrations presented here were determined using the trichloroacetic acid method in which MDA in the sample reacts with thiobarbituric acid (TBA) to form red MDA-TBA adducts that are colorimetrically quantified at 530 nm. Therefore, increased urinary MDA levels observed in this study could be due to higher WFS exposure concentration and/or different analytical methods for urinary MDA.

In comparison with a population-based study that used gas chromatography–mass spectrometry to determine urinary MDA level, crude urinary MDA concentrations among healthy adults in a woodsmoke-impacted community were 1.5–2.5 times lower than those measured among WLFFs in this study.²⁰ Furthermore, an increased MDA level in exhaled breath condensate collected from healthy adults following a 4-hour woodsmoke exposure was observed in two controlled human exposure studies.^{36 37} The results described above, along with our findings, suggest that woodsmoke exposure could lead to both local and systemic oxidative effects among exposed individuals. In this study, we observed that pre-shift to post-shift changes in MDA levels were correlated positively with BC concentration (table 5). Similarly, a positive effect of indoor BC exposure on urinary MDA among smokers with diagnosed chronic obstructive pulmonary disease was reported in a recent study.³⁸

Table 5 Association between air pollutant concentrations in wildland fire smoke emission during prescribed burns and cross-shift changes (ie, post-shift or next-morning vs pre-shift) in creatinine-corrected values of urinary biomarkers in WLFFs

	PM _{2.5} (mg/m ³)		Black carbon (μg/m ³)	
	β (95% CI)	P value	β (95% CI)	P value
Pre-shift to post-shift*				
Mutagenicity	0.01 (−0.28 to 0.31)	0.93	−0.04 (−0.28 to 0.20)	0.74
8-isoprostane	0.12 (−0.78 to 1.03)	0.78	0.42 (−0.30 to 1.14)	0.24
MDA	0.05 (−0.32 to 0.42)	0.80	0.36 (0.10 to 0.63)	0.01
Ox-GS	0.21 (−0.69 to 1.11)	0.64	−0.36 (−1.08 to 0.37)	0.32
Pre-morning to next-morning*				
Mutagenicity	0.05 (−0.30 to 0.40)	0.77	−0.27 (−0.53, to 0.01)	0.04
8-isoprostane	0.50 (−0.75 to 1.74)	0.42	0.20 (−0.83 to 1.23)	0.70
MDA	0.26 (−0.24 to 0.76)	0.30	0.31 (−0.09 to 0.70)	0.12
Ox-GS	−0.62 (−1.31 to 0.08)	0.08	−0.32 (−0.92 to 0.27)	0.27

*Cross-shift changes were defined as log (post-shift or next-morning) versus log (pre-shift) and results were backlog-transformed.

MDA, malondialdehyde; Ox-GS, oxidised guanine species; WLFFs, wildland firefighters.

Different analytical methods used to determine different damaged nucleic acid species make a direct comparison difficult between our study and the other studies. We measured the sum of damaged nucleic acid species (8-OHdG, 8-OHG and 8-OHGua) in the urine sample using ELISA, whereas the others often reported one of the species determined by high-performance liquid chromatography.^{16,22} However, our results were consistent with previous studies, showing that DNA/RNA damage decreased non-significantly by ~20% after WFS exposure but increased nearly twofold the next morning (table 1). Similarly, in a Southeastern WLFF study, post-exposure levels of 8-Oxo-dG dropped ~14% compared with pre-exposure level.¹⁶ In an experimental woodsmoke exposure study, urinary excretion of 8-OxoGua in healthy adults increased non-significantly ~2-fold following 20 hours after leaving the exposure chamber.²²

PM-mediated oxidative stress and/or inflammation are postulated to induce oxidative DNA damage.³⁹ This hypothesis is supported by the significant difference of pre-morning to next-morning changes in oxidative DNA/RNA damage between burn and non-burn days in this study (table 3). These results suggest that oxidative DNA/RNA damage might be a delayed response to the effect of biomass smoke exposure compared with other oxidative effects.

In conclusion, urinary biomarkers used in this study reflected the effect of WFS exposure on acute health responses among exposed individuals. Our results suggest that WLFFs working in the Midwestern region of the USA may have an increased risk of systemic exposure to mutagens and oxidative injury due to repeated exposure to elevated levels of WFS compared with those working in the Southeastern and Western USA.

Acknowledgements Our sincere thanks go to Ryan Sundberg, Jason Bew, and the Wayne National Forest crew and wildland firefighters who participated in this study. We also thank W. Kyle Martin (University of North Carolina at Chapel Hill) for training Chieh-Ming Wu on how to perform the urinary organic extractions.

Contributors OA and C-MW obtained research funding, designed the study, recruited wildland firefighters and collected spot urine samples. C-MW also performed biochemical and statistical analyses with the advice from OA and CS. DMD and SHW performed urinary mutagenicity using the *Salmonella* (Ames) mutagenicity assay. The final manuscript was prepared by C-MW and critically revised by the coauthors.

Funding This study was supported by: (1) the Grant or Cooperative Agreement Number, T42-OH008455, funded by the Centers for Disease Control and Prevention. (2) Alumni Grants for Graduate Research and Scholarship (AGGRS) at The Ohio State University. The organic extractions and urinary mutagenicity experiments were performed at the US Environmental Protection Agency, Research Triangle Park, North Carolina, and supported by the intramural research program of the Office of Research and Development of the US EPA, Research Triangle Park, North Carolina.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The protocol was reviewed and approved by The Ohio State University Institutional Review Board (2017H0075). Informed consent was obtained from each participant prior to their enrolment.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. De-identified data are available upon request to the corresponding author (adetona.1@osu.edu).

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