Evaluation of Interventions to Reduce Firefighter Exposures

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Objective: Evaluate the effectiveness of firefighter exposure reduction interventions. **Methods:** Fireground interventions included use of self-contained breathing apparatus by engineers, entry team wash down, contaminated equipment isolation, and personnel showering and washing of gear upon return to station. Urinary polycyclic aromatic hydrocarbon metabolites (PAH-OHs) were measured after structural fire responses before and after intervention implementation. Separately, infrared sauna use following live-fire training was compared to standard postfire care in a randomized trial. **Results:** The fireground interventions significantly reduced mean total urinary postfire PAH-OHs in engineers (−40.4%, 95%CI −63.9%, −2.3%) and firefighters (−36.2%, 95%CI −56.7%, −6.0%) but not captains (−11.3% 95%CI −39.4%, 29.9%). Sauna treatment non-significantly reduced total mean PAH-OHs by −43.5% (95%CI −68.8%, 2.2%). **Conclusions:** The selected fireground interventions reduced urinary PAH-OHs in engineers and firefighters. Further evaluation of infrared sauna treatment is needed.

Keywords: exposure reduction, firefighter, intervention, polycyclic aromatic hydrocarbon, sauna, SCBA, wash down

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Source of Funding: US Federal Emergency Management Agency Assistance to Firefighters Grant program, EMW-2014-FP-00200.

Burgess, Hoppe-Jones, Griffin, Zhou, Gulotta, Wallentine, Moore, Valliere, Weller, Beitel, Flahr, Littau, Dearmon-Moore, Zhai, Jung, Garavito, and Snyder have no relationships/conditions/circumstances that present potential conflict of interest.

The JOEM editorial board and planners have no financial interest related to this research.

Supplemental digital contents are available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.joem.org).

Clinical Significance: Firefighters have a higher risk of cancer than the general population and are exposed to multiple known and suspected carcinogens including some polycyclic aromatic hydrocarbons (PAHs). Use of SCBA by engineers and wash down for entry teams, but not infrared sauna use, significantly reduce urinary PAH metabolites after fire exposures.

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DOI: 10.1097/JOM.0000000000001815

Learning Objectives

- Discuss the increased cancer risk in firefighters compared to the general population and the role of hydroxylated metabolites of polycyclic aromatic hydrocarbons (PAH-OHs) as biomarkers of exposure.
- Summarize the new findings on effectiveness of specific fireground interventions to reduce exposure during structural fire responses.
- Summarize the findings on the effectiveness of post-exposure infrared saunas.

INTRODUCTION

Firefighters are at higher risk for multiple cancers than the general population,[1,2] with cancer incidence and mortality increasing with time spent at the fire scene and number of fire runs for lung cancer and leukemia, respectively.[3] Exposure to multiple known and suspected human carcinogens, including some polycyclic aromatic hydrocarbons (PAHs), benzene, and formaldehyde, have been documented in products of combustion at the fireground.[4,5] The measurement of hydroxylated metabolites of PAHs in urine (PAH-OHs) has been used extensively as a biomarker of firefighter exposure[6–14] and reflects exposure from inhalation, skin exposure, and ingestion. Many urinary PAH-OHs have biological half-lives of the order of several hours or less and generally serve as a marker of short-term exposures.[15]

Fire departments are increasingly putting into practice strategies to reduce or mitigate exposure to carcinogens, focusing on both inhalation and dermal exposure routes. These include increased use of self-contained breathing apparatus (SCBA) during overhaul, more rapid dermal decontamination at the fire scene, postfire personal protective equipment (PPE) decontamination to reduce firefighters' exposure to combustion products via off-gassing from contaminated gear and dermal transfer, taking showers and changing clothes as soon as possible upon return to the station, and washing turnout gear after each fire.[16–18] In addition, some fire departments are providing saunas for use after returning to the station postfire incident.

Only limited information is available on the effectiveness of fire department interventions on reducing the concentration of toxicant biomarkers in the body of firefighters, and uncertainty over the efficacy of these practices limits their implementation.[17–19] In addition, even well-intended interventions may have adverse effects. For example, use of air purifying respirators during overhaul led to poorer respiratory outcomes than use of no respiratory protection at all, resulting in the recommendation to use SCBA during overhaul.[5] The purpose of the current study was to evaluate the effectiveness of specific interventions chosen by the fire service, including fireground interventions put in place by the Tucson Fire Department (TFD) for structural fire responses and the use of postexposure infrared saunas by the Scottsdale Fire Department (SFD).

METHODS

The fireground exposure reduction intervention study implemented by the TFD was part of a larger cancer prevention research project approved by the UA IRB, Protocol # 1509137073. The sauna intervention study implemented by the SFD was separately approved by the UA IRB. All subjects provided informed consent prior to entry into the study.

Fireground Intervention Study

The fireground intervention study included baseline, pre-intervention postexposure, intervention training and post-intervention postexposure components. All TFD firefighters were eligible for inclusion in the study. Participating subjects completed a survey on their occupational and medical history at baseline, when they entered into the study. Biological samples collected at this time included urine, blood, and buccal cells. Collection of baseline samples started in October, 2015 and extended through July, 2018. During the pre- and post-intervention postexposure periods, firefighters were monitored for exposure to products of combustion by collecting urine 2 to 3 hours following cessation of their fire response. TFD predominantly selected residential structural fires for evaluation as this was the most common type of fire event. Industrial fires were excluded from evaluation. The pre-intervention exposure evaluation period began in February, 2016 and extended through January, 2017. The post-intervention period extended from November, 2017 to March, 2019. All TFD personnel were trained on the new interventions from October to November of 2017, with continuing reminders thereafter.

TFD used the results of the pre-intervention urinary PAH-OH analyses to plan multiple exposure reduction interventions to minimize both inhalation and dermal exposure. These included use of SCBA by engineers (“engineers on air”) and fire cause investigators, surface contamination reduction (“wash down”) of turnout gear and SCBA predominantly worn by entry teams by cleaning the gear with soap and water prior to doffing, additional skin decontamination, and segregation of contaminated gear prior to transport and additional cleaning of gear upon return to the station. TFD focused the post-intervention evaluation on fire crews expected to have followed the recommended interventions.

The engineers on air intervention was selected based on the assumption that their exposure was primarily due to a lack of respiratory protection, as they did not participate in interior firefighting and generally did not show evidence of soot deposition on their turnout gear or skin. Prior to the intervention, first-in engineers operating at the pump panel or aerial and/or securing utilities generally operated without an SCBA. The intervention involved the recommendation that, as soon as practical, engineers should don their SCBA and be on positive pressure air while exposed to smoke.

The postfire wash down was selected to reduce self-contamination by soot deposition on turnout gear and SCBA, exposure to off-gassing during and after doffing of gear and contamination of other personnel treating the exposed firefighter and/or subsequently handling the gear. This intervention was chosen based on a previous study demonstrating that two minutes of brushing with soap and water removed a median of 85% of PAHs from the turnout ensemble.[18] The intervention involved gross external decontamination of turnout gear prior to removing the firefighting ensemble (including SCBA regulator) worn in the hot zone. The firefighters brushed off large debris first and then sprayed each other with water to remove loose particulates. The wash down kit included a bucket with a lid, 2.5″ to green line reducer, hose, nozzle, brush, and soap (Dawn Ultra Dishwashing Liquid, Procter & Gamble, Cincinnati, OH). The gear was washed for ~ 2 minutes, with water pressure limited to avoid drenching of the gear. After the wash down, the turnout gear was removed prior to reporting to rehabilitation (“rehab”) at the fire scene. Following return to their station, the firefighters were encouraged to shower as soon as possible, ideally within an hour, and put on clean clothing.

The practice of bagging of gear and maintaining a “clean cab” was selected based on the premise that products of combustion should be treated in a similar fashion as any other biohazard. Contaminated hose, tools, SCBAs, or any other contaminated equipment were to be decontaminated on scene and/or transported in a manner as to not contaminate the cab of the truck. Clear plastic bags were carried by each executive captain so that gear could be bagged and easily identified. Fire hose and any other dirty gear were bagged or transported separately from the cab. Upon arrival back at the station, the bags were opened outside the bays and allowed to off-gas before cleaning.

In the station, all contaminated gear was washed in an extractor (UniMac and Wascomat) with turnout manufacture approved mild detergent (ECOLAB Tri-Star Flexylite), using nitrile gloves and eye protection. The outer shells were separated from the inner liners and washed separately with the manufacturer recommended extractor wash cycles for each with parameters determined by the device specifications, calibrated by the vendor. Boots were scrubbed and gloves, helmet pieces and SCBA facepieces were hand washed with warm water. During the intervention TFD also increased the number and size of the station extractors, allowing for more turnouts to be cleaned with each wash cycle.

Two separate surveys, one on-scene and another following return to the station (in-station), were completed using tablet computers during the pre- and post-intervention periods. The on-scene survey was taken during rehab and collected information on the subject’s role during the response, extinguishing agents used, PPE worn, medical symptoms, how long it had been since they were involved in fire suppression prior to this response and how long it had been since their turnout gear had been washed. The in-station survey, completed two to three hours after the end of the fire response, included questions on soot on their gear and/or skin, bagging their gear prior to leaving the fire scene, showering within an hour and cleaning and storage of gear. In addition, questions were asked concerning exposures in the past two weeks not related to their firefighting activities, including smoking, grilling foods, beverages, refueling vehicles, and bicycling.

Sauna Intervention Study

SFD firefighters scheduled for annual live-fire fire training were eligible for study participation. Exclusion criteria included current smoking (including cigarettes, cigars, and e-cigarettes) and contraindication to ingestion of the core body temperature monitor probe, including impairment of the gag reflex, a swallowing disorder, diseases or disorders of the esophagus, previous gastrointestinal surgery or obstructive disease of the gastrointestinal tract, a low motility disorder of the gastrointestinal tract, a cardiac pacemaker or other implanted electromedical device and undergoing nuclear magnetic resonance or magnetic resonance imaging scanning less than 3 days after swallowing the sensor. Urine was collected for 12 hours prior to the anticipated annual fire training start time and for 12 hours following completion of the live-fire training. The firefighters ingested a temperature probe and wore a core body temperature monitor (CorTemp Data Recorder, HQ, Inc., Palmetto, FL) and a chest heart rate belt (Polar Heart Rate Chest Belt, Polar, Bethpage, NY) during the fire and for 8 hours afterward, with the exception of removal during showering.

The sauna intervention study was conducted over three days in 2018 (May 8th, May 10th, and September 5th) with two crews of three subjects on each of the first two dates and four crews of three subjects each on the final date. The study utilized live fire training evolutions conducted annually for every SFD firefighter as a continuing education requirement. The evolutions were conducted in a fire service training burn building with each burn utilizing one wooden pallet, a 1/4 bale of hay, and 1.44 square meters of oriented strand board. The evolutions simulated a residential structure fire with crews wearing SCBA being deployed interior for the tactical objectives of fire attack and search and rescue. The approximate time operating on the interior was 10 to 15 minutes for each evolution. The subjects participated in two evolutions with a 30-minute break in between to rehydrate, refill SCBA bottles, and a quick critique of the first evolution. No decontamination efforts were conducted during the break. The second evolution was conducted with the same burn materials and a similar scenario, except crews alternated assignments for deployment and arrival order. After completion of the second scenario, the crews went to full rehab and conducted their standard decontamination protocols including cleaning their face, neck, hands, and arms with wipes soaked in a dish soap and water solution. Water and electrolytes were provided for rehydration and mister fans were utilized to facilitate cooling. All PPE was taken out of service for cleaning. During rehab, three of the six firefighters were randomly selected for sauna treatment. All firefighters showered after rehab, usually within 20 to 30 minutes of completion of the second evolution. The firefighters not assigned to the sauna treatment left the training yard after showering. Both groups had been instructed not to eat grilled meat 24 hours before and during the 12-hour postexposure urine collection period.

The firefighters randomly selected for sauna treatment entered the sauna (Dynamic Palermo 3-person FAR Infrared Sauna, Model DYN-6330-01, Dynamic Saunas, Ontario, CA) immediately after showering. The mean time between exiting the burn building at the end of the firefighters' second evolution and entering the sauna was 42 minutes, with a range of 36 to 47 minutes. The subjects rested in the sauna for 20 minutes at a temperature setting of 49°C (120 °F), wearing standard fire department station physical training clothing (shorts and t-shirt) and sitting on and utilizing clean towels to absorb sweat. The subjects then took an additional shower immediately after exiting the sauna and then left the training yard. The sauna treatment protocol was determined by SFD based on a time interval that would be reasonable when utilized on duty and a temperature which would be acceptable to the firefighters. While in the sauna the subjects drank water *ad libitum* on the first 2 days of testing but did not have access to water on the final day of testing.

Urine Collection and Analysis

The firefighters were instructed to wash their hands prior to urine collection and to collect each full void using as many urine cups as needed. Samples were stored at 0 to 8°C until they could be transported on ice to the laboratory for processing. For the sauna intervention study, 50 mL 12 hour pre-exposure and postexposure composite urine samples were created by calculating the sum of each full void collected and adding them together to determine the total volume, dividing each time point's volume by the total volume and multiplying by 50 mL. The volume calculated for each time point was then added to a 50 mL conical tube, centrifuged at 1900 rpm for 10 minutes and the supernatant frozen at −20°C until analyzed. Specific gravity was measured on the 12-hour composite baseline, 2 to 4 hour postexposure, and 12-hour the postfire composite samples by refractometry (Atago "Pocket" Urine Specific Gravity Refractometer, Atago Co., Bellevue, WA).

Urine samples were analyzed for 10 PAH-OHs (1-naphthol, 2-naphthol, 2-fluorenol, 3-fluorenol, 4-fluorenol, 1-phenanthrol, 2-phenanthrol, 3-phenanthrol, 4-phenanthrol, and 1-hydroxypyrene) as previously described.[20] In short, 3 mL urine samples were spiked with a mix of isotopically labeled PAH-OHs (1-hydroxynaphthalene-*d*7, 9-hydroxyphenanthrene-*d*8, 2-hydroxyphenanthrene-*d*9, 2-hydroxyfluorene-*d*9, and 1-hydroxypyrene-*d*9). After the addition of 10 uL β-glucuronidase from *Helix pomatia* (Sigma Aldrich, Milwaukee, WI) and 5 mL of sodium acetate buffer, the samples were incubated at 37°C for 16 to 18 hours and extracted using Bond Elut Focus SPE cartridges (Agilent Technologies, Santa Clara, CA). After loading and drying of the cartridges, they were eluted with 6 mL dichloromethane. The solvent in the extracts was exchanged to nonane and the samples derivatized with MSTFA. The derivatized extracts were analyzed on a GC-MS 7890A (Agilent Technologies, Santa Clara, CA).

Statistical Analyses

Non-detectable levels of individual PAH-OHs were replaced by half of their detection limits (175/2 ng/L for naphthols, 100/2 ng/L for fluorenols, 150/2 ng/L for phenanthrols and 200/2 ng/L for 1-hydroxypyrene). Non-detectable PAH-OH sums were replaced by their machine limits (175 ng/L for sum of naphthols, 100 ng/L for sum of fluorenols and 225 ng/L for sum of phenanthrols). All PAH-OH concentrations were log transformed. For fireground interventions multivariable analyses were performed using a linear mixed effects model with random intercept to assess mean differences of log-transformed PAH-OHs, comparing pre- and post-interventions stratified by job types. For the analysis of sauna intervention effects on PAH-OHs, a linear mixed effect model was also adopted. The effect of sauna intervention compared with control treatment on PAH-OHs over time (ie, at baseline, after 2 to 4 hours, after 12 hours) was estimated by adding a "treatment" by "time" interaction. The mean difference of PAH-OH measurements between those with sauna intervention and controls after 12 hours was estimated and the proportion of this difference over the mean PAH-OH measurements after 12 hours with no sauna intervention was reported. Assessment of model fit was performed by the analysis of residuals. All statistical analyses were performed using R version 3.6.0 (https://www.r-project.org). Longitudinal analyses were conducted by the R package "lme4." Confidence intervals of ratio were assessed by Fieller's theorem and R package "mratio."[21] A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

The participating firefighters from both departments were predominantly non-Hispanic white males (Table 1). The average ages were 38.1 and 38.6 years at baseline for the TFD firefighters providing postexposure urines and SFD firefighters, respectively. Despite randomization, the SFD firefighters in the control group were significantly older than the sauna intervention group. Body mass index averaged 27.9 ± 3.4, 27.8 ± 3.3, and $27.2 \pm 3.0\,kg/m^2$ in the TFD baseline, pre-intervention and post-intervention groups, respectively. Height and weight information were not available for the SFD firefighters. For TFD subjects, 242 of the 255 total subjects provided a baseline urine sample, 104 provided at least one postexposure pre-intervention urine and 54 provided at least one postexposure post-intervention urine. Thirty-six firefighters provided more than one pre-intervention urine, ranging up to six samples, and eight firefighters provided more than one post-intervention urine, ranging up to three samples. Eleven firefighters provided at least one pre-intervention urine and at least one post-intervention urine. SFD firefighters participated only once in the sauna intervention study. The analysis was limited to the results of urine testing of engineers, firefighters, and captains, as only these groups had sufficient numbers of pre- and post-intervention subjects for statistical comparison.

The TFD firefighters responded to 15 fires in the pre-intervention period and 13 fires in the post-intervention period. The fires in each group were similar, consisting of residential and commercial structure fires. The pre-intervention fires included ten homes, one house and car combination, one apartment, two commercial structures, and one school. Three of the fires were mostly defensive following an interior fire attack. Four fires involved two or more rotations of firefighters returning to the involved structure after rehab to perform additional firefighting functions and overhaul. Average response time for the 13 fires for which this information was complete was 36 minutes. The post-intervention fires included

TABLE 1. General Characteristics of Fireground and Sauna Intervention Subjects

Intervention Groups	Fireground Baseline ($n = 242$)*	Fireground Pre-intervention ($n = 104$)*	Fireground Post-intervention ($n = 54$)*	Sauna Intervention Control Group ($n = 12$)*	Sauna Intervention Treatment Group ($n = 12$)
Male *n* (%)	226 (96.6%)	98 (98.0%)	52 (100%)	11 (91.7%)	11 (91.7%)
Female *n* (%)	8 (3.4%)	2 (2.0%)	0 (0%)	1 (8.3%)	1 (8.3%)
Ethnicity and Race					
White, Non-Hispanic *n* (%)	201 (85.9%)	81 (81.0%)	43 (82.7%)	7 (63.6%)	10 (83.3%)
White, Hispanic n (%)	29 (12.4%)	18 (18.0%)	7 (13.5%)	1 (9.1%)	2 (16.7%)
Other *n* (%)	4 (1.7%)	1 (1.0%)	2 (3.9%)	3 (27.3%)	0 (0%)
Age, yrs					
Mean (SD), yrs	39.0 (8.4)	38.1 (9.1)	36.6 (8.6)	43.8 (10.7)†	33.3 (9.26)†
<30 yrs *n* (%)	37 (15.7%)	23 (23.0%)	9 (17.3%)	1 (8.33%)	5 (41.7%)
30–39 yrs *n* (%)	87 (36.9%)	36 (36.0%)	26 (50.0%)	3 (25.0%)	5 (41.7%)
≥40 yrs *n* (%)	112 (47.5%)	41 (41.0%)	17 (32.7%)	8 (66.7%)	2 (16.6%)
Smoking status *n* (%)					
Never	220 (93.6%)	95 (95.0%)	44 (84.6%)	12 (100%)	12 (100%)
Occasional	9 (3.8%)	3 (3.0%)	4 (7.7%)	0 (0%)	0 (0%)
Current	6 (2.6%)	2 (2.0%)	4 (7.7%)	0 (0%)	0 (0%)

*Total *n* for each variable may be less based on unanswered survey questions.
†$P < 0.05$ by two sample *t* test comparing sauna intervention control and treatment groups.

eight homes, two apartments and two commercial structures, including one hotel. One of the fires was mostly defensive following an interior fire attack and one involved two or more rotations of firefighters returning to the involved structure after rehab to perform additional firefighting functions and overhaul. The average response time for the 13 fires was 38 minutes.

For fireground interventions, engineers showed a statistically significant 40.4% reduction in urinary mean concentration of all naphthol, fluorenol, and phenanthrol metabolites and 1-hydroxypyrene combined (Σ sums) comparing post-intervention to pre-intervention time periods (Table 2). Firefighters showed a significant 36.2% mean reduction in Σ sums, and captains showed a non-significant 11.3% mean reduction. The distribution of urinary PAH-OHs at baseline, pre-intervention, and post-intervention are shown in Figure 1. The statistical significance of reduction in specific isomer groups of PAH-OHs comparing pre-

TABLE 2. Fireground and Sauna Intervention Effectiveness (Geometric Means and Standard Deviations, ng/L)

	Baseline			Pre-intervention			Post-intervention			
Fireground	***n* (% ND)**	**Mean**	**SD**	***n* (% ND)**	**Mean**	**SD**	***n* (% ND)**	**Mean**	**SD**	**% Change (95% CI)***
Σ naphthols										
Engineer	39 (0%)	6968.2	2.0	24 (0%)	14790.1	2.8	13 (0%)	8706.8	2.0	−39.6 (−64.1, 1.5)
Firefighter	82 (0%)	6840.5	3.2	57 (0%)	19235.1	2.8	30 (0%)	12235.3	2.8	**−36.4 (−58.6, −2.4)**
Captain	66 (0%)	6151.7	2.9	33 (0%)	12666.7	2.5	17 (0%)	10516.2	2.9	−10.0 (−41.2, 37.8)
Σ fluorenols										
Engineer	39 (74.4%)	198.7	1.7	24 (41.7%)	477.9	3.4	13 (38.5%)	269.8	1.7	**−65.1 (−82, −32.1)**
Firefighter	82 (67.1%)	206.4	1.7	57 (21.1%)	762.8	3.0	30 (33.3%)	459.0	3.0	**−40.7 (−60.9, −10.2)**
Captain	66 (75.8%)	195.5	1.7	33 (48.5%)	367.3	2.5	17 (41.2%)	430.9	2.9	31.8 (−16.4, 107.9)
Σ phenanthrols										
Engineer	39 (41.0%)	478.7	1.7	24 (16.7%)	1140.9	2.9	13 (30.8%)	428.7	1.5	**−68.2 (−86.7, −24.0)**
Firefighter	82 (40.2%)	481.7	1.7	56 (8.9%)	1488.1	2.6	30 (33.3%)	639.3	2.4	**−63.1 (−74.8, −46.0)**
Captain	66 (56.1%)	434.3	1.7	33 (27.3%)	955.9	2.3	17 (35.3%)	599.7	2.2	**−43.7 (−66.7, −4.8)**
Σ sums†										
Engineer	39 (0%)	8239.4	1.8	24 (0%)	17297.4	2.8	13 (0%)	10197.4	1.9	**−40.4 (−63.9, −2.3)**
Firefighter	82 (0%)	8396.8	2.7	57 (0%)	22706.9	2.7	30 (0%)	14454.7	2.7	**−36.2 (−56.7, −6.0)**
Captain	66 (0%)	7436.6	2.5	33 (0%)	15332.9	2.3	17 (0%)	12544.3	2.7	−11.3 (−39.4, 29.9)

Sauna‡	**Pre-intervention**			**Post-intervention control**			**Post-intervention sauna treatment**			**% Change (95% CI)***
Σ naphthols	24 (0%)	6894.6	2.4	12 (0%)	35712.2	2.7	12 (0%)	19667.7	1.7	−44.9 (−71.1, 4.8)
Σ fluorenols	24 (37.5%)	266.6	3.0	12 (8.3%)	1000.4	1.5	12 (0%)	834.1	1.7	−32.7 (−40.5, 49.0)
Σ phenanthrols	24 (8.3%)	572.9	1.8	12 (0%)	1405.9	1.4	12 (0%)	1177.2	1.6	−16.3 (−41.9, 20.6)
Σ sums†	24 (0%)	8495.7	2.2	12 (0%)	40012.1	2.6	12 (0%)	22604.3	1.6	−43.5 (−68.8, 2.2)

CI, confidence interval; ND, non-detectable; SD, standard deviation.
*For fireground interventions comparing pre- and post-intervention means and for sauna intervention comparing post-intervention control and sauna treatment means.
†Includes sum of Σ naphthols, Σ fluorenols, Σ phenanthrols, and 1-hydroxypyrene.
‡All sauna intervention study values are from 12-hour composite urine samples.

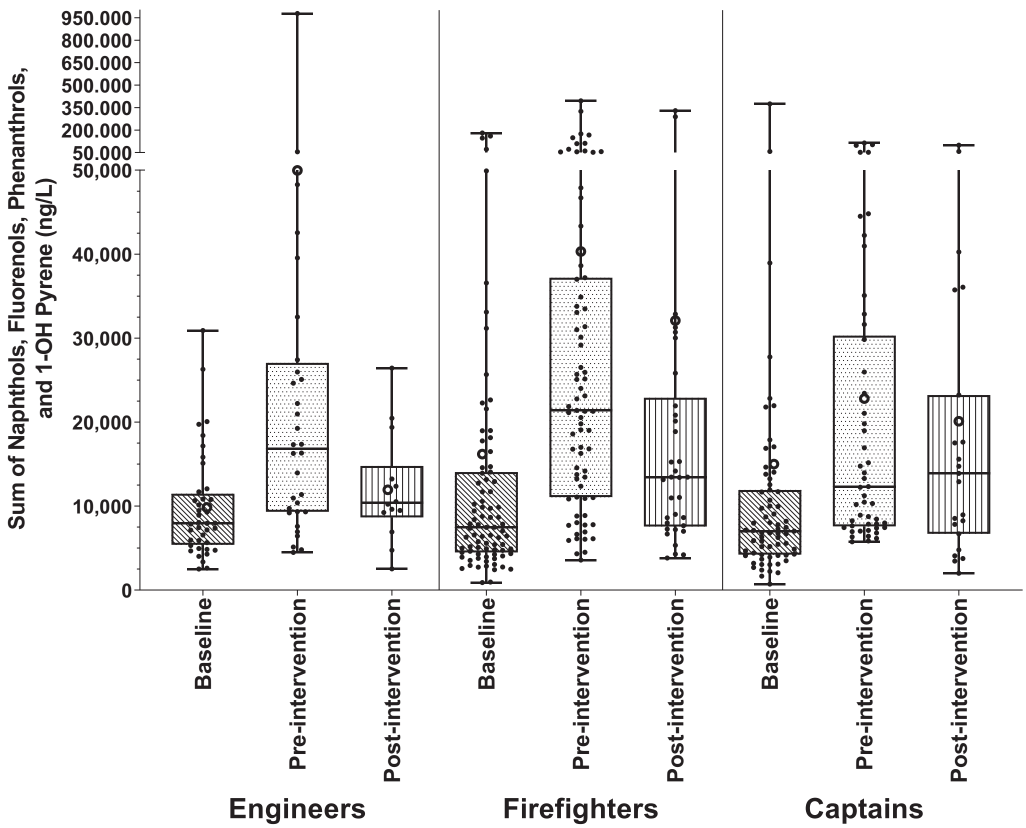

FIGURE 1. Fireground urinary PAH-OH measurements by job classification (open circle = mean). PAH-OH, polycyclic aromatic hydrocarbon metabolites.

and post-intervention periods varied by fire service activity: sum of naphthols, only firefighters; sum of fluorenols, engineers and firefighters; and sum of phenanthrols, engineers, firefighters, and captains. The results for individual PAH-OHs are listed in Supplementary Table 1, http://links.lww.com/JOM/A707. There was a wide range of urinary PAH-OH concentrations within each group; for example, one engineer in the pre-intervention group had a urinary 1-naphthol measurement (585,300 ng/L, confirmed by reanalysis) more than twice the level of the second highest measurement.

For the pre-intervention phase, 180 on-scene and 120 in-station surveys were completed for individuals that also provided a postexposure urine (Table 3). For the post-intervention phase, 67 on-scene and 60 in-station surveys were completed. These show a 15% increase during the post-intervention period in having clean gear before the response and a smaller increase in various PPE worn during fire attack (range 8% to 13%) and overhaul (range 3% to 6%). In regards to respiratory protection, SCBA use increased 13% during fire attack, which included both interior and exterior attack, and 8% during overhaul. Use of skin wipes/washing with water on-scene and replacing hoods on scene, both practices put in place prior to the study interventions, increased 14% and 2%, respectively. Wash down of turnout gear and SCBA on-scene, both new interventions, increased 58% and 35%, respectively. There was less emphasis on wash down for engineers given that they did not do interior fire response or ventilation. Excluding the 10 engineers with survey responses from the question "washed/rinsed/or replaced the following on scene," the percentages of subjects responding positively increased to 82% for hoods and 76% for turnout gear and stayed at 75% for SCBA. For all subjects combined (including engineers), bagging dirty gear and storing it outside of the cab increased 28% and 15%, respectively, while there was a 10% decline in both showering and washing/replacing clothes within an hour after the response. All four of these activities were the focus of the new interventions.

For the sauna intervention, there was a non-significant 43.5% decrease in the geometric mean PAH-OH Σ sums concentration in the 12-hour postexposure composite urine sample for those firefighters randomized to infrared sauna treatment compared to the controls (Table 2). While also not statistically significant, there were greater reductions in sum of naphthols than sum of fluorenols and sum of phenanthrenes. As with the fireground intervention groups, there was a wide range of urinary PAH-OH concentrations within each group (Supplementary Table 2, http://links.lww.com/JOM/A708). The highest individual urinary PAH-OH measurement was a 2-naphthol level of 295,808 ng/L in a control subject, confirmed by reanalysis. For the 2 to 4 hour postexposure geometric mean urinary PAH-OH concentrations, comparing the and control and sauna treatment groups respectively, there were non-significant reductions in sum of naphthols (36,431 $\pm$ 2.2 and 25,982 $\pm$ 2.1 ng/L, $P = 0.08$), sum of fluorenols (1,367 $\pm$ 1.8 and 1,048 $\pm$ 1.8 ng/L, $P = 0.39$), sum of phenanthrols (1,730 $\pm$ 1.6 and 1,359 $\pm$ 1.5 ng/L, $P = 0.48$) and Σ sums (41,832 $\pm$ 2.0 and 29,522 $\pm$ 2.0 ng/L, $P = 0.07$). The 12-hour composite pre-exposure, 2 to 4 hour postexposure and 12-hour composite postexposure urinary sums are shown graphically in Figure 2A to D. The mean urine specific gravities in the control and sauna treatment groups were 1.012 $\pm$ 0.004 and 1.015 $\pm$ 0.006 ($P = 0.13$) at baseline, 1.014 $\pm$ 0.007 and 1.018 $\pm$ 0.008 ($P = 0.17$) at 2–4 hours and 1.012 $\pm$ 0.007 and 1.016 $\pm$ 0.007 ($P = 0.18$) for the 12-hour postexposure composite samples, respectively.

TABLE 3. Fireground Intervention Survey Results

Status or Activity	Pre-intervention n (%)*	Post-intervention n (%)*
Turnout gear clean before the response	97 (54%)	46 (69%)
Personal protective equipment worn during fire attack:		
Turnout gear	112 (62%)	47 (70%)
Firefighting boots	110 (61%)	47 (70%)
SCBA	109 (61%)	49 (73%)
Helmet	112 (62%)	49 (73%)
Firefighting gloves	108 (60%)	47 (70%)
Eye/face protection other than SCBA	38 (21%)	23 (34%)
Personal protective equipment worn during overhaul:		
Turnout gear	48 (27%)	21 (31%)
Firefighting boots	49 (27%)	21 (31%)
SCBA	46 (26%)	21 (31%)
Helmet	48 (27%)	21 (31%)
Firefighting gloves	48 (27%)	20 (30%)
Eye/face protection other than SCBA	16 (9%)	10 (15%)
Used skin wipe or washed with water while on scene	77 (64%)	47 (78%)
Washed/rinsed/or replaced the following on scene:		
Turnout gear	12 (10%)	41 (68%)
SCBA	48 (40%)	45 (75%)
Hood	85 (71%)	44 (73%)
Bagged dirty gear before transporting it from the fire scene	5 (4%)	19 (32%)
Stowed dirty gear for transport outside of the truck cab	55 (46%)	37 (62%)
Took a full body shower within an hour after the response	69 (57%)	28 (47%)
Washed or replaced clothes within an hour after the response.	72 (60%)	30 (50%)

*The total number of responses varies based on how many subjects answered each question.
SCBA, self-contained breathing apparatus

There were no significant differences in mean core temperature comparing the control and sauna treatment groups during the 20-minute segments before (37.3 ± 0.3 and 37.5 ± 0.3°C, $P = 0.99$), during (37.4 ± 0.3 and 37.5 ± 0.3°C, $P = 0.95$) and after (37.5 ± 0.3 and 37.4 ± 0.3°C, $P = 0.95$) sauna treatment, respectively (Fig. 3). Mean heart rate was similar in both groups during the 20 minutes prior to sauna treatment (105 ± 34.9 and 111 ± 10.4 beats per minute (bpm), $P = 0.41$), respectively, but compared to the control group increased in the sauna treatment group in the 20 minutes during (99.7 ± 33.2 and 134 ± 27.6 bpm, $P = 0.004$) and after (93.1 ± 30.6 and 126 ± 21.5 bpm, $P = 0.006$) sauna treatment, respectively (Fig. 4).

DISCUSSION

The study results support the effectiveness of the selected fireground interventions for engineers and firefighters, and provide some initial measurements of the effects of the sauna treatment following live-fire exposure. The fireground interventions were associated with a roughly 40% reduction in urinary PAH-OHs in engineers and a slightly lower reduction in firefighters. However, no significant change was measured in captains. Sauna treatment non-significantly reduced mean urinary PAH-OHs by over 40%, with the largest reduction in urinary naphthols.

A primary fireground intervention was use of SCBA for engineers in the presence of smoke. Respiratory protection in the fire service is predominantly provided through the use of pressure demand SCBA with a full facepiece which has an assigned protection factor of over 10,000,[22] a value supported by testing under high exertion levels in firefighters assuming reasonable facepiece fit.[23] Firefighters generally wear SCBA where immediately dangerous to life and health concentrations of combustion products exist (the hot zone) such as during interior fire responses and ventilation. SCBA use is much less common in the warm zone where engineers operate but where combustion products from the fire can still collect.[24] As TFD engineers had substantially less visual deposition of soot on their gear than entry teams and therefore were less likely to participate in wash down (reported in only 30% of engineers completing post-intervention surveys), the reduction in their urinary PAH-OHs is likely primarily due to increased SCBA use.

Unlike the current study, Fent et al[25] did not observe changes in urinary PAH-OH for pump operators (engineers) when comparing samples collected pre-exposure and three hours postfire. However, their pump operator personnel had a non-significant 33% increase in benzene measured in their exhaled breath comparing postexposure to pre-exposure. Atmospheric conditions and personnel positioning relative to the fire are important factors that can contribute to an engineer's inhalation exposure.[26] The importance of SCBA use to prevent inhalation exposures of PAHs and other contaminants is supported by a study of training fuel packages and exposure effects on instructors and firefighters.[13] Air purifying respirators are not recommended for conditions with potentially elevated concentrations of products of combustion, as their use during overhaul has been associated with adverse respiratory effects,[5] and certain chemicals such as formaldehyde may break through even chemical, biological, radiological, and nuclear canisters.[27–30]

A primary fireground intervention for entry teams in the current study was wash down. Gear was cleaned using soap and water prior to doffing in order to reduce surface contamination and the potential for self-contamination as well as cross-contamination of other fire service personnel potentially coming in contact with the turnout gear, such as paramedics operating in the rehab area. Scrubbing turnout gear with dish soap and water has been shown to reduce surface PAH contamination by 85%.[18] Naphthalene, the most volatile PAH, may penetrate the protective layers of turnout gear more than other PAHs,[31] indicating that postfire decontamination may not prevent or minimize all potential PAH dermal exposure equally.

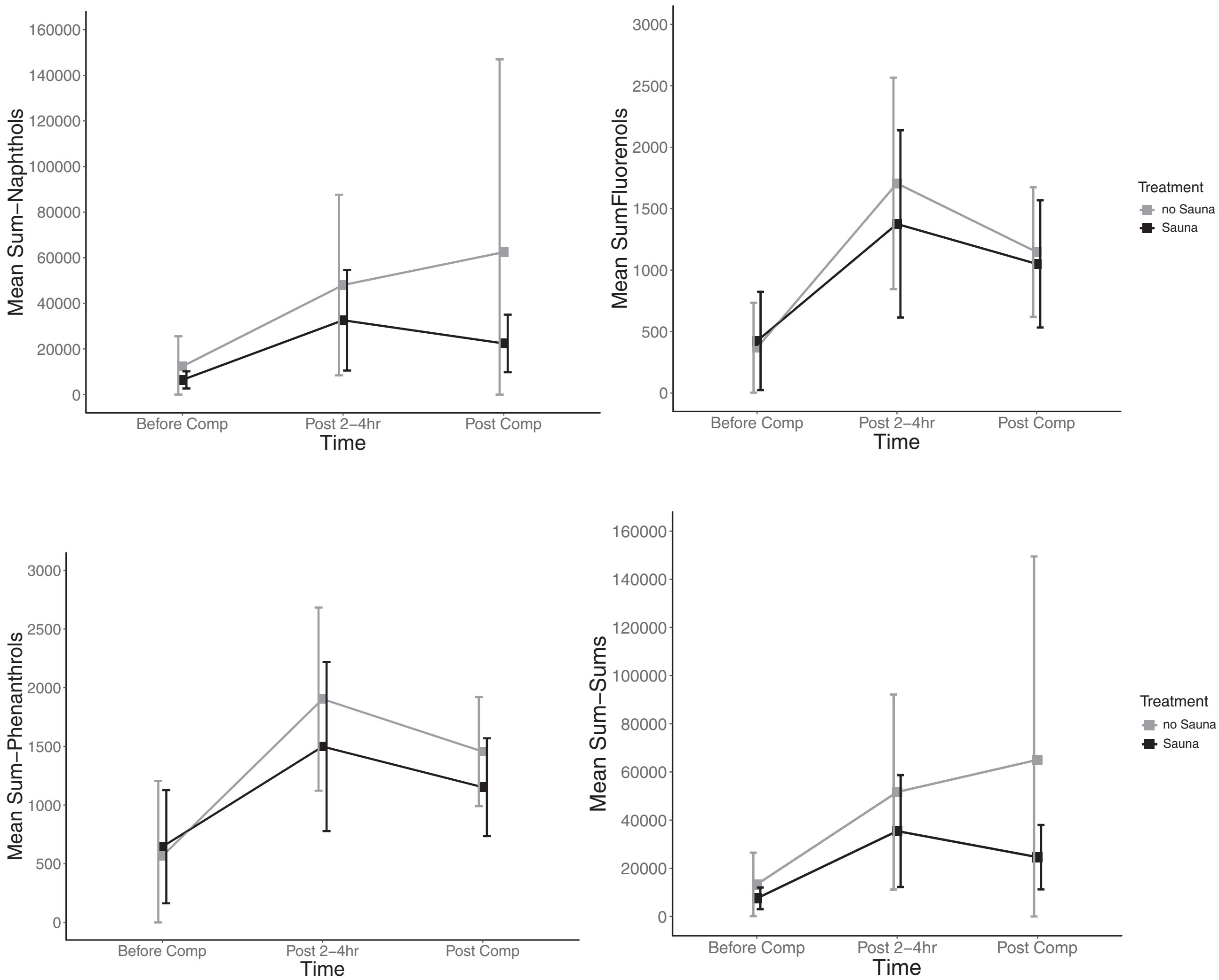

FIGURE 2. A–D: Mean (SD) of PAH-OHs (ng/L) before and after (2 to 4 hours and 12 hour composite) firefighting by treatment group. PAH-OH, polycyclic aromatic hydrocarbon metabolites.

One unanticipated finding of the current study was the lack of effectiveness of the fireground interventions for captains. A potential explanation is increased inhalation exposure for captains in comparison to firefighters. The role of the captain at a fire scene includes radio communications between the crew and dispatchers and later the incident commander. While the firefighters gear up and don their SCBA on arrival at the scene, the captain conducts the incident size-up and radios reports to dispatchers and incoming crews. This fireground function can place the captain in the area of the working fire, resulting in a possible inhalation exposure before donning his or her SCBA. In addition, there are times when a captain removes the SCBA regulator for communication purposes as he or she exits the involved structure to communicate with the incident commander, thus exposing the captain to higher contaminant levels in comparison to the firefighters who continually use their SCBA. The unique job functions of the captain could thereby contribute to the differences observed in intervention effectiveness.

TFD practices predating the new fireground interventions included use of skin wipes and exchange of contaminated hoods on-scene. TFD personnel used soap and water or hypoallergenic alcohol and scent-free skin wipes (eg, Huggies Natural Care® Plus Wipes, Kimberly-Clark Corporation, Irving, TX) to clean off their neck, face, arms, legs and anywhere else with visible contamination both before and after the implementation period. These methods have been previously demonstrated to reduce skin PAH contamination by 54%.[18] Laundering contaminated hoods has been shown to reduce PAH contamination by 76%.[11]

The current study survey results showed an increase in the fireground activities promoted by TFD as part of their interventions and included in their training activities prior to intervention implementation and in subsequent reminders. Additional improvement in intervention compliance would be expected to yield further reductions in fireground exposures. Organizational culture change and behavioral interventions increase the likelihood of success of programs including gear decontamination.[19,32] However, complete compliance may not be possible, as fatigue, heat, or other factors may prevent the wash down step, and the condition of the firefighter at the time should be considered. It is also important to note that the added time on scene for postfire wash down, decontamination of equipment and bagging of gear is a likely explanation for the 10% decline in firefighters reporting showering within an hour after the response.

The sauna intervention results were equivocal with substantial but non-significant reductions in mean urinary PAH-OH Σ sums

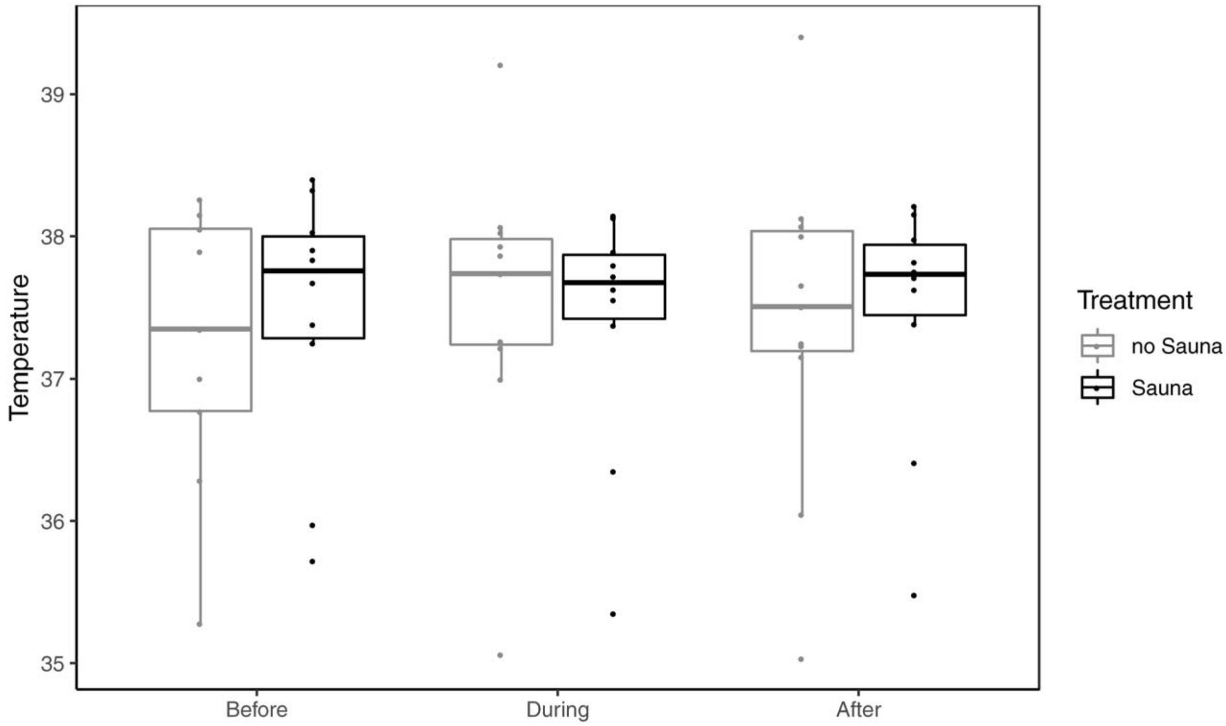

FIGURE 3. Core temperature (°C) by treatment group.

with sauna treatment compared to the control group. The standard deviation in urinary PAH-OHs was larger in the control than the sauna group. The reason for this difference is not clear, although PAH-OH from dietary sources could not be excluded as adherence with the instructions to avoid grilled meat during the study period was not confirmed. The largest reduction associated with the sauna intervention, also non-significant, was in the sum of naphthol metabolites with smaller non-significant reductions in sum of

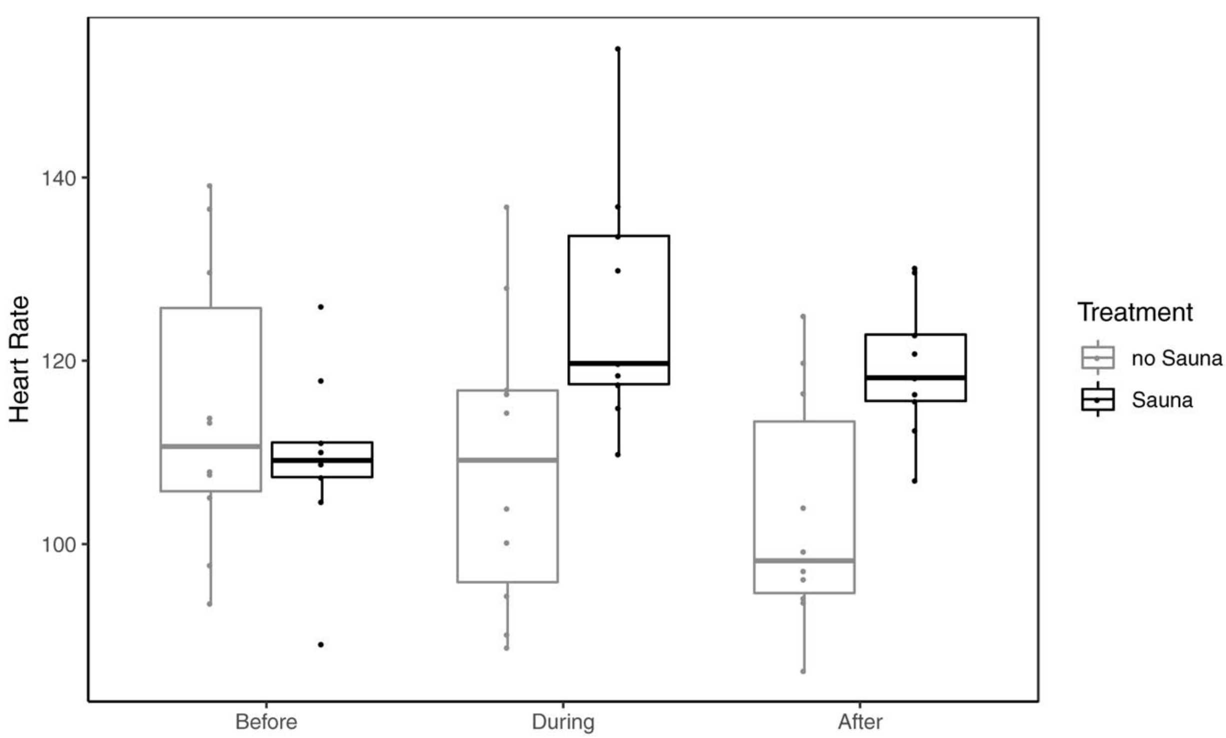

FIGURE 4. Heart rate (beats per minute) by treatment group.

fluorenols and sum of phenanthrols. A potential explanation for this difference could be the higher volatility of naphthalene.

Little is known about the ability of heat exposure, for example, sauna, to alter the excretion of organic molecules through sweat. A study with 20 participants found that induced perspiration facilitated the excretion of PBDE congeners in sweat, although the effectiveness depended on the type of sweat-inducing intervention and the PBDE congener.[33] A study of seven World Trade Center rescue workers evaluated the effects of the Hubbard sauna detoxification method, including multiple hours of sauna a day for at least a month, vitamin and mineral supplements and a balanced lifestyle.[34] The study found a reduction of polychlorinated biphenyls (PCBs) in the blood of the participants while other contaminants like polychlorinated dibenzodioxins and polychlorinated dibenzofurans remained unchanged. Another study found urinary excretion of tetracycline decreased immediately after heat exposure, although the total amount of tetracycline in the 24-hour postexposure composite urine was similar to the control group,[35] demonstrating the need for analysis of extended composite or multiple time periods of urine analysis following sauna treatment to fully measure effectiveness. If the hypothesized mechanism for sauna treatment is the release of chemicals absorbed into skin or pores, then it would also be useful in future studies to measure PAH concentrations on the skin using wipe samples after initial showering but before sauna treatment, again after sauna treatment and at similar time intervals in the control group.

A concern with any treatment, including saunas, is the potential to cause harm. The elevated heart rate seen in the current study during and after sauna treatment is an indication of heat stress. However, the foremost concern is elevated core temperature, which was not found with the current study but which has been associated in past studies of live-fire training with altered coagulation and in studies of non-firefighters with fatigue and decreased cognitive function.[36–38] Additional firefighter sauna treatment studies are needed, potentially involving a range of sauna types, temperatures, durations and exercise conditions as well as outcome measures beyond urinary PAH-OHs, core temperature and heart rate monitoring.

This study had a number of important limitations. As the fireground interventions were not randomized, potential differences in the fires in the pre- and post-intervention periods could explain some of the reductions found in the urinary PAH-OHs. Based on the survey results, the recommended interventions were not fully implemented, suggesting that additional reductions in urinary PAH-OHs could be achieved with more complete compliance. Firefighters may have occupational exposure to PAHs that are not-fire related such as from ambient air pollution[10] or food eaten on shift that may not be controlled by targeted interventions such as those described here. The sauna intervention study involved idealized treatment conditions which included shorter intervals between exiting the fire and entry into the sauna than could likely be achieved with actual structural fires, and it was not possible to separate the effects of the sauna itself from the additional shower taken after the sauna. The sauna intervention effect on toxicity from smoke exposure is not known. In addition, other adverse or beneficial effects not measured in the current study could potentially occur with sauna treatment.

In conclusion, the study results directly support the use of SCBA by engineers while operating at a fire incident and indirectly suggest the need for additional use of respiratory protection for other fire service personnel operating in the warm zone. The study results also support the use of wash down for entry teams, particularly as part of a broader dermal exposure reduction and contaminated gear segregation program. The infrared sauna intervention did not yield a statistically significant reduction in urinary PAH-OHs, although the number of subjects was limited. However, within the protocols developed by SFD, sauna treatment did not elevate core temperature, so there was also no clear evidence that their sauna treatment was detrimental. Further research on firefighter postexposure sauna treatment is needed.

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RESEARCH ARTICLE

DNA methylation among firefighters

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Abstract

Firefighters are exposed to carcinogens and have elevated cancer rates. We hypothesized that occupational exposures in firefighters would lead to DNA methylation changes associated with activation of cancer pathways and increased cancer risk. To address this hypothesis, we collected peripheral blood samples from 45 incumbent and 41 new recruit non-smoking male firefighters and analyzed the samples for DNA methylation using an Illumina Methylation EPIC 850k chip. Adjusting for age and ethnicity, we performed: 1) genome-wide differential methylation analysis; 2) genome-wide prediction for firefighter status (incumbent or new recruit) and years of service; and 3) Ingenuity Pathway Analysis (IPA). Four CpGs, including three in the *YIPF6*, *MPST*, and *PCED1B* genes, demonstrated above 1.5-fold statistically significant differential methylation after Bonferroni correction. Genome-wide methylation predicted with high accuracy incumbent and new recruit status as well as years of service among incumbent firefighters. Using IPA, the top pathways with more than 5 gene members annotated from differentially methylated probes included Sirtuin signaling pathway, p53 signaling, and 5' AMP-activated protein kinase (AMPK) signaling. These DNA methylation findings suggest potential cellular mechanisms associated with increased cancer risk in firefighters.

Citation: Zhou J, Jenkins TG, Jung AM, Jeong KS, Zhai J, Jacobs ET, et al. (2019) DNA methylation among firefighters. PLoS ONE 14(3): e0214282. https://doi.org/10.1371/journal.pone.0214282

Editor: Osman El-Maarri, University of Bonn, Institute of Experimental Hematology and Transfusion Medicine, GERMANY

Received: August 29, 2018

Accepted: March 11, 2019

Published: March 26, 2019

Data Availability Statement: Data cannot be shared publicly because the subject consent form limits the use of the collected data for specified purposes. Data requests can be sent to Dr. Burgess (jburgess@email.arizona.edu), the corresponding author for this study. Data requests can also be sent directly to the OPB and the University of Arizona IRB. Casey Grant, the Director of the OPB, can be contacted through the official study email (firefightercastudy@miami.edu). Mariette Marsh, the Director of the University of Arizona IRB, can be contacted through email (vpr-IRB@email.arizona.edu).

Introduction

Epidemiologic studies of firefighters from multiple countries have demonstrated an elevated rate of cancer incidence and/or mortality for a number of cancer types [1–8]. For example, in a recent study of three large fire departments in the United States, overall cancer incidence and mortality was significantly increased by 9% and 14%, respectively, as compared with the general population, and significant increases in cancer incidence and mortality were noted specifically for cancers of the esophagus, intestine, lung, and kidney [2]. Firefighters are

Funding: This study was supported by a grant from the US Federal Emergency Management Agency Assistance to Firefighters Grant program (https://www.fema.gov/assistance-firefighters-grant-awards), #EMW-2014-FP-00200. This grant was awarded to the corresponding author, JLB. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

occupationally exposed to multiple products of combustion and other substances containing carcinogens through inhalation and/or skin contamination [9–11], including but not limited to polycyclic aromatic hydrocarbons (PAHs), benzene, per- and polyfluoroalkyl substances (PFAS) and diesel exhaust [11–15]. However, other risk factors such as shift work may also contribute to this elevated cancer risk [16].

Epigenetic modifications are critical steps in carcinogenesis and cancer prevention [17, 18]. We have previously shown that microRNAs are differentially expressed between incumbent and new recruit firefighters [19], but published information on DNA methylation in firefighters to our knowledge has been limited to four genes [20]. DNA methylation refers to the addition of a methyl group to cytosine within 5'-C-phosphate-G-3' (CpG) dinucleotides, which are often concentrated in large clusters called CpG islands. Inactivation of certain tumor-suppressor genes occurs as a consequence of hypermethylation within the promoter regions and numerous studies have demonstrated a broad range of genes silenced by DNA methylation in different cancer types [21–24]. Global hypomethylation, inducing genomic instability, also contributes to cell transformation. Apart from DNA methylation alterations in promoter regions and repetitive DNA sequences, this phenomenon is associated with regulation of expression of noncoding RNAs such as microRNAs that may play a role in tumor suppression. Furthermore, DNA methylation has shown promise in putative translational use in patients and hypermethylated promoters may serve as disease-related biomarkers [25, 26]. Importantly, while every effort is made by previous studies to put identified methylation signatures in context, it should be noted that the landscape of methylation alterations and the associated impact on gene activity is extremely complex. Thus, changes in methylation signatures are not always clearly linked to specific alterations in gene activity.

We hypothesized that compared to new recruits without previous firefighting experience, incumbent firefighters would show differential DNA methylation patterns that had been previously associated with cancer. We analyzed DNA methylation in peripheral blood by microarray and compared the results between new recruits and incumbent firefighters to address this hypothesis.

Methods

Subjects

Study protocols were approved by the University of Arizona Institutional Review Board (approval No.1509137073) and all subjects provided written informed consent. The study subjects were selected from a larger group of incumbent firefighters within the Tucson Fire Department (Tucson, Arizona, United States of America) and new recruit firefighters prior to any live-fire exposures or other occupational exposures to fire and smoke. All subjects completed questionnaires regarding their age, body weight, height, working duration as firefighters, and tobacco use.

Initially, blood for methylation analysis was collected from 47 male incumbents and 48 male and one female new recruits. Subjects who either had current smoking exposure or missing smoking information and the sole female recruit were excluded, leaving 86 (45 incumbents and 41 recruits) subjects for methylation data analysis. Body mass index (BMI) (kg/m^2) was classified as normal (18.5–24.9), overweight (25.0–29.9), and obese ($\geq$ 30) following World Health Organization (WHO) classifications.

DNA methylation measurement

Blood samples were collected in one 6.0 ml dipotassium ethylenediaminetetraacetic acid (K_2EDTA) tube (Becton, Dickinson and Company, Franklin Lakes, NJ) for DNA methylation analyses. As an alternative to the ethylenediaminetetraacetic acid (EDTA) tube, eight samples were also collected in cell preparation tubes (CPTs) (Becton, Dickinson and Company, Franklin Lakes, NJ). The EDTA tube was processed within 30 minutes of collection, which consisted of centrifugation at 3200 rpm for 15 minutes and separation of the plasma from the cells. All aliquots were stored at -20 ˚C until transfer under Arizona Department of Transportation guidelines to the University of Arizona for storage at -80 ˚C for subsequent processing by the University of Arizona Genetics Core. The CPT was processed according to the product guidelines and the cell pellet was stored at -80 ˚C until processed.

Genomic DNA from the EDTA tubes and CPTs was isolated using the FlexiGene DNA Kit (Qiagen, Valencia, CA). Genomic DNA was extracted from 9 additional packed cell pellets from CPTs using the Qiagen DNeasy Blood and Tissue Kit. DNA quantity was assessed with the QuantiFluor dsDNA System (Promega, Madison, WI) on the Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT) and 96 of the highest yield samples were normalized to 250ng in 30uL. The samples then underwent bisulfite conversion using the Zymo EZ DNA Methylation Kit (Zymo Research Corp., Irvine, CA) with a genomic DNA input of 250ng. The recommended modification to the protocol using alternative incubation conditions for the Illumina assays was performed. Upon bisulfite conversion completion, samples were sent to the University of Utah DNA Sequencing and Genomics Core Facility (Salt Lake City, Utah) for Infinium HD Methylation using the Illumina MethylationEPIC kit (Illumina, Inc., San Diego, CA) scanning on the iScan instrument, and raw data export.

Raw intensity data were processed by Bioconductor package minfi (version 1.22.1) [27] which included normalization of data using Illumina's reference factor-based normalization methods (preprocess Illumina) and Subset-quantile Within-Array Normalisation (SWAN) [28] for type I and II probe bias correction. All samples passed quality control. A detection p-value is returned for every genomic position in every sample, with small p-values indicating good quality probes. Probes with detection p-value $>$ 0.05 in one or more samples, and probes with single-nucleotide polymorphisms (SNPs) inside their body or at the nucleotide extension were excluded, leaving 834,912 probes. DNA methylation levels (M-values) were determined by calculating the logarithm of the ratio of intensities between methylated (signal A) and unmethylated (signal B) alleles, log (A/B) [28, 29]. Potential batch effects were investigated using principal component analysis using M-values.

Statistical analyses

Differential methylation analysis. Differentially methylated probes were detected using the *limma* package [30]. A linear model with Empirical Bayes estimator was adopted [31], with adjustment for age, ethnicity, and BMI. Probes were considered to be differentially methylated if the resulting adjusted p-value was $<$0.05. The Bonferroni correction method was used to adjust the p-values and ensure that the familywise error rate was less than 0.05 [32]. The *DMRcate* package was used to identify differentially methylated regions (DMR) based on tunable kernel smoothing of the differential methylation signal, adopting the default setting [33]. *DMRcate* uses limma-derived statistics for calculation of individual CpG site methylation differences and it can assess all 850K probes as candidates for DMR constituents. The corresponding gene list was derived from the gene annotations associated with the probes. Because our DNA samples were derived from blood, we estimated white blood cell type composition for every individual using the Houseman method [34]. We corrected the analysis by including the estimated cell type composition as covariates in the linear model. Only results that were significant first without and then also with adjustment for cell type composition were reported as it has been shown that when cell composition and age are confounded, adjustment of cell-type composition can lead to false positives [35].

Genome-wide methylation prediction. Genome-wide methylation prediction was performed with the *glmnet* package using elastic-net penalization [36]. Years of service information was collected for both incumbent and new recruit firefighters. Since the newly recruited firefighters' years of service measures were zero, we carried out a two-stage prediction model to incorporate this excess of zeros in the distribution of years of service. In the first stage we used genome wide methylation profile, age, BMI, and ethnicity to predict job status, i.e., recruit vs incumbent firefighter, which is equivalent to exposed to fire or not. In the second stage, for firefighters predicted to be incumbents, we then predicted their years of service. We employed a 10-fold cross validation strategy to repeatedly perform trainings on 90% of our sample set while holding out 10% of the samples for a test set. This procedure was repeated 10 times on unique subgroups of the entire data set.

Pathway analysis. We performed pathway analysis for the top probes differentially methylated between new recruits and incumbent firefighters using a p value of $< 10^{-4}$ selected based on the published literature [37], and an empirically selected 1.5-fold change between the two groups. These probes were annotated to genes according to the closest transcription start site (TSS) [38]. The gene list was uploaded to QIAGEN Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City) for assessing overrepresentation relative to all human gene functions [39]. The *Pathway Build* and *Relationship Summary* tools in IPA were used to build the gene regulatory networks, including expression regulation, protein-protein/DNA interaction, activation and inhibition. Genes were ranked by their connectivity in the regulatory networks, and genes with the top 10% connectivity were chosen as hubs. Hub genes play important roles in gene regulation due to their multiple interactions with other genes [40]. Two analyses were then performed to reveal the related canonical pathways and human diseases. First, using the *Canonical Pathways* tool, we identified canonical signaling (or metabolic) pathways with associated input genes and ranked the pathways by the number of gene members. Pathways that included more than five gene members were defined as top canonical pathways in this regulatory network. Second, using the IPA scientific literature-based *Diseases and Functions overlay* tool we annotated the genes enriched within human diseases and biological functions. The software is backed by highly structured, detail-rich biological and chemical findings derived from top journals and reviewed using full text and is also supported by third-party information, including but not limited to GO, TarBase, ClinicalTrials.gov, and BIND. It retrieves a wealth of experimental evidence for genes and explores the association with diseases or phenotypes by leveraging the depth of the Ingenuity Ontology and the Human Phenotype Ontology. With the IPA application, the significance of each enriched disease module is calculated as follows: (1) the number of input genes mapped to a given disease module in the IPA literature database, denoted by m; (2) the number of genes included in the disease module, denoted by M; (3) the total number of input genes mapped to the IPA's literature database, denoted by n; and (4) the total number of known genes included in the IPA's literature database denoted by N. The significance of gene enrichment in the disease module is then calculated using a one-tailed Fisher's exact test [41]. Genes with no regulatory relationship with any other genes were excluded from analysis.

As IPA does not take the direction of the effects into consideration, directionality of methylation alteration was not assessed separately. Instead all alterations (whether gain of methylation or loss of methylation) were included in the analysis. The rational for this approach is that the methylation signature and associated alterations are a reflection of a cell's transcriptional activity. Thus, regardless of direction, all methylation states in our samples of interest in theory contribute to the activity of cellular pathways.

Results

Subjects

All subjects were white, and a similar percentage of incumbent and new recruit firefighters were of Hispanic ethnicity (Table 1). The subjects' mean age in years was significantly higher in incumbents (40.6 ± 7.7) than in recruits (28.9 ± 6.3) ($p<0.001$). The incumbent firefighters and recruits had similar distribution of BMI. For incumbents, the mean number of years serving as a firefighter was 14.0 ± 7.2 years, and number of years of service was significantly correlated with age (Pearson's r = 0.804, $p<0.0001$). Distribution of cell type composition across job status is shown in Fig 1. There were no significant differences comparing the incumbent and new recruit firefighters.

Differential methylation analysis

Comparing incumbents to recruits and adjusting for multiple comparisons, age, BMI, and ethnicity, as well as cell type composition, four CpGs (cg00287370, cg05236728, cg12253469 and cg24034992) demonstrated statistically significant differential methylation exceeding 1.5-fold (Table 2). These four CpGs included one that was hypermethylated and three that were hypomethylated in incumbent firefighters compared to new recruits. Two of the three hypomethylated CpGs were annotated to promoter regions. One additional CpG (cg07897354) demonstrated significantly reduced methylation in incumbents as compared with recruits when adjusting for multiple comparisons, age and ethnicity, but lost significance when BMI was added to the model. In order to further assess the effects of age on differential methylation between incumbent and new recruit firefighters, we also investigated whether any of the CpG sites in Table 2 varied significantly by age group (<40 years old vs > 40 years old). None of these sites was significantly associated with age after correction for multiple comparisons (data not shown). In a separate analysis, 41 differentially methylated regions were identified, of which seven were still significant after adjustment for cell type compositions (Table 3).

Genome-wide methylation prediction

Using a 10-fold cross validation procedure, we applied machine learning algorithms to determine which CpGs had variable methylation associated with firefighters' service status, i.e., new recruit or incumbent, and the years of service each individual had performed. In each stage of cross validation, the CpGs that were determined by the training to be predictive were noted. A total of 91 CpGs were selected at least once during the 10 rounds of training associated with firefighters' years of service. However, only 11 CpGs (cg09544149, cg24034992, cg22280238, cg00287370, cg02932780, cg13753209, cg15304928, cg07897354, cg22433210, cg20821958, and cg03177084) were selected in more than half of the trainings. The best-performing model was chosen based on the lowest misclassification rate in the first stage and the lowest mean squared error of years of service in the second stage in the test set. This model was then applied to the entire data set and predictions were compared to the actual years of service (Fig 2). The resulting misclassification rate between predicted incumbents and actual incumbent firefighters was 2% in the first stage and within incumbent firefighters the correlation of predicted and actual years of service was robust with an R^2 of 0.889. We also evaluated whether including DNA methylation could increase predictive power compared to only using the covariates age, BMI, and ethnicity. By adding methylation levels to the prediction model, r^2 increased from 0.533 to 0.889 and the misclassification rate was reduced from 8% to 2% (data not shown).

Table 1. General characteristics of subjects.

Variable	Recruits (n = 41)	Incumbents (n = 45)	P-value
Age (years)			
≤ 29	23 (56.1%)	3 (6.67%)	<0.0001
30–39	14 (34.1%)	15 (33.3%)	
≥ 40	4 (9.76%)	27 (60.0%)	
Mean (SD)	28.9 (6.3)	40.6 (7.7)	<0.0001
Body Mass Index (kg/m^2)			
Normal (18.5–24.9)	13 (31.7%)	9 (20.0%)	0.39
Overweight (25.0–29.9)	22 (53.7%)	26 (57.8%)	
Obese (≥ 30)	6 (14.6%)	10 (22.2%)	
Race/Ethnicity			
White, Hispanic	6 (14.6%)	6 (13.3%)	1.0
White, Non-Hispanic	35 (85.4%)	39 (86.7%)	
Years of Service			
Mean (SD)	0.85 (1.5)	14.0 (7.2)	< 0.0001
Missing	0	1 (2.2%)	

https://doi.org/10.1371/journal.pone.0214282.t001

Pathway analysis

Five hundred and twelve CpG sites demonstrated differential methylation with a p-value $< 10^{-4}$ and at least 1.5-fold differences between incumbent firefighters and new recruits. They were annotated to 443 unique genes which were used to build a gene regulatory network (Fig 3). There were 93 genes that had at least one connection with other genes in the regulatory network. All hub genes had at least 20 connected relationships. They included *STAT3*, *TP63*, *TP73*, *FOXO1*, *PML*, *DAXX*, *RUNX2*, *INSR*, and *PCNA*. Top pathways with more than 5 gene members annotated from differentially methylated probes included the Sirtuin signaling pathway (3 hubs of 8 gene members: *FOXO1*, *STAT3* and *TP73*), molecular mechanisms of cancer (2 hubs of 7 gene members: *DAXX* and *FOXO1*), p53 signaling (4 hubs of 7 gene members: *PCNA*, *PML*, *TP63* and *TP73*), and 5' AMP-activated protein kinase (AMPK) signaling (2 hubs of 6 gene members: *FOXO1* and *INSR*). Enriched diseases (disease annotation) included abdominal cancer (9 hubs of 88 genes), colon tumor (8 hubs of 44 genes), skin cancer (6 hubs of 51 genes), and lung tumor/cancer (5 hubs of 49 genes), all with p-values $<10^{-6}$ in IPA (Table 4). To address the effect of using a different fold change criteria, we performed a sensitivity analysis by using the same p-value $< 10^{-4}$ but with a two-fold change limit (data not shown). The sensitivity analysis identified 293 CpG sites annotated to 282 unique genes (reduced from 512 CpG sites annotated to 443 genes in the primary analysis). Among them, 67 genes had at least one connection with other genes in the regulatory network based on IPA databases. Using the same hub gene criterion as in the primary analysis (i.e., with >20 connections with other genes in the regulatory network), six hub genes were identified, including *STAT3*, *PML*, *RUNX2*, *DAXX*, *PCNA*, and *INSR*. All of them were also reported in the primary analysis. The Sirtuin signaling pathway remained the top pathway with 5 annotated gene members, and the molecular mechanisms of cancer, p53 signaling, and AMPK signaling pathways all had at least 3 gene members.

Discussion

The results of this study support our hypothesis that, compared to new recruits, incumbent firefighters would show differential DNA methylation associated with cancer pathways. This

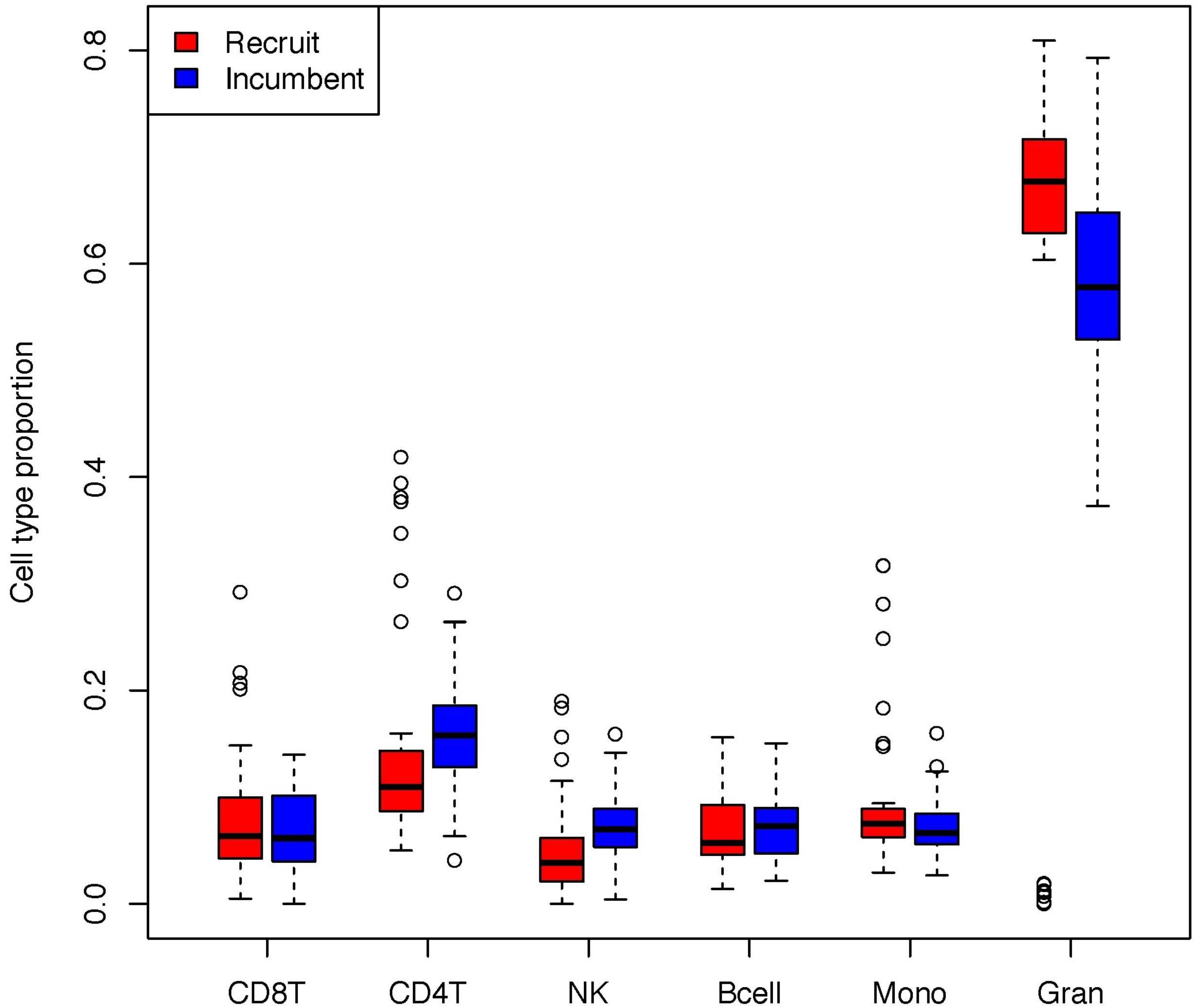

Fig 1. Cell type proportion among new recruit and incumbent firefighters.

https://doi.org/10.1371/journal.pone.0214282.g001

Table 2. Differentially methylated positions.

CpG	Recruits[a]	Incumbent[a]	FC[b]	95% CI		Chr	UCSC RefGene Name	CpG Site Location	Regulatory Feature Group
				Lower	Upper				
cg12253469	98.7% (0.4%)	99.1% (0.3%)	2.40	1.81	3.20	22	MPST	Gene Body	
cg00287370	5.5% (0.9%)	3.7% (0.8%)	0.49	0.40	0.60	1			Promoter Associated
cg24034992	8.4% (1.3%)	5.2% (1.7%)	0.43	0.34	0.55	X	YIPF6	Gene Body	Promoter Associated Cell type specific
cg05236728	3.1% (0.9%)	2.0% (0.8%)	0.40	0.34	0.55	12	PCED1B	Gene Body; 5'UTR	
cg07897354[c]	4.4% (1.2%)	2.7% (0.9%)	0.44	0.34	0.58	18	SPIRE1		Promoter Associated

[a]Group mean (SD) of % methylation (Beta values).

[b]Fold changes (FC) of M values of CpG sites in incumbents compared to recruits with adjustment for age, ethnicity, and body mass index (BMI).

[c]Fold changes shown with adjustment for age and ethnicity; statistical significance lost when also adjusting for BMI.

https://doi.org/10.1371/journal.pone.0214282.t002

Table 3. Differentially methylated regions.

Coordinate	Number of CpGs within region	Mean Beta FC within region[a]
chr19:37825009–37826008	12	0.07292553
chr19:52390810–52392100	15	0.05981331
chr12:47219626–47220197	13	0.08163149
chr19:12305392–12306303	10	0.03812897
chr15:29562049–29562633	10	-0.0089748
chrX:67719027–67719066	2	-0.0178829
chr14:64108940–64109325	5	-0.0089722

[a]Fold change comparing incumbents to new recruits after adjustment for age, body mass index (BMI), ethnicity and cell type composition.

https://doi.org/10.1371/journal.pone.0214282.t003

adds to the currently sparse body of literature describing the epigenetic effects among firefighters, a population occupationally exposed to known carcinogens with documented increased cancer risk [2].

Our differential methylation analysis identified five CpGs assigned to both promoter and non-promoter regions. Promoter hypermethylation frequently leads to silencing of tumor-suppressor or DNA repair genes in cancers while hypomethylation of CpGs often results in overexpression of genes [22, 42–44]. However, recent investigations of broader methylation patterns suggest that non-promoter (intragenic) methylation may also affect transcription regulation and efficiency; while CpG hypermethylation in non-promoter regions does not impede transcription (as it does in promoter regions), it has been correlated with increased or ectopic gene expression [45–48].

Four of the five differentially expressed CpG sites are located in genes with known functions and reported associations with cancer and metastatic potential. However, all five differentially expressed CpGs identified in this study represent novel epigenetic markers that have not previously been reported in the limited body of literature describing differential DNA methylation in firefighters or those with similar occupational exposures. One CpG with decreased methylation among incumbent firefighters was located on the *YIPF6* gene, annotated to the promoter region. *YIPF6* has been associated with prostate cancer, and amplification and overexpression of *YIPF6* protein has been posited to indirectly stimulate tumor progression [49, 50]. Another CpG with decreased methylation in incumbent firefighters is located in the gene body of *PCED1B*. This gene encodes a protein that belongs to the GDSL/SGNH-like acyl-esterase family, hydrolases thought to function in modification of biopolymers on the cell surface. High expression of this gene has significant associations with renal (unfavorable) and urothelial cancer (favorable) patient survival based on Cancer Genome Atlas (TCGA) data (https://www.proteinatlas.org/ENSG00000179715-PCED1B/pathology). One CpG with decreased methylation in incumbent firefighters, that was statistically significant until additionally adjusted for BMI (Table 2), is located in the promoter region of *SPIRE1*. The dysregulated expression of the protein encoded by this gene, SPIRE1, has been associated with cellular potential for extracellular matrix degradation, which may impact the invasive and metastatic behavior of cancer cells [51]. The hypermethylated CpG identified in our analysis was located on the *MPST* gene body. The *MPST* encoded protein is associated with cysteine degradation, cyanide detoxification and likely other metabolic processes, given observed *MPST* deficiency in individuals with the heritable disorder, mercaptolactate-cysteine disulfiduria [52]. As part of its cysteine degradation pathway, *MPST* produces enzymes involved in formation of sulfane sulfur containing compounds. Sulfur metabolism dysregulation in cancer cells and anti-cancer effects *in vivo* of

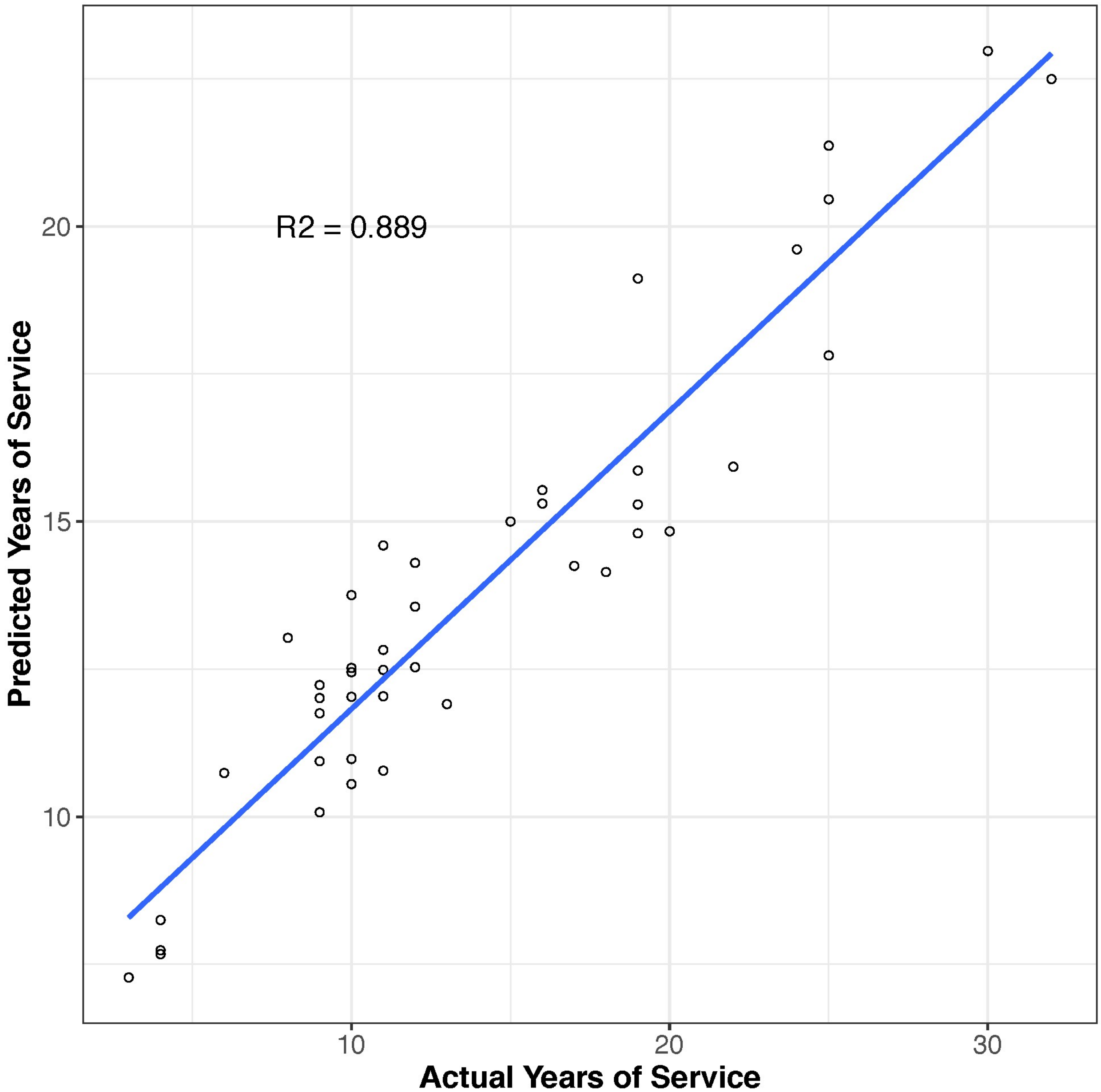

Fig 2. Predictive model for 'years of service' based on CpG level DNA methylation signals (n = 91).

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sulfane sulfur precursors suggest that proliferation of malignant cells may be related to a deficiency of sulfane sulfur and the uncontrolled operation of a set of enzymes normally inactivated by sulfane sulfur [53].

Of the seven differentially methylated regions that remained significant after correction for cell type composition, three were located on genes (*SYNE2*, *AR*, and *PCED1B*) with known functions and disease associations. *SYNE2* encodes a protein involved in maintaining the structural integrity of the nucleus. *AR*, the androgen receptor gene, encodes a member of the steroid hormone nuclear receptor family that regulates gene expression. AR signaling is reported to be involved in prostate, bladder, liver, kidney and lung tumorigenesis and metastasis [54, 55]. Differential methylation patterns of *AR* are also associated with prostate cancer,

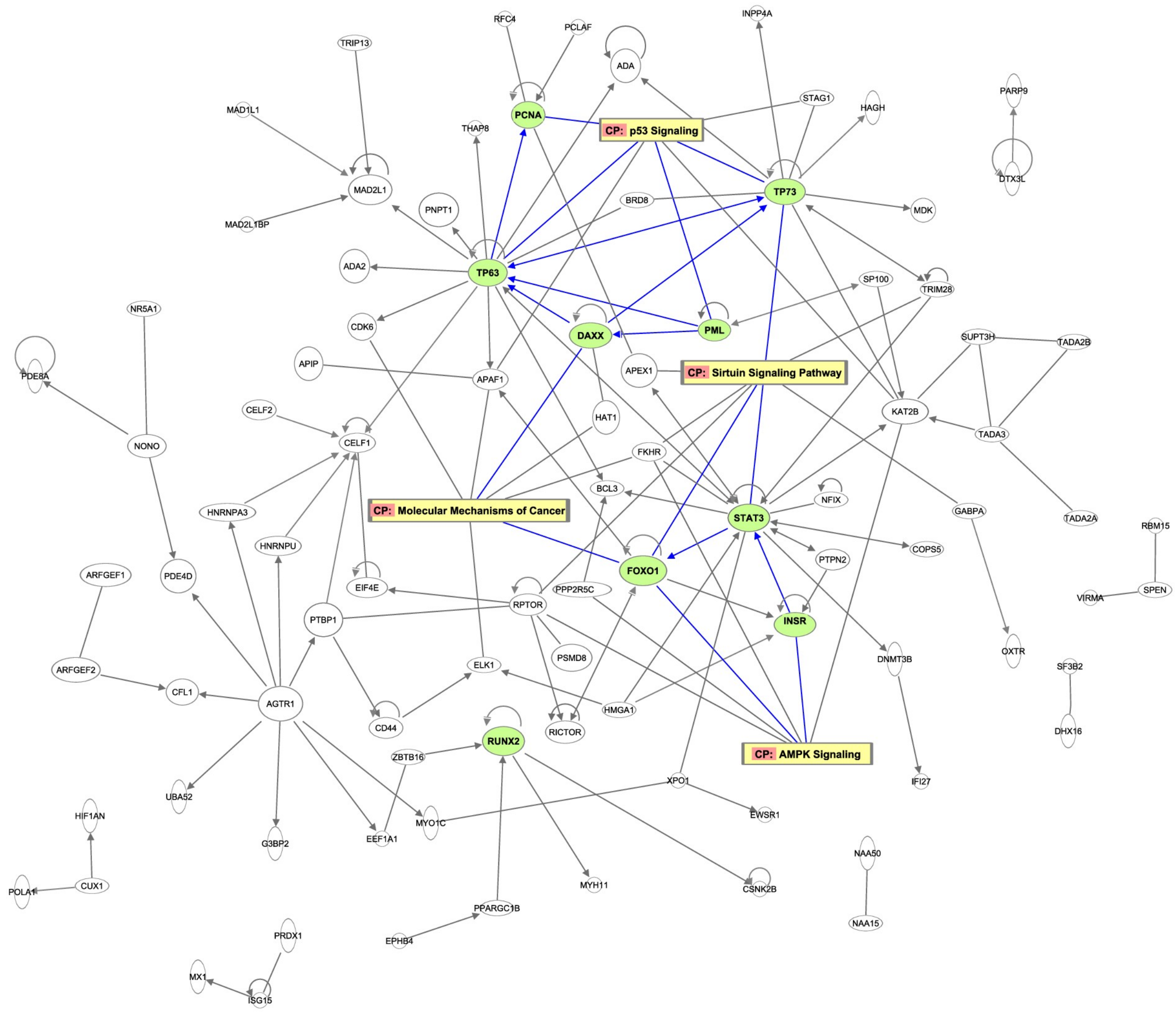

Fig 3. The gene regulatory network and pathways of enriched differential probes between new recruit and incumbent firefighters. Hub genes are highlighted in green. Top background and canonical pathways are highlighted in yellow. Connections between hub genes and top pathways are marked with blue lines.

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Table 4. Disease annotation, number of related genes, and the corresponding hubs.

Disease annotation	*p*-value	# of genes	Hub genes
Abdominal cancer	5.1e-18	88	*STAT3*, *TP63*, *TP73*, *FOXO1*, PML, *DAXX*, *RUNX2*, *INSR*, *PCNA*
Abdominal neoplasm	2.2e-19		
Abdominal carcinoma	1.1e-11		
Adenocarcinoma	5.4e-16		
Colon tumor	5.9e-09	44	*STAT3*, *TP63*, *TP73*, *FOXO1*, *DAXX*, *RUNX2*, *INSR*, *PCNA*
Skin cancer	2.9e-07	51	*STAT3*, *TP63*, *PML*, *DAXX*, *RUNX2*, *INSR*
Lung tumor	6.6e-07	49	*INSR*, *PCNA*, *STAT3*, *TP63*, *TP73*
Lung cancer	1.0e-06		

https://doi.org/10.1371/journal.pone.0214282.t004

non-Hodgkin's lymphoma, and ovarian cancer [24, 56–58]. Variants of *SYNE2* have been associated with p21 expression and reduced overall survival in hepatitis B-related hepatocellular carcinoma [59]. p21 is a cell cycle regulator reported to downregulate TP53, a tumor suppressor [60, 61]. Known functions of *PCED1B*, which also contained a differentially methylated CpG as shown in Table 2, were previously discussed above. Information for the four remaining regions were sparse. One region was located on the protein coding gene *FAM189A1*, which is reported to have tissue-specific expression in brain and colon, but no known disease associations [62]. No information about function or disease associations was found for *ZNF528-AS1*. One region was located on or near uncharacterized genes (*AC016582.2* and *CTD-2554C21.2*) and for the region containing chr19:12,305,392–12,306,303 no further information was available.

Because DNA methylation signatures are tightly correlated to transcriptional activity throughout the genome, they provide a powerful platform for prediction of complex traits or diseases [63–65]. Our machine learning analyses were used to predict whether or not an individual was an incumbent firefighter (and had thus had a certain anticipated level of environmental exposures) and how long that individual had been in the service. Five of the 11 CpGs identified in our best-performing predictive model, cg24034992, cg02932780, cg15304928, cg07897354, and cg03177084, were located on or near genes *YIPF6*, *VARS*, *TMEM9*, *SPIRE1*, and *PSME3*, respectively. *YIPF6*, *TMEM9*, and *PSME3* have been associated with cancer [49, 50, 66–69] and *SPIRE1* reportedly contributes to metastatic potential [51]. *VARS* encodes a multi-domain protein that catalyzes the aminoacylation of tRNA and has been associated with neurodevelopmental disorders [70]. No information was available for the remaining 6 CpGs.

The top identified canonical pathways with differentiated methylated genes included many associated with cancer. The sirtuins, which regulate a large number of cellular pathways and protect the age-associated diseases, regulate processes in cancer cells such as DNA repair and cancer metabolism [71, 72]. More than half of all cancers may involve p53-inactivating mutations, and downstream p53 signals result in cell cycle arrest, apoptosis or senescence [72–74]. AMPK, a highly conserved kinase through evolution, regulates energy-consuming biosynthetic pathways, and activation of AMPK by pharmacological or other means might reduce cancer incidence [75, 76]. The *STAT3* gene, the top identified hub, is a component of essential chemical signaling pathways within cells and an ideal target for chemoprevention and cancer therapy [77, 78]. *STAT3* acetylation silences gene expression and enhances DNA methylation of key tumor-suppressor gene promoters, and inhibition of *STAT3* acetylation reverses aberrant CpG island methylation and leads to the reactivation of several tumor-suppressing gene promoters [79]. Overexpression of *STAT3* leads to continued growth of tumor cells and promotes other malignant properties such as tumor angiogenesis [80, 81]. Tumor proteins p63 and p73, encoded by the *TP63* (on p53 pathway) and *TP73* genes (on both p53 and Sirtuin pathway), provide a complex contribution to tumorigenesis as they regulate cell cycle and apoptosis after DNA damage. For example, *TP73* has been found to be transcriptionally silenced in lymphoblastic leukemias and lymphomas induced by CpG island methylation [82–84]. p63 genomic amplification may have an early role in lung tumorigenesis and may act as a biomarker for lung cancer progression [84]. *INSR*, has been used as a biomarker for prognosis of non-small cell lung cancer and an INSR protein inhibitor, Zykadia, has been authorized by U.S. Food and Drug Administration (FDA) [85] and European Medicines Agency [86] as a treatment of advanced ALK-positive non-small cell lung cancer [87].

Increased risk of many of the enriched diseases identified in our pathway analysis (abdominal cancer, adenocarcinoma, colon tumor, skin cancer, lung cancer) have been previously reported among firefighters. A study examining firefighters from Nordic countries reported excess risk of adenocarcinomas among firefighters aged 70 years and older [6]. In a pooled cohort of US firefighters, excess cancer mortality and incidence were reported for digestive and respiratory sites, including colorectal, mesothelioma and lung cancers [2]. A higher risk of colorectal cancer was also observed in a 2006 meta-analysis of 32 studies on firefighters [5]. Several studies have also reported higher prevalence and risk of non-melanoma and melanoma skin cancer among firefighters [5–7, 88].

Firefighters are exposed to elevated concentrations of multiple products of combustion and other toxic substances, including PAHs, benzene, and PFAS, many of which are carcinogenic, genotoxic or mutagenic [11, 13, 89–92]. Studies among other highly exposed populations have reported associations between PAH exposure and global or gene promoter-specific DNA methylation changes, suggesting that these epigenetic changes may reflect a history of exposure to PAHs [93, 94]. Firefighters also generally work in shifts, typically 24 hours, and shiftwork that disrupts circadian rhythms has been concluded to be "probably carcinogenic" [16]. Studies in non-firefighter populations have also found that long-term shiftwork is associated with differential DNA methylation and whole-genome methylation [95, 96] and there is increasing evidence that long-term shiftwork may increase the risk of breast cancer via epigenetic mechanisms [96–98]. Additional studies are needed, ideally prospective cohort studies with a larger number of firefighters, to help validate the specific CpG sites identified in the current study and to determine which exposures are associated with altered methylation at those sites.

Prior published studies on the relationship between firefighter occupational exposures and epigenetic changes are scarce. We could find only one other study focused on differential DNA methylation among firefighters. This study assessed promoter methylation in four *a priori* genes comparing firefighters to non-firefighting controls and reported significant decreased methylation for one of the four genes, *DUSP22*, as well as a correlation between duration of firefighting service and decreased methylation [20]. However, in our analysis we were unable to detect significant differential DNA methylation at the *DUSP22* promoter region. Additionally, the previous study demonstrated that the decreased *DUSP22* promoter methylation was inducible in cultured human cells by low-dose exposure of benzo[a]pyrene, a highly carcinogenic PAH [20]. In our previous analysis based on many of the same Arizona firefighters evaluated in the current study, we identified nine miRNA markers differentially expressed in incumbent firefighters compared to new recruits [19]. Notably, the six miRNAs with reduced expression in incumbent firefighters have reported tumor suppressor activities while two of the three miRNAs with increased expression are reported to participate in cancer promoting activities, consistent with the hypothesis that firefighters are at increased cancer risk.

The results of our study provide potential mechanisms linking firefighter exposures and the excess risks of specific cancer types identified in epidemiologic studies of cancer in the fire service [1–8]. Given the long latency between exposures and the development of cancer, ranging from less than 5 years to greater than 30 depending on the type of cancer, DNA methylation biomarkers have the potential to be used to both identify the cumulative effect of exposures and to identify firefighters at increased risk of disease susceptibility. In addition to its use in helping to predict future disease, DNA methylation could potentially be used to assist in determining cancer diagnosis and prognosis, as has been demonstrated in groups other than firefighters [99–101]. For example, the methylation signature identified can be used as an "epigenetic clock" of firefighting. If the magnitude and/or length of exposures is both predictive of cancer risk and detectable in methylation signatures, it is likely that prediction of future cancer risk may eventually be attainable. If this is true, it is possible that preventative efforts and close monitoring can be put in place for firefighters at particularly high risk. Identification of epigenetic markers both associated with exposures in firefighters and diseases also have the potential to assist in determining occupational causation in workers' compensation cases.

Limitations of the current study include a relatively small sample size, a cross-sectional design, and inclusion of firefighters from a single geographic region. There was also a significant age difference between the incumbent and new recruit firefighters, although we adjusted for age in our analyses. To further ensure that age differences were not driving the differential methylation identified in our study, we assessed the CpGs known to be altered with age and compared them to the CpGs identified in our study. We did not identify any overlap in these significant regions (data not shown). Future longitudinal studies of a larger number of firefighters across geographic regions are needed to determine the extent to which the findings can be generalized to other firefighter populations, ideally with an external comparison group of similar age to the incumbent firefighters. Information on lifestyle exposures was limited to smoking; no information on diet was available, and occupational history was limited to years as a firefighter. It will also be important in future studies to determine the association among this broader group of exposures with the identified DNA methylation markers, as well as to determine whether the markers are predictive of disease outcomes in firefighters.

Conclusions

In conclusion, DNA methylation varied among male non-smoking incumbent firefighters and new recruits after adjusting for age, BMI and ethnicity. Furthermore, DNA methylation markers were also able to predict with high accuracy the number of years worked as a firefighter. Based on pathway analysis, many of the DNA methylation markers were associated with cancer, supporting the potential for these changes to help explain the mechanism for increased cancer risk among firefighters.

Acknowledgments

This study was supported by a grant from the US Federal Emergency Management Agency Assistance to Firefighters Grant program, EMW-2014-FP-00200.

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MicroRNA Changes in Firefighters

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Objectives: Firefighters have elevated cancer incidence and mortality rates. MicroRNAs play prominent roles in carcinogenesis, but have not been previously evaluated in firefighters. **Methods:** Blood from 52 incumbent and 45 new recruit nonsmoking firefighters was analyzed for microRNA expression, and the results adjusted for age, obesity, ethnicity, and multiple comparisons. **Results:** Nine microRNAs were identified with at least a 1.5-fold significant difference between groups. All six microRNAs with decreased expression in incumbent firefighters have been reported to have tumor suppressor activity or are associated with cancer survival, and two of the three microRNAs with increased expression in incumbent firefighters have activities consistent with cancer promotion, with the remaining microRNA associated with neurological disease. **Conclusion:** Incumbent firefighters showed differential microRNA expression compared with new recruits, providing potential mechanisms for increased cancer risk in firefighters.

Keywords: firefighter, cancer, microRNA

Cancer is a leading cause of death among firefighters in the United States. In a recent large study, overall cancer incidence and mortality among firefighters were 9% and 14% higher than the general public, respectively, with increased mortality rates of 30% or more for mesothelioma and cancers of the esophagus, intestine, and rectum.[1] In addition, elevated incidence and/or mortality have been reported in firefighters for cancers of the bladder, kidney, lung, prostate, skin (melanoma and non-melanoma), stomach, and testes, as well as leukemia, multiple myeloma, and non-Hodgkin lymphoma.[1–7]

Firefighters are occupationally exposed to carcinogens and other toxicants, including benzene, polycyclic aromatic hydrocarbons, formaldehyde, arsenic, 1–3 butadiene, cadmium, chromium compounds, asbestos, flame retardants, and particulates.[5,8–12] Furthermore, most firefighters work prolonged shifts associated with sleep disruption, and shiftwork with circadian disruption has been classified as a probable human carcinogen (group 2A) by the International Agency for Research on Cancer (IARC).[11,13]

While exposure to carcinogens and elevated cancer risk have been well established for firefighters, there is limited information on the cellular mechanisms involved. Greater understanding of these mechanisms is critical to identify potentially reversible cellular changes before the development of cancer, and to help determine causation with regard to firefighter worker's compensation cancer claims. Based in part on the lack of data regarding mechanistic changes in firefighters leading to carcinogenesis, in 2010, the IARC classified occupational exposures to firefighters as only possibly carcinogenic to humans (group 2B), despite multiple epidemiologic studies demonstrating elevated cancer incidence rates in firefighters.[11,13]

Epigenetic changes, including histone modifications, DNA methylation, and microRNA (miRNA) mediated pathways, play prominent roles in carcinogenesis and cancer prevention, and have been associated with activation of oncogenes or inhibition of tumor suppressor genes.[14,15] MiRNAs are small (18 to 22 nucleotide) noncoding RNAs involved in regulating cell cycle progression, apoptosis, and differentiation. Some miRNAs act as oncogenes by inducing oncogene expression or tumor-suppressor genes through regulation of DNA methylation and histone modification. These epigenetic changes serve as molecular biomarkers of environmental exposures and carcinogenesis.[16–19]

We hypothesized that occupational exposures in firefighters would lead to changes in miRNA expression associated with activation of cancer pathways and increased cancer risk. As a first step in testing this hypothesis, we designed this study to compare miRNAs in incumbent firefighters and new recruits.

METHODS

This study was a part of larger firefighter cancer prevention study working in partnership with the Tucson Fire Department. All study protocols were approved by the University of Arizona Institutional Review Board (approval No. 1509137073). To identify epigenetic changes associated with occupational carcinogen exposures in firefighters, we recruited newly employed (new recruit) firefighters before occupational exposure to fire and smoke and incumbent firefighters. After receiving a detailed explanation of the study design and potential risks, all subjects provided written informed consent. We surveyed general characteristics using questionnaires to collect information regarding age, body weight, height, working duration as firefighters, and tobacco use. Body mass index (BMI) (kg/m^2) was classified as normal (18.0 to 24.9), overweight (25.0 to 29.9), and obese ($\geq$30) following World Health Organization (WHO) classifications.

At the time of sample selection for the current analyses, the study subjects consisted of 55 male recruits and 117 male incumbents who had completed baseline blood sampling. One recruit was excluded because of an inadequate blood draw. The 54 remaining recruits were then matched by race/ethnicity to 54 incumbents for sample processing, for a total of 108 subjects. One recruit sample was not adequate for miRNA analysis, and five subjects (four recruits and one incumbent) were later excluded for current smoking

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US Federal Emergency Management Agency Assistance to Firefighters Grant program, EMW-2014-FP-00200.
The authors have no conflicts of interest.
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DOI: 10.1097/JOM.0000000000001307

as well as another five (four recruits and one incumbent) subjects for not completing the smoking-related questions on the questionnaire, leaving 97 (45 recruits and 52 incumbents) subjects for miRNA data analysis.

Whole blood samples were collected in Tempus™ Blood RNA tubes (Applied Biosystems, Foster City, California). Immediately after collection, the tube was vigorously shaken for 10 seconds and aliquoted into two 5 mL cryogenic tubes (VWR International, Radnor, Pennsylvania, Cat. # 89094–820). All aliquots were stored at −20°C until transfer under Arizona Department of Transportation guidelines to the University of Arizona for storage at −80°C for subsequent processing by the University of Arizona Genetics Core (Arizona Research Laboratories).

An aliquot for each subject was thawed for 20 to 30 minutes on ice. RNA isolation was achieved using MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Life Technologies, Carlsbad, California, Catalog #4451893) following the manufacturer's protocol. Purified total RNA qualities and quantities were measured with the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware) and a subset was additionally quality checked using the Advanced Analytical High Sensitivity RNA assay with the Fragment Analyzer Automated CE System (AATI, Ankeny, Iowa).

MiRNA expression was measured using the nCounter® Human v3 miRNA expression panel (NanoString Technology Inc., Seattle, Washington) with 800 miRNAs from miRBase v21 as well as 5 housekeeping genes and 20 assay controls (six positive, eight negative, and six ligation controls). The panel includes greater than 95% of human miRBase reads (https://hdmzlive.nanostring.com/application/files/7014/8943/1030/LBL-10112-01_Human_miRNA.pdf). One hundred nanogram of the purified RNA was prepared by multiplexed annealing of specific tags to each target miRNA, followed by a ligation reaction, and enzymatic purification to remove the unligated tags. Five microliters of the cleaned reaction was hybridized with the Human miRNA Code Set (Nanostring Technologies part #CSO-MIR3-12) at 65°C overnight. Purification and binding of the hybridized probes to the optical cartridge were performed on the nCounter Prep Station, and the cartridge scanned on the nCounter Digital Analyzer (NanoString Technologies, Inc., Seattle, Washington). Raw counts from each gene were normalized against background genes, and overall assay performance was assessed through evaluation of built-in positive controls.

For comparison of age and BMI between recruits and incumbents, the Chi-square test was used. The mean comparisons of age and BMI were done by the Student *t* test. To evaluate the correlation between age and working duration as a firefighter, Pearson correlation was used. These statistical analyses were performed using R (version 3.4.1). MiRNAs sites with mean counts that were less than 2 were filtered, leaving 821 genes for analysis. Filtered miRNAs raw counts were first transformed and quantile normalized by Voom package[20] in preparation for linear modeling and then analyzed by the limma package.[21] A linear model with Empirical Bayes estimator was adopted,[22] with adjustment for age, ethnicity, and BMI. Probes were considered to be differentially expressed if the resulting *P* value was less than 0.05/m applying Bonferroni correction for multiple comparisons. The corresponding gene list was derived from the gene annotations associated with the probes.

Both K-means clustering and hierarchical clustering using the ''factoextra'' package in R 3.4.1 were used to discover miRNA clusters discriminating between the incumbent and new recruit groups. Both analyses were restricted to miRNAs differentially expressed between the two groups, adjusted for age, BMI, and ethnicity, with *P* values less than 0.05. The optimal cluster size was determined by minimizing within sum of squares in K-means clustering analysis. Hierarchal clustering was carried out based on complete linkage and person correlation.

TABLE 1. General Characteristics of Subjects

Variables	New Recruits ($n=45$)	Incumbents ($n=52$)	*P*
Race/Ethnicity			
White, non-Hispanic	39 (86.7%)	44 (84.6%)	1.0
White, Hispanic	6 (13.3%)	8 (15.4%)	
Age, years			
≤29	26 (57.8%)	4 (7.7%)	<0.001
30–39	14 (31.1%)	16 (30.8%)	
≥40	5 (11.1%)	32 (61.5%)	
Mean (standard deviation)	28.8 (6.21)	40.8 (8.70)	<0.001
BMI			
Normal	14 (31.1%)	10 (19.2%)	0.39
Overweight	24 (53.3%)	32 (61.5%)	
Obese	7 (15.6%)	10 (19.2%)	

The miRNA enrichment analysis and annotation tool miEAA (https://ccb-compute2.cs.uni-saarland.de/mieaa_tool/),[23] which relies on the GeneTrail framework (https://genetrail2.bioinf.uni-sb.de/),[24] was employed to investigate downstream effects of the miRNA clusters. Effects of single miRNAs on pathways and organs were determined by miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/holistic.html).[25] The miRNA-disease association was evaluated using the Human microRNA Disease Database (HMDD v2.0, http://www.cuilab.cn/hmdd).[26] Both miRWalk and HMDD are integrated in miEAA. Unless mentioned explicitly, all tools were used with standard parameters.

RESULTS

All subjects were white, and a similar percentage of incumbent and new recruit firefighters were of Hispanic ethnicity (15.4% and 13.3%, respectively). The subjects' mean age in years was significantly higher in incumbents (40.8 ± 8.7) than in recruits (28.8 ± 6.2) ($P < 0.001$) (Table 1). The incumbent firefighters and recruits had similar BMI distributions. None of the new recruit firefighters had any previous firefighting experience. For incumbents, the mean number of years serving as a firefighter was 14.1 ± 7.3 years, and number of years of service was significantly correlated with age (Pearson $r = 0.818$, $P < 0.001$). The 45 new recruits and 52 incumbent firefighters in our study did not significantly differ in terms of age, race, ethnicity, or BMI from the larger group of 89 new recruits and 352 incumbent firefighters, respectively, currently enrolled in the larger Tucson Fire Department cancer prevention study (data not shown).

Comparing incumbents to recruits and adjusting for multiple comparisons, nine miRNAs demonstrated statistically significant differences in expression at a level of at least 1.5-fold (Table 2). Among these, only two miRNAs differed significantly by age group and none by BMI group. Expression levels of all nine miRNAs remained significantly different between incumbents and recruits after adjusting for age, BMI, and ethnicity. Comparing within incumbent and recruit groups, there were no significant differences by age for these nine miRNAs (data not shown).

Three incumbent firefighters reported a previous diagnosis of non-melanoma skin cancer, while none of the new recruits reported this diagnosis. To address this difference, we performed a sensitivity analysis by excluding the three firefighters with skin cancer, and all nine miRNAs remained significant (data not shown). Although all firefighters in the study were current nonsmokers, significantly

TABLE 2. Fold Change (FC) of miRNAs Between Groups by Job Status, Age, and Body Mass Index (BMI)

	Incumbents vs Recruits			Age: ≥40 vs 21–39			BMI: Overweight and Obese vs Normal			Incumbents vs Recruits Adjusted for Age, BMI, and Ethnicity		
		95% CI			95% CI			95% CI			95% CI	
Gene Name	FC	Lower	Upper	FC	Lower	Upper	FC	Lower	Upper	FC	Lower	Upper
hsa-miR-1260a	**0.54**	**0.44**	**0.66**	0.69	0.55	0.87	0.94	0.71	1.24	**0.55**	**0.43**	**0.71**
hsa-miR-548h-5p	**0.55**	**0.43**	**0.72**	0.82	0.60	1.12	0.84	0.60	1.18	**0.59**	**0.51**	**0.69**
hsa-miR-145-5p	**0.57**	**0.51**	**0.65**	0.75	0.64	0.87	0.93	0.77	1.12	**0.44**	**0.32**	**0.61**
hsa-miR-4516	**0.59**	**0.52**	**0.66**	0.75	0.64	0.87	0.96	0.80	1.16	**0.56**	**0.48**	**0.65**
hsa-miR-331-3p	**0.59**	**0.52**	**0.67**	**0.72**	**0.62**	**0.84**	0.87	0.72	1.06	**0.60**	**0.52**	**0.70**
hsa-miR-181a-5p	**0.60**	**0.53**	**0.68**	0.76	0.65	0.89	0.96	0.79	1.15	**0.62**	**0.53**	**0.72**
hsa-miR-5010-3p	**1.56**	**1.41**	**1.72**	1.25	1.10	1.41	1.12	0.96	1.30	**1.59**	**1.41**	**1.81**
hsa-miR-374a-5p	**1.57**	**1.33**	**1.85**	1.09	0.90	1.32	1.18	0.95	1.47	**1.72**	**1.40**	**2.13**
hsa-miR-486-3p	**3.51**	**2.88**	**4.28**	**2.22**	**1.64**	**3.01**	1.23	0.85	1.78	**3.35**	**2.59**	**4.33**

Significantly differentially expressed genes (after Bonferroni correction) are highlighted in bold font.
BMI, body mass index; CI, confidence interval; FC, fold change.

more new recruits (11) than incumbents (four) previously smoked over 100 cigarettes (Pearson Chi-square $P = 0.034$).

Cluster analysis was based on 234 miRNAs differentially expressed between incumbent and new recruit firefighters. The optimal number of clusters for K-mean analysis was determined to be three, with centroids hsa-miR-525-3p (cluster 1), hsa-miR-52 (cluster 2), and hsa-miR-376b-3p (cluster 3) (Fig. 1). There were 103, 80, and 51 miRNAs in these three clusters, respectively. An enrichment analysis was performed to investigate whether the miRNA sets within the three clusters belonged to a pathway, gene ontology, organ, or other functional category, with FDR adjusted P value less than 0.05 (Table 3). MiRNAs in the first cluster were associated with stem cells and three pathways: inflammation mediated by chemokine and cytokine signaling; cytokine-cytokine receptor interaction; and cell adhesion molecules. MiRNAs in the second cluster were also associated with stem cells. The third cluster yielded miRNAs associated with carcinoma, Burkitt lymphoma, melanoma, and 10 targeted genes.

DISCUSSION

The study results supported our hypothesis that incumbent firefighters, compared with new recruits, would show differences in expression of miRNAs associated with cancers or cancer pathways. This adds to the scarce published literature on epigenetic effects in firefighters, to our knowledge limited to hypomethylation of dual specificity phosphatase 22 promoter,[27] and suggests potential mechanisms for the association between firefighting and cancer.

Of the nine differentially expressed miRNAs identified in our study, all six (miR-548h-5p, miR-145-5p, miR-4516, miR-331-3p, miR-181a-5p, and miR-1260a) with decreased expression in incumbent firefighters have been reported to have tumor suppressor activity or are associated with cancer survival, and two (miR-374a-5p and miR-486-3p) of the three miRNAs with increased expression in incumbent firefighters have activities consistent with cancer promotion. The miR-548 family suppresses tumor cell growth and development by increasing apoptosis and regulating reactive oxygen species.[28] MiR-4516 and miR-145-5p play a role in tumor suppression by controlling p53.[29–31] MiR-331-3p acts as a tumor suppressor in colorectal and gastric cancer.[32–34] MiR-181a-5p has a tumor suppressor effect in nonsmall cell lung cancer through reduction of K-ras expression.[35] Increased levels of miR-1260a are associated with survival in glioblastoma patients.[36] MiR-374a-5p promotes cell proliferation, migration, and invasion in esophageal and gastric cancer,[37,38] and is overexpressed in

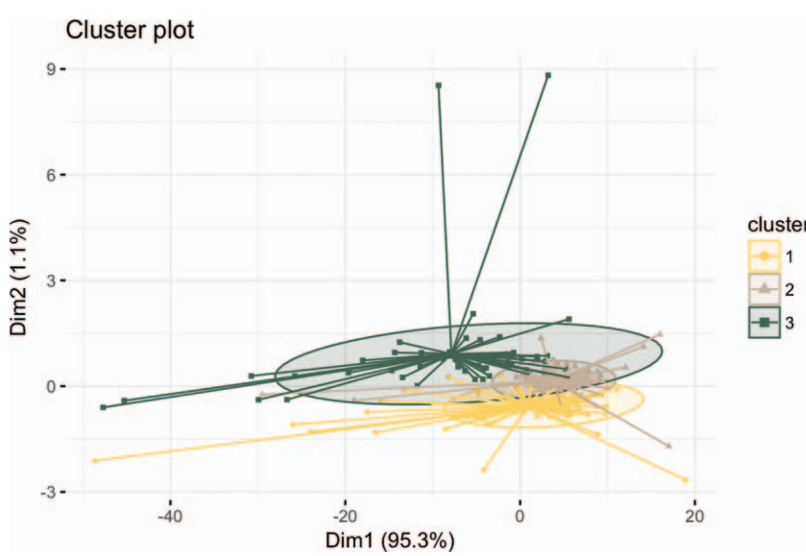

FIGURE 1. K-mean clustering using 234 MiRNAs.

TABLE 3. MiRNA Enrichment Analysis Results for Three K-Mean Clusters

Cluster	Category	Subcategory	*N*	Observed miRNAs	*P* (FDR)
1	Organs	Stem Cells	8	miR-126-3p; miR-133b; miR-15a-5p; miR-195-5p; miR-29b-3p; miR-302a-3p; miR-302b-3p; miR-326	0.048
	Pathways	Inflammation mediated by chemokine and cytokine signaling (P00031)	19	miR-126-3p; miR-135a-5p; miR-15a-5p; miR-193b-3p; miR-195-5p; miR-196b-5p; miR-218-5p; miR-22-3p; miR-29b-3p; miR-302a-3p; miR-302b-3p; miR-30e-3p; miR-320c; miR-326; miR-337-3p; miR-451a; miR-7-5p; miR-935; miR-96-5p	0.048
		Cytokine–cytokine receptor interaction (hsa04060)	9	miR-126-3p; miR-133b; miR-15a-5p; miR-193b-3p; miR-195-5p; miR-22-3p; miR-29b-3p; miR-302b-3p; miR-7-5p	0.048
		Cell adhesion molecules (hsa04514)	6	miR-126-3p; miR-15a-5p; miR-193b-3p; miR-196b-5p; miR-218-5p; miR-29b-3p	0.048
2	Organs	Stem cells	6	miR-137; miR-138-5p; miR-214-3p; miR-224-5p; miR-27a-3p; miR-302d-3p	0.021
3	Diseases	Burkitt lymphoma	2	let-7a-5p; let-7b-5p	0.033
		Carcinoma	19	let-7a-5p; let-7b-5p; let-7c-5p; miR-106b-5p; miR-1226-3p; miR-125a-5p; miR-141-3p; miR-145-5p; miR-151a-5p; miR-181a-5p; miR-181c-5p; miR-185-5p; miR-197-3p; miR-19a-3p; miR-19b-3p; miR-21-5p; miR-223-3p; miR-376b-3p; miR-93-5p	0.033
		Melanoma	2	let-7a-5p; let-7b-5p	0.033
	Target genes	*AIDA*	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		ANKRD17	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		CCNB2	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		CSNK2A1	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		DHX9	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		IPO7	5	let-7a-5p; let-7b-5p; let-7c-5p; miR-106b-5p; miR-1226-3p	0.010
		LTA4H	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		NME4	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		PTGES2	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019
		RPS24	3	let-7a-5p; let-7b-5p; let-7c-5p	0.019

colorectal cancer.[39] Upregulation of miR-486-3p is associated with K-ras mutation in colorectal cancer.[40] The one miRNA for which we did not find an association with cancer, miR-5010-3p, is increased in patients with Alzheimer disease.[41] In addition to their previously described roles in cancer, miR-374a-5p is overexpressed with high altitude, hypoxia, and oxidative stress,[42] miR-486-3p is increased in ST-elevated acute myocardial infarction,[43] and miR-1260a is increased in children with asthma.[44]

Aging and obesity are major risk factors for cancer,[45] and they are also associated with epigenetic changes.[46–53] Specific to miRNAs, age, BMI, and sex are associated with miRNA expression.[54] In our study, only two miRNAs showed significant differences in expression by age group, and there were no significant differences in miRNA expression by BMI group. Furthermore, all nine miRNAs continued to show significant differences between incumbents and new recruits following adjustment for age and BMI.

In addition to documented associations with cancer pathways, some of the miRNAs identified in our study have also been evaluated for relationships with exposures common to firefighters. In a study examining gaseous formaldehyde exposure and miRNA expression in human bronchial epithelial cells, miR-181a was one of the most significantly downregulated.[55] MiR-181a-5p expression, decreased in incumbent firefighters in our study, was downregulated in Lin-c-Kit+ cells obtained from mice exposed to benzene.[56] MiR-313-3p expression, decreased in incumbent firefighters in our study, was reduced following short-term PM_{10} exposure in a population of overweight/obese subjects,[57] but was increased in lung adenocarcinoma patients exposed to asbestos compared with nonexposed patients with adenocarcinoma.[58] MiR-4516 expression was decreased in our incumbent firefighters, but was upregulated in a study of A549 cells exposed to $PM_{2.5}$ as well as the serum of persons living in a Chinese city with moderate air pollution.[59] MiR-145-5p expression was decreased in our incumbent firefighters, but increased in service members with polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzofuran (PCDF) exposures from open air burn pits comparing pre- and post-deployment.[60] However, to evaluate for dose–response relationships specific to firefighters, longitudinal studies including exposure assessment and measurement of miRNA changes are needed.

Cluster analysis comparing the incumbent firefighters to new recruits identified miRNA groupings associated with stem cells, inflammation, cytokine–cytokine receptor interactions, cell adhesion molecules, cancers, and a number of target genes. MiRNAs control stem cell self-renewal and differentiation,[61] and through this role have been implicated in the etiology of a variety of cancers.[62,63] For example, hsa-miR-302a-3p and hsa-miR-302b-3p, enriched in cluster 1, and hsa-miR-302d-3p, enriched in cluster 2, belong to the miR-302 family, which has important roles relative to stem cells.[64] MiR-302 inhibits human pluripotent stem cell tumorigenicity by enhancing multiple G1 phase arrest pathways.[65] Moreover, the miR-302 family functions to reprogram skin cancer cells into a stem cell-like pluripotent state.[66] MiR-137, enriched in cluster 2, is down-regulated in colon cancer stem cells compared with normal colon stem cells.[67] MiR-124 and 137 also regulate the differentiation and proliferation of neural stem cells and glioblastoma-multiforme tumor cells.[68]

Beyond stem cells, all three pathways identified in cluster 1 were related to inflammation. Chronic inflammation has long been linked with cancers.[69] Inflammation mediated by the chemokine and cytokine signaling pathway (P00031) includes chemokine-induced adhesion and migration of leukocytes[70,71] and miRNAs differentially expressed in lung cancer,[72,73] bladder cancer,[74] senescence, and aging.[75] The cytokine–cytokine receptor interaction pathway (CCRI, hsa04060) is a large pathway that includes 270 related genes.[76] Cytokines that act through receptors are released in response to infection, inflammation, and immunity, and cytokines and cytokine receptors can function to inhibit tumor development and progression. Cancer cells also respond to host-derived cytokines that promote growth, attenuate apoptosis, and facilitate invasion and metastasis.[77,78] Cell adhesion molecules (CAMs, hsa04514), constituting the third pathway, play a critical role in a wide array of biologic processes, including immune response and inflammation, contributing to cancer development.[79–81]

The third cluster identified in our analyses included specific cancer types and genes. Of the 19 miRNAs enriched in carcinomas, three of them were from the miRNA let-7 family and are reported to be downregulated in human lung carcinomas, where reduced let-7 expression is associated with a poor cancer prognosis.[82] Also among these 19 miRNAs, miR-21 and miR-181 are potential diagnostic or prognostic biomarkers for nonsmall cell lung cancer,[83] and miR-106 and miR-93 are expressed in hepatocellular carcinoma.[84] The enriched let-7 family in cluster 3 targets 10 genes, and let-7 is highly conserved in human organs and associated with colon adenocarcinoma, kidney renal clear cell carcinoma, esophageal carcinoma, lung squamous cell carcinoma, and liver hepatocellular carcinoma, among others.[23] Specifically, CSNK2A1, IP07, and DHX9 are coexpressed in kidney renal clear cell carcinoma and lung squamous cell carcinoma.[24]

Our study has a number of limitations. The number of firefighter participants was limited and cross-sectional in nature, and larger prospective longitudinal studies are needed to validate the epigenetic changes observed. Although we adjusted for age, there were only a limited number of new recruits older than 40 years of age, and similarly only a limited number of incumbent firefighters less than 30 years old. We did not validate the miRNA findings using a second technique, given the strong correlation reported among multiple miRNA platforms including NanoString.[85] Although we did not have complete information on toxic exposures outside of firefighting, previous use of cigarettes was lower in the incumbent firefighters, so we do not believe that this past exposure could explain the miRNA findings. We were not able to adjust for other potential confounders due to incomplete responses to survey questions such as but not limited to diet and exercise. Additional studies in other geographic regions are also needed to determine whether the results of our study are generalizable to firefighters elsewhere.

In conclusion, this study identified multiple miRNAs with significantly different expression levels comparing incumbent firefighters with new recruits. These findings suggest potential mechanisms for development of cancer in firefighters.

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Environment International 135 (2020) 105207

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Assessment of the toxicity of firefighter exposures using the PAH CALUX bioassay

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ARTICLE INFO

Handling Editor: Zhen Jason He

Keywords:
Bioassay
PAH
Firefighters
Aryl hydrocarbon receptor

ABSTRACT

Firefighters can be exposed to a complex set of contaminants while at a fire scene. Identifying new ways to monitor and assess exposure, particularly relating to toxicity is essential to determine the effectiveness of intervention techniques to reduce exposure. This study investigated the use of the polycyclic aromatic hydrocarbon (PAH) CALUX® bioassay for the assessment of exposure and associated toxicity firefighters might encounter. This was done through analysis of extracts of dermal wipes and urine samples collected from firefighters before and after a controlled fire. An increased bioassay response was observed from post-fire neck and calf samples, indicating a greater concentration of PAH-like compounds on the skin. The use of a baby wipe to clean the face and neck during rehab resulted in the attenuation of the observed bioassay response from the neck post-fire. Though a correlation was observed between the bioassay response and hydroxylated PAH concentrations found in the urine, the increased bioassay response from the post-fire urine samples was likely due to unknown compounds other than the hydroxylated PAHs tested. Our results suggest that this bioassay provides a useful measure of firefighter exposure, particularly relating to the potential toxicity of contaminants.

1. Introduction

There are many products of combustion with known toxic effects, including but not limited to carbon monoxide, hydrogen cyanide, benzene, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs) (IARC, 2010; Fabian et al., 2014; Kirk and Logan, 2015). Firefighters and associated personnel at a fire scene may be exposed to these contaminants through inhalation, dermal, and ingestion routes. Numerous studies have concluded a greater cancer incidence and mortality in firefighters overall and/or for specific cancers compared to the general population (Daniels et al., 2014; Monash University, 2014; Pukkala et al., 2014; Daniels et al., 2015; Glass et al., 2016), and the International Agency for Research on Cancer (IARC) has classified firefighting as possibly carcinogenic to humans (Group 2B) (IARC, 2010).

PAHs are formed as a result of incomplete combustion of organic material and include chemicals known to be mutagenic and/or carcinogenic (Boffetta et al., 1997; Papa et al., 2008; IARC 2010 vol 92; Fernando et al., 2016; Andersen et al., 2017). Previous studies have quantified the concentrations of different PAHs in smoke from both training and active fires, along with extracts from swabs of the gear and skin (Baxter et al., 2014; Fabian et al., 2014; Fernando et al., 2016; Fent et al., 2014; Keir et al., 2017; Wingfors et al., 2017; Stec et al., 2018). Dermal exposure is thought to be an important route of exposure of PAH-like compounds to firefighters particularly if inhalation of these compounds is minimized due to the use of a self-contained breathing apparatus (SCBA) (VanRooij et al., 1993; Stec et al., 2018). Exposure to these compounds have been observed not only while actively fighting fires, but also during overhaul and at the firehouse (Baxter et al., 2014; Oliveira et al., 2017). In addition to ambient exposure to PAHs, researchers have quantified PAH metabolites in the urine of firefighters before and after fires as biomarkers of exposure (Edelman et al., 2003; Fent et al., 2014; Fernando et al., 2016). The primary method of quantification of both PAHs and metabolites has been through the use of targeted mass spectroscopy, which is a strong tool when investigators are interested in specific PAHs or specific metabolites. However, it can be very difficult to assess the potential toxicity or overall exposure when a limited number of PAHs and related compounds are being quantified, as each PAH can have a different toxic potential and

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https://doi.org/10.1016/j.envint.2019.105207
Received 9 March 2019; Received in revised form 27 August 2019; Accepted 20 September 2019
Available online 04 December 2019

exposures often involve complex mixtures.

Toxicity of PAHs, along with other dioxin-like compounds, is primarily caused through the binding to the aryl hydrocarbon receptor (AhR), induction of AhR-related genes and subsequent transformation to toxic metabolites (Behnisch et al., 2001; Tuyen et al., 2014). The *in vitro* PAH CALUX® bioassay can be used to assess the overall AhR mediated toxicity from PAHs and related compounds. A shorter incubation time is used to maximize the observed response from compounds metabolized at a faster rate, such as PAHs. This bioassay reports the total AhR mediated toxicity from dioxins, furans, PAHs, polychlorinated biphenyls, among others, in the form of Benzo[a]Pyrene (B[a]P) equivalence. The assay has been used to assess the presence and associated toxicity of PAH-like compounds from sediment, crude oil and from particulate matter from wood combustion (Pieterse et al., 2013; Gauggel-Lewandowski et al., 2013; Radovic et al., 2014). To date, the PAH CALUX® assay has not previously been used to assess the potency of hydroxylated PAHs or the overall exposures that firefighters encounter.

This study investigated whether the PAH CALUX® bioassay could be used to aid in the assessment of toxicity of exposure that firefighters and associated personnel encounter at a fire scene. This bioassay was used to assess the overall AhR activity from extracts of skin wipes before and after a controlled burn to identify the overall load of AhR active compounds on the skin that have the potential to enter the body through dermal absorption. Additional endpoints within the dermal sampling campaign included investigation into whether a prototype particulate blocking hood could help decrease exposure to PAHs and other AhR active compounds, and investigation into whether the use of a baby wipe to clean the face and neck post-fire could remove AhR active compounds from the skin. Urine samples collected before and after the control fire were also tested with this bioassay. Investigation into the bioactivity of a suite of hydroxylated PAHs and related compounds commonly used as predictors of exposure was conducted to identify if hydroxylated PAHs responded in this bioassay and what proportion of the bioactivity observed in the urine extracts can be explained. Understanding if other unmeasured compounds are primarily responsible for the bioactivity related to fire exposure will help determine the need for evaluation of other toxic contaminants in urine.

2. Materials and methods

2.1. Test subjects

The study was approved by the University of Arizona Institutional Review Board (approval No. 1509137073, and all subjects signed informed consent forms prior to participation in the research. A total of 11 non-smoking male Tucson Fire Department incumbent firefighters participated in this study involving one controlled fire. The average age, weight and height of the participants ± standard deviation were 39 ± 9 yr, 84.4 ± 7.3 kg and 175 ± 5 cm, respectively. To reduce PAH exposure from dietary sources, participants were asked not to eat grilled or charred food 12 hrs before the control fire and until after their final urine collection post-fire.

2.2. Test chemicals

Benzo[a]Pyrene (BaP) was supplied by BioDetection Systems (Amsterdam, The Netherlands). 1-Hydroxyphenanthrene, 2-Hydroxyphenanthrene, 3-Hydroxyphenanthrene, 4-Hydroxyphenanthrene, 3-Hydroxychrysene, and 3-Hydroxy-Benzo[a]Pyrene were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). 9-Hydroxyphenanthrene, Eugenol, 4-Ethylguiacol, 2–6-Dimethoxyphenol, 2-Methoxy-4-methylphenol, 2-Methoxy-4-propylphenol, 2-Hydroxyfluorene, 1-Hydroxypyrene, 2-Hydroxynaphthalene and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (Milwaukee, WI). 3-Hydroxyfluorene was purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA). 6-Hydroxycrysene was purchased from Crescent Chemical Co. Inc. (Islandia, NY). All chemicals tested for response in the bioassay were dissolved in DMSO.

2.3. Controlled fire

The controlled fire took place at the Tucson Public Safety Academy in Tucson, AZ, USA. The building used for this test fire had a total of 3 rooms: a burn room and maze room on the ground floor with 10 ft ceilings, and one room on the second floor containing two windows and two doors (all closed during the burn). Ten firefighters were in the building, with 5 in either the maze room or burn room, and 1 individual outside of the building in full gear who did not enter the building. Half way through the fire, the individuals switched rooms and activities. The burn room was heated to 425–500 °F, with the maze room having smoke and residual heat ranging from 100 to 175 °F. The burn time was 14 min to resemble the average time of response activities during a basic residential structural fire. The items burned were chosen to represent a room and contents fire and consisted of wood with a pallet base, a padded arm chair, particle board shelving, a 4′ × 6′ carpet and padding, and miscellaneous objects (books, a clock radio, and a plastic vase). When smoke production in the room decreased, additional materials were added to the fire to maintain 14 min of smoke production. During the fire, firefighters simulated firefighting activities. They carried a hose up and down the 19 steps to the second floor, swung a sledge hammer against a tire, and crawled around the maze to simulate search and rescue. Upon exiting the fire, the firefighters did not remove their SCBA until they were away from the structure, and they received assistance removing their turnout gear and hood to avoid cross contamination from their gear to their skin. New turnout gear and hoods (never before used) were utilized for this study to avoid contamination from past fires. Two different hood types were used within this study. Five participants wore prototype particulate blocking hoods (not commercially available) meant to provide improved protection against particulates while the other 5 participants wore a traditional non-particulate hood comprised of blended PBI, Kevlar and Lenzing fibers.

2.4. Sample collection

Dermal wipes were collected to measure the PAHs and other AhR active compounds present on the neck and calf. Dermal samples were collected on the right side of the body at each location pre-fire, and the left side of the body at each location post-fire. Texwipe™ AlphaWipe™ polyester wipes (Fisher Scientific, Denver, CO) were prepared for use by being cut to 2″ × 4″ in size, submerged in GC grade Methylene Chloride (DCM) (Fisher Scientific, Chino, CA) and sonicated for 30 min, removed from DCM, and allowed to dry. Once dry, the wipes were sonicated for a second time in fresh DCM for 30 min, dried and placed into scintillation vials (Fisher Scientific, Chino, CA). Prior to the sampling campaign, 15 mL of LC/MS grade isopropanol (IPA) (Fisher Scientific, Chino, CA) was added to each scintillation vial to saturate the wipe prior to use. For dermal sampling, wipes were removed from the scintillation vial with forceps and a 3″ × 3″ section of skin at each sample site was wiped in a circular motion 10 times. The IPA in the vial was removed, and the processed wipe was placed back into the vial. Wipes were stored at 4 °C prior to extraction. Dermal sampling was collected first from the neck, followed by the calf for each sampling event. An additional dermal wipe of the neck was taken after each subject cleaned their neck and face with a baby wipe containing no alcohol or aloe. This final neck sample was collected from the right side of the body.

Urine samples were collected in 120 mL Coviden urine collection cups (Fisher Scientific, Pittsburgh, PA) to measure exposure to PAHs and other AhR active compounds. Urine was collected just before the control fire and 2, 4, and 6 hrs post-fire. Urine samples were held on ice until transported according to ADOT guidelines to the laboratory where they were processed immediately.

2.5. Sample preparation and extraction

Dermal wipes were extracted to be analyzed using *in vitro* bioassays. Wipes were sonicated with 20 mL of DCM for 30 min, followed by the DCM being transferred through a sodium sulfate (Sigma Aldrich) packed column. This sonication and transfer step was completed a second time, with the extracts being combined. The extract was then evaporated under a stream of nitrogen below 1 mL, then filtered through a Millex 13 mm PFTE 0.45 µm filter (Fisher Scientific) using a glass syringe. Finally, 85 µL of DMSO was added to the extract, evaporated under nitrogen, and brought up to 100 µL DMSO by weight for analysis with *in vitro* bioassays. All extracts were stored at −20 °C until analyzed.

Urine samples were well mixed prior to processing. One 10 mL vial of each urine sample was kept in neat form, specific gravity (S.G.) was measured using an Atago urine specific gravity refractometer (Atago USA, Inc., Bellvue, WA), and the remaining urine was centrifuged for 10 min at 1500–2000 rpm (400–600 × g). The supernatant was aliquoted into 10 mL aliquots. All 10 mL aliquots were stored at −20 °C.

Extraction of urines for bioassay analysis began with a deconjugation of the urine which was conducted in a similar manner as (Fernando et al., 2016). Briefly, 10 mL of pre-centrifuged urine was added to 16.5 mL of 0.100 M sodium acetate buffer (pH 5.5) and 33.3 µL of β-glucuronidase from *Helix pomatia* (Sigma Aldrich, Milwaukee, WI). This mixture was incubated at 37 °C for 16–18 hrs. Waters HLB cartridges (150 mg, 6 mL) (Waters, Milford MA) were conditioned with 5 mL of HPLC grade methyl-tert butyl ether (MtBE) (Fisher Scientific) followed by 5 mL of HPLC grade Methanol (MeOH) (Fisher Scientific) and then 5 mL of ultra-pure water. The sample was loaded onto the cartridge followed by 5 mL of water. The cartridge was dried by aspirating nitrogen through to remove all residual water. Elution was done with 5 mL of MeOH followed by 5 mL of a solution containing 90% MtBE and 10% MeOH. Eighty five microliters of DMSO was added to the eluent, MtBE and MeOH evaporated under nitrogen, and then brought up to 100 µL DMSO by weight. Extracts were stored at −20 °C until analyzed.

Extraction of urines for analytical analysis of hydroxylated PAHs on an Agilent Gas Chromatography-Triple Quadrupole Mass Spectrometer (GC-QQQ) followed a similar extraction procedure as above. A detailed description is provided in the supplemental material.

2.6. In vitro bioassay culture and exposure

PAH CALUX® assays were cultured and exposed according to the manufacturer's guidelines (BioDetection Systems). Briefly, cells were seeded in 96-well plates at a density of 400,000 cells/mL, incubated at 37 °C for 16–18 hrs prior to adding the test chemicals and reference compound at a final concentration of 0.8% DMSO. Benzo[a]pyrene was used as the model PAH for this assay and for all relative potency (REP) calculations. All concentrations were tested in triplicate on each plate. Once exposed, cells were incubated for 4 hrs, then washed, lysed, luminescence reagent added and luminescence read using a Molecular Devices FlexStation 3 multi-mode microplate reader (Molecular Devices, Sunnyvale, CA). Relative potency of the PAHs and hydroxylated PAHs were calculated by first subtracting the solvent control (DMSO), then the maximum signal from the reference compound (B[a]P) was set to 100%, with the signal observed from the compounds of interest being illustrated as percentage of max response. Response curves are based on testing the compounds a total of 2 times for compounds with response < 15% the max response and 3 times for compounds with responses > 15% max response. When analyzing urine and dermal wipe samples, results were calculated as B[a]P equivalence in order to allow for comparison among samples, and method blank samples subtracted. S.G. was used to calculate a concentration factor to standardize the urine samples for how hydrated the subjects were at the time of collection. The concentration factor was calculated as follows: Concentration factor = (1.02 − 1.0)/(S.G. − 1.0).

2.7. Statistical analysis

All statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA) and data expressed as mean ± standard deviation. Data were analyzed by one-sample Kolvogrov-Smirnov test for normality, and by Levene's test for homogeneity of variance. Data from skin wipes of the calf were analyzed by a student's *t*-test for comparison of samples within individuals, and a paired *t*-test when individuals were grouped. Data from skin wipes of the neck were analyzed by analysis of variance (ANOVA), followed by a Tukey's test. Data from the urine samples were analyzed by ANOVA, followed by a one-tailed Dunnett's test, comparing 0 hr to the 4 post-fire time points. A one-way ANCOVA was conducted to determine if there was a statistically significant difference between the type of hood on the relative PAH concentration on the skin post-fire, with the pre-fire response being the covariate. Correlations were assessed using the Spearman correlation coefficient. Levels below the limit of detection (LOD) were substituted with half the LOD for statistical analyses.

3. Results and discussion

3.1. Dermal wipes

Dermal wipes were collected before and after the fire at locations of the body found previously to have soot post-fire to address the hypothesis that dermal exposure contributes to the overall exposure of PAHs and related compounds to firefighters, and for the neck to investigate whether a prototype particulate blocking hood helped decrease exposure to PAHs and other AhR active compounds. Analysis of the dermal calf wipes showed a statistically greater response from the calf post-fire ($M = 5.58\ ng/cm^2$, $SD = 2.76\ ng/cm^2$) compared to pre-fire ($M = 2.64\ ng/cm^2$, $SD = 2.61\ ng/cm^2$) for the group of 10 firefighters who entered the control fire ($t(9) = -2.690$, $p = 0.025$), (Fig. 1). As expected, there was no statistical difference in the observed bioassay response post-fire between the group wearing the hood with specialized particulate blocking material and the group wearing a hood without specialized particulate blocking material for calf wipes ($F(1,7) = 0.704$, $p = 0.429$). The variability observed among the individuals pre-fire could be due to any number of activities they might have been involved in that would cause deposition of compounds on their skin. This could include use of skin care products or standing near any type of exhaust, among others. It is hypothesized the variability observed post-fire is likely due to how fitted the turnout gear was for each individual, providing more or less space for the particulates in the smoke to get underneath the turnout gear while they were simulating firefighting activities. It is important to note that the majority of individuals showed a statistical increase in deposition of compounds on the skin post-fire that interact with the AhR, regardless of the inherent variability of human participants.

The increased bioassay response observed from the calf wipe extracts post-fire indicates that PAHs and other AhR active compounds from the fire were deposited on the skin. This is in alignment with previous studies that demonstrated that a select number of PAHs were found at greater concentrations on the skin underneath PPE post-fire, and contribute to the overall exposure of PAHs and related compounds to firefighters (Laitinen et al., 2010; Baxter et al., 2014; Fent et al., 2014; Fernando et al., 2016; Stec et al., 2018). Laitinen et al (2010) illustrated that the hands are one area that PAHs can be deposited, and that wearing undergloves can decrease the amount of PAHs on the hands by up to 80%. Fernando et al (2016) found an increase in PAHs post-fire on the wrist, neck, forehead, back, and fingers of firefighters exposed to wood smoke. Stec et al (2018) found an increase in PAHs post-fire on the front of the neck, back of the neck, the jaw and the hands; and through a cancer risk characterization using PAH concentrations concluded that there is an elevated risk primarily through dermal exposure. Fent et al (2014) looked at the forearms, hands, neck,

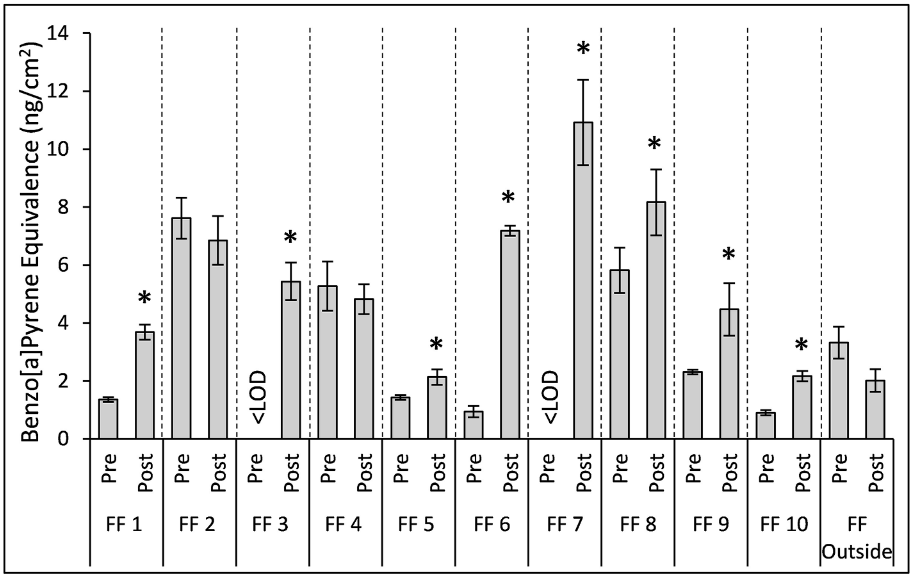

Fig. 1. Benzo[a]pyrene equivalence of dermal wipe samples taken pre-fire and post-fire from the calf of firefighters. '*' represents a post-fire sample being statistically greater than pre-fire (*t*-test, $p < 0.05$).

face, and scrotum as areas of concern, with the neck being the only site that was statistically greater post-fire in one of the conducted rounds. The neck is also of increased concern as the skin is thinner and in general, sites with thinner skin tend to have faster absorption rates (VanRooij et al., 1993). Finally, the neck is also the only location in which the PPE does not have a vapor barrier built into it, and therefore could lead to a greater deposition of contaminants and increased exposure compared to other areas. With this in mind, two different hood types were tested within this study, one with and one without specialized particulate blocking material.

When evaluating the neck for the presence of AhR active compounds using the *in vitro* bioassay, it was found that the majority of individuals saw greater concentrations of AhR active compounds post-fire (Fig. 2). Four of the five individuals wearing the particulate blocking hoods had greater bioassay response post-fire (Fig. 2A), as did 4 of the 5 individuals wearing the hoods without particulate blocking material (Fig. 2B). The individual who did not enter the fire was also wearing the non-particulate blocking hood and was found to have elevated AhR active compounds on the neck post-fire. It is uncertain if this increased bioassay response resulted from exposure to smoke within the vicinity of the fire, or if this was due to contamination during sampling. When comparing the two types of hoods, it was found that there was no statistically significant difference of post-fire bioassay response from the neck between the group wearing the hood with particulate blocking material and the group wearing a hood without particulate blocking material ($F(1,7) = 1.948$, $p = 0.206$). When this study was conducted, the hood containing the particulate blocking material was a prototype design which was ultimately not commercially produced.

The use of a baby wipe to wash the head and neck area during rehab was tested to see if this type of intervention would result in decreasing the concentration of PAHs and other AhR active compounds on the neck. It was found that there was a statistically significant decrease in the majority of individuals that had increased AhR active compounds on their skin post-fire (Fig. 2). This illustrates that the use of a baby wipe is beneficial to remove AhR active compounds from the skin soon after a fire as there is usually a time delay before the firefighters are able to shower and clean their skin. Additional studies designed to evaluate how effective skin wipes are at reducing the concentrations of PAHs, other AhR active compounds, and their metabolites in blood and/or urine would be beneficial to link the observed decrease of concentrations on the skin to what could be absorbed and enter the body.

3.2. Urine extracts

Urine collected pre- and post-fire was analyzed to investigate the degree of exposure firefighters received from this fire in the form of the AhR response in the PAH CALUX® assay. While dermal wipes give an estimation of skin deposition, measuring metabolites in the urine can help measure absorbed dose from inhalation, dermal and ingestion exposure. Two of the 5 individuals that wore the hood containing the particulate blocking material had a greater AhR-mediated response post-fire when compared to pre-fire (Fig. 3A), while only 1 of the 5 individuals who wore the hood without particulate blocking material had a greater AhR-mediated response post-fire compared to pre-fire (Fig. 3B). It was found that 2 to 4 hr post-fire was the optimal time to observe an increase in AhR active compounds in the urine (Fig. 3A and B). This is in accordance with a previous pilot study that was conducted by this research group, where urine samples were collected before and after a similar training fire (Fig. S.1) and from urine samples collected from firefighters responding to structural fires in the community (Fig. S.2). It is hypothesized that the variability in the intensity of the fire could be one reason only a few firefighters in this current study showed a significant increase in bioassay response in 2–4 hr post-fire urine extracts compared to the majority of firefighters from the pilot study and structural fires in the community.

3.3. Relative potency of PAH and hydroxylated PAHs standards in PAH CALUX bioassay

Of the 20 different compounds tested on the PAH CALUX® assay, 7 were found to have a quantifiable agonistic response with relative potencies (REPs) less than that of B[a]P (Table 1). The particular isoform of the compound was found to be important with respect to the observed response. Although metabolism usually leads to a decrease in biological activity of a compound, this was not the case for 4-hydroxyphenanthrene and 3-hydroxyfluorene. In both of these cases, the potency of the metabolite was greater than that of the parent compound (Table 1). It should be noted that the potency of some of the parent compounds could not be determined and therefore the REPs are being based off of the maximum concentration tested.

Since an agonistic response was observed from some of the hydroxylated PAHs, it is likely that the response observed from the urine extract has a proportion of the response coming from the hydroxylated PAHs in the mixture. In order to determine how much of the bioactivity

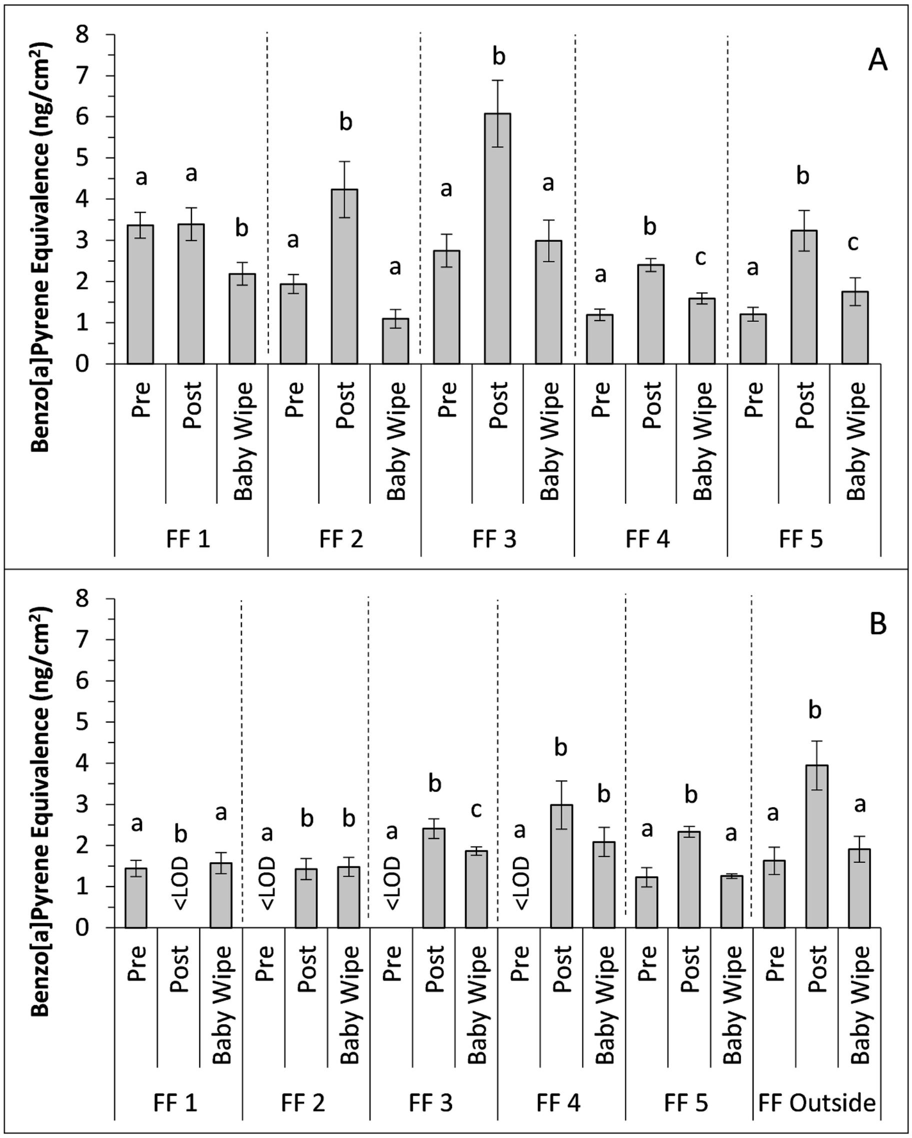

Fig. 2. Benzo[a]pyrene equivalence of dermal wipe samples taken pre-fire, post-fire and post-baby wipe from the neck of individuals wearing A) hoods with prototype particulate blocking material and B) hoods without particulate blocking material. A different letter represents a statistical difference ($p < 0.05$) among the different dermal samples collected pre-fire, post-fire or post-baby wipe for each individual when analyzed with ANOVA followed by a Tukey's test.

in the urine extracts was from the known hydroxylated PAHs, and how much was attributed to unknown metabolites, analytical analysis of these 20 hydroxylated PAHs was conducted on extracts of the same urine sample. Understanding what proportion of biological response was from the commonly quantified hydroxylated PAHs is important as current analytical tests use a select number of hydroxylated PAHs to represent the complete mixture of all PAH metabolites in the urine. Although correlations have been found with increased concentrations of some hydroxylated PAHs and exposure to fires (Fent et al., 2014; Keir et al., 2017; Wingfors et al., 2017), being able to get a measurement of the complete mixture is of great importance in evaluating overall exposure. This study also looked at the correlation of the sum of the quantified hydroxylated PAHs to the bioassay response from a set of urine samples collected from firefighters who responded to structural fires in the community. Baseline urine samples were collected to represent a no-exposure sample; and 2 hr post-fire urine samples were collected after responding to the structural fire. The select set of samples was chosen in order to provide a range of quantified hydroxylated PAHs post-fire, to identify if there is a correlation between quantifiable PAHs and bioassay response. It was shown that there was a statistically significant relationship between the hydroxylated PAHs known to be elevated post-fire and the *in vitro* bioassay response ($r(14) = 0.638$, $p = 0.008$).

Concentrations of the hydroxylated PAHs were quantified in extracts of the urine samples from the control fire (Table S.1) and were used along with the REPs to predict a B[a]P equivalence response. This predicted B[a]P equivalence was compared to the response observed from the urine samples using the PAH CALUX® assay. Of the PAH-OHs that were responsive in the bioassay and used to calculate the predicted B[a]P equivalence, 2-hydroxyphenanthrene, 3-hydroxyfluorene, 1-hyroxypyrene, 6-hydroxychrysene and 2-hydroxynaphthalene were detected in the urine samples, where 4-hydroxyphenanthrene and 3-hydroxychrysene were below the detection limit in all samples. This comparison between predicted- and observed B[a]P equivalence showed that less than 1% of the response was able to be accounted for by the quantified hydroxylated PAHs, and therefore greater than 99% is from unknown compounds. This is not surprising as only a few of the likely vast number of metabolites in the urine were tested, and the AhR is known to interact with a diverse set of compounds. It must be noted that only a few of the hydroxylated metabolites of PAHs were

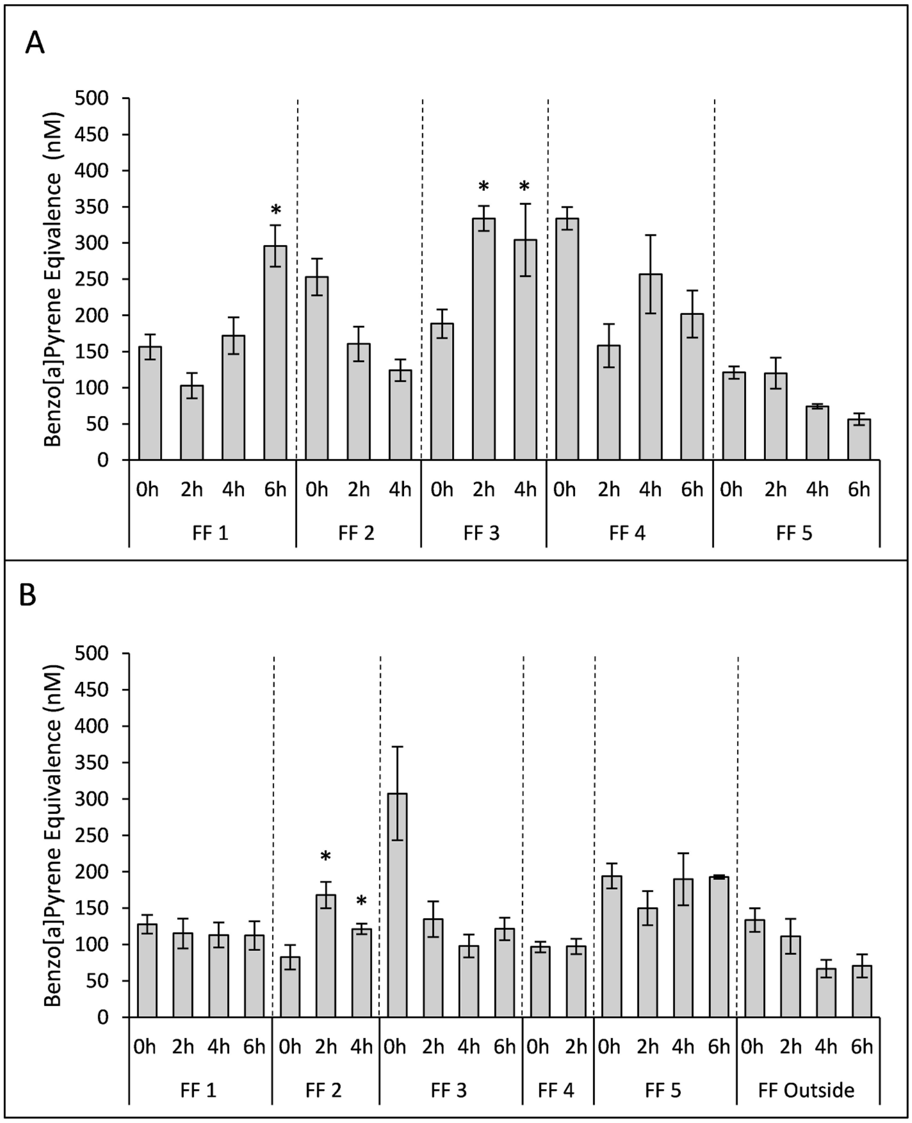

Fig. 3. Benzo[a]pyrene equivalence of urine samples taken pre-fire (0 hr) and at 2, 4, and 6 hr post-fire for individuals wearing A) hoods with prototype particulate blocking material and B) hoods without particulate blocking material. '*' represents a post-fire sample being statistically greater than 0 hr (ANOVA, $p < 0.05$).

quantified in the urine and the hydroxylated metabolites are a result of only one metabolic pathway, of which there are multiple.

There are sub classes of PAHs and related compounds which include alkylated-, heterocyclic- and nitro-PAHs that have been shown to be important groups to analyze in order not to underestimate the overall load of PAH contamination in environmental samples (Talaska et al., 1996; Titaley et al., 2016; Lam et al., 2018). Specifically, methylated PAHs have been shown to have greater potency in terms of AhR response than parent PAHs (Myers and Flesher, 1991; Pieterse et al., 2013; Lam et al., 2018) and have been shown to be mutagenic and carcinogenic. One study found that 5-methylchrysene was responsive in the PAH CALUX® assay, having a REP of 1.4 compared to B[a]P (Pieterse et al., 2013), which is between 280× and 170,000× of greater potency compared to the responsive hydroxylated PAHs in this study. Nitro PAHs and other unsubstituted PAHs have AhR-mediated activity, and have also been shown to have mutagenic activity (Pitts, 1987; Talaska et al., 1996; Ciganek et al., 2004; Amakura et al., 2016). In fact, some of these PAHs have been shown to respond in the PAH CALUX® bioassay with greater REPs than B[a]P. These include benzo[*j*]fluoranthene, dibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene, benzo[*k*]fluoranthene, and benzo[*b*]fluoranthene, having REPs of 1.3, 1.3, 1.3, 3.7, and 5.0, respectively (Pieterse et al., 2013). Finally, poly aromatic ketones are a class of polar compounds found in wood smoke at equal abundances as PAHs that also have been shown to cause mutagenicity (Ramdahl, 1985). Unfortunately, the relative potencies of the metabolites of these compounds are not known and would need to be investigated to determine if they are present in the urine post-fire and to what extent they would respond. In addition to PAHs, some metabolites of PCBs have been shown to have agonist responses with the AhR (Machala et al., 2004). These are all metabolites of the variety of compounds that firefighters are exposed to and therefore could be in the urine and responsible for some of the observed bioassay response. Unfortunately, quantification of all metabolites in order to gain a comprehensive view of the exposure profile is nearly impossible, which is why the bioassay is advantageous as its strength lies in assessing mixture effects. Additional research should be conducted to identify the compounds present in the urine that are responsible for the majority of the bioassay activity, which could result in new, more prominent biomarkers of exposure. It is suggested that future research use an effect directed analysis approach to aid in the separation and identification of possible bioactive compounds.

Additional *in vitro* bioassays designed to assess toxicity endpoints such as genotoxicity or oxidative stress could also be used in addition to the PAH CALUX® assay to characterize the toxicity of the exposures that

Table 1
Overview of relative potencies (REP) of PAHs, hydroxylated PAHs and methoxyphenols in relation to Benzo[a]pyrene in the PAH CALUX® assay.

PAH derivative	EC20 ± S.D (M)	EC50 ± S.D (M)	REP EC20	REP EC50	Reference
1-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
2-Hydroxyphenanthrene	3.93E−5 ± 4.57E−6	8.11E−5 ± 4.00E−6	2.69E−5	5.63E−5	This Study
3-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
4-Hydroxyphenanthrene	1.83E−6 ± 2.19E−7	7.75E−6 ± 1.26E−6	5.78E−4	5.89E−4	This Study
9-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
Eugenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
Guaiacol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
4-Ethylguaiacol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
2,6-Dimethoxyphenol	> 1.00E−3	> 1.00E−3	N/A	N/A	This Study
2-Hydroxyfluorene	> 8.00E−4	> 8.00E−4	N/A	N/A	This Study
3-Hydroxyfluorene	2.38E−6 ± 8.7E−7	8.43E−6 ± 2.75E−6	4.44E−4	5.41E−4	This Study
4-Hydroxyfluorene	> 3.00E−5	> 3.00E−5	N/A	N/A	This Study
1-Hydroxypyrene	1.09E−4 ± 1.38E−5	> 1.00E−4	9.70E−6	N/A	This Study
2-Methoxy-4-Methylphenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
2-Methoxy-4-Propylphenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
3-Hydroxychrysene	2.46E−7 ± 4.72E−9	1.03E−6 ± 1.31E−7	4.30E−3	4.43E−3	This Study
6-Hydroxychrysene	3.06E−6 ± 3.71E−7	1.34E−5 ± 1.42E−6	3.45E−4	3.40E−4	This Study
1-Hydroxynaphthalene	> 2.40E−4	> 2.40E−4	N/A	N/A	This Study
2-Hydroxynaphthalene	3.54E−5 ± 4.56E−6	7.50E−5 ± 1.25E−5	2.99E−5	6.08E−5	This Study
3-Hydroxybenzo[a]pyrene	> 8.00E−6	> 8.00E−6	N/A	N/A	This Study
Benzo[a]pyrene	1.06E−9 ± 1.31E−10	4.56E−9 ± 8.66E−10	1.00	1.00	This Study
2,3,7,8-Tetrachlorodibenzodioxin				5.0	Pieterse et al, 2013
5-Methylchrysene				1.4	Pieterse et al, 2013
Chrysene				0.8	Pieterse et al, 2013
Naphthalene				< 1.00E−4	Pieterse et al, 2013
Fluorene				< 1.00E−4	Pieterse et al, 2013
Phenanthrene				< 1.00E−4	Pieterse et al, 2013
Pyrene				< 1.00E−4	Pieterse et al, 2013

firefighters receive. For example, a recent study found increased mutagenic potential of post-fire urine compared to pre-fire urine collected from firefighters (Keir et al., 2017). These endpoints would be of importance in addressing the targeted toxicity pathways that contaminants entering the body might take. This would add to the comprehensive view of the exposure firefighters receive, and ultimately these endpoints could be used to monitor and assess intervention techniques with the primary goal of decreasing the exposure firefighters and associated personnel receive.

4. Conclusion

The PAH CALUX® bioassay was shown to be useful in assessing firefighter's exposure to PAHs and other AhR active compounds by quantifying bioassay responses from extracts of dermal wipes and of urine samples collected before and after a fire. The majority of individuals had an increase of AhR active compounds on the skin of the calf and neck post-fire. Wearing a prototype hood with particulate blocking materials as compared to a standard hood did not affect the increase in AhR active compounds on the neck post-fire. The use of a baby wipe to wipe the skin of the face and neck during rehab post-fire decreased the observed bioactivity and therefore the amount of AhR active compounds on the skin. Although some hydroxylated PAHs were found to be agonistic of the AhR, the majority of bioassay response observed in the urine extracts was likely from compounds other than the hydroxylated PAHs quantified. More research is needed to identify which compounds are primarily responsible for the increased bioassay response in the urine post-fire, which might lead to a new biomarker of exposure.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

This study was funded by the Federal Emergency Management Agency, grant number EMW-2014-FP-00200. The authors would like to thank the Tucson Fire Department along with other staff and students who assisted with the training fires and sample processing. We would like to thank Biodetection Systems for donation of the cell line and reagents used in this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105207.

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Journal of Exposure Science & Environmental Epidemiology (2021) 31:900–912
https://doi.org/10.1038/s41370-021-00306-8

ARTICLE

Longitudinal evaluation of whole blood miRNA expression in firefighters

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Received: 18 September 2020 / Revised: 18 January 2021 / Accepted: 28 January 2021 / Published online: 18 February 2021

Abstract

Background Dysregulated microRNA (miRNA) expression could provide a mechanism linking firefighter exposure to increased cancer risk.

Objective To determine if changes in longitudinal miRNA expression in firefighters are associated with occupational exposures.

Methods Whole blood MiRNA was evaluated in 52 new recruits prior to live-fire training and 20–37 months later. Linear mixed effects models adjusted for age, ethnicity, BMI, and batch effects were used to determine associations separately for all fires and structure fires only between employment duration, cumulative fire-hours and fire-runs, and time since most recent fire with (1) nine a priori and (2) the full array of 799 miRNAs.

Results For multivariable models including all fires, two a priori miRNAs were associated with employment duration and four with time since most recent fire. For multivariable models restricted to structure fires, three a priori miRNAs were associated with employment duration and one with fire-runs. Additional miRNAs from the full array were associated with employment duration for all fires and/or structure fires. In general, tumor suppressive miRNAs decreased and oncogenic miRNAs increased with exposure.

Significance Changes in miRNAs may serve as biomarkers of exposure effects and a mechanism for increased cancer risk in firefighters.

Keywords Cancer · Epidemiology · Workplace Exposures

Introduction

Firefighting is a hazardous occupation involving exposure to toxic combustion byproducts, including many known and probable carcinogens [1–5]. Within the past decade, the epidemiological evidence that cancer risk is elevated among firefighters compared to the general population has grown [6–10]. Notably, an evaluation of ~30,000 career firefighters in the United States (US) found excess overall cancer mortality and incidence (increases of 9 and 14%, respectively), in addition to increased incidence and mortality for cancers of the lung, digestive tract, and kidney [6]. Associations between lung cancer and leukemia mortalities and surrogate fireground exposures were also reported, specifically cumulative fire-hours and cumulative fire-runs, respectively [7]. A meta-analysis study reported elevated risk in firefighters of cancers of the prostate, testes, colon, rectum, bladder, thyroid, and pleura as well as non-Hodgkin lymphoma and melanoma [11]. Other studies using cancer registry data from California and Florida have also observed elevated cancer risks among firefighters, including melanoma, multiple myeloma, leukemia, and esophageal, prostate, brain, and kidney, testicular, thyroid and colon cancers [9, 10].

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41370-021-00306-8.

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Despite the number of epidemiologic studies linking firefighting to excess cancer risk, there is limited information on the cellular mechanisms that lead to cancer in firefighters. Partly due to this lack of mechanistic evidence, an assessment by the International Agency for Research on Cancer concluded that firefighting was only possibly carcinogenic in humans [12]. Epigenetic modifications, such as dysregulated microRNA (miRNA) expression and DNA methylation, are changes in gene expression that are heritable, reversible (do not affect DNA sequence), and critical steps in the cancer pathway that have been associated with the regulation of oncogenes and tumor suppressor genes [13–15]. These epigenetic modifications have been proposed as biomarkers of cancer risk and exposure, and a limited number of studies have previously examined epigenetic modifications among firefighters [16–18].

MiRNAs are small non-coding RNAs involved in the regulation of cell cycle progression, apoptosis, and cell differentiation, with reported oncogenic or tumor-suppressive roles, though these may vary depending on which gene is targeted and which cancer is considered [19–21]. Our previous research comparing incumbent and new recruit firefighters identified nine dysregulated miRNAs with previously published associations with cancer or cancer pathways [16]. However, there are no prior published studies evaluating potential associations between longitudinal changes in miRNA and interim firefighting exposures. This information could be useful to advance future efforts to identify firefighters at increased cancer risk, to provide intermediate endpoints for cancer prevention interventions, and to provide evidence for causation in firefighter workers' compensation cancer claims.

In our current analysis we evaluated whole blood samples, collected from newly recruited firefighters prior to live-fire training and again ~2 years later, for miRNA expression levels and determined if changes in expression were associated with measures of occupational exposure. We hypothesized that after ~2 years of employment in the fire service, we would be able to identify differential miRNA expression patterns among newly recruited firefighters that were associated with employment duration, surrogate measures of fire exposure (cumulative hours at fire and cumulative number of fires), and/or most recent fire exposure, and that changes in these miRNAs would be associated with increased cancer risk based on published studies in the general population.

Materials and methods

Study participants

All study protocols and materials were approved by the University of Arizona Institutional Review Board (IRB). Study participants provided written informed consent. New-recruit firefighters with no previous live-fire exposures were recruited from the Tucson Fire Department (TFD) between 2015 and 2016. Participant age, race, ethnicity, height, and weight were collected from health records and current tobacco usage was assessed at study enrollment via survey. Participants who reported current tobacco usage were excluded from analyses. Height and weight were used to calculated body mass index (BMI) (kg/m^2) and classified using World Health Organization categories: normal (18.5–24.9), overweight (25.0–29.9) and obese (≥30). Biological samples including blood, urine and buccal cells were collected at baseline and blood samples were collected again after a period of 20 to 37 months, at which time a follow-up survey was administered. Employment duration, the total months between baseline and follow-up, was calculated.

Measures of fireground exposures

Cumulative hours at fire (fire-hours) and cumulative number of fires (fire-runs) were collected from TFD response records and assessed as surrogate measures of chronic fireground exposure. This information included the type of fire (structure fire, vehicle fire, other), date and time of the fire, and duration of the fire response (minutes). The time between the blood draw at follow-up and the new recruit's most recent response to a fire call (days since most recent fire exposure) was calculated. Fire-hours, fire-runs, and days since most recent fire exposure were determined for: (1) all fire types (structure, vehicle, and other); (2) structure fires only; and (3) non-structure fires only. Other fires, or non-structure non-vehicle fires, consisted of outside vegetation (i.e., grass, brush, forest, crop) fires, outside trash fires, outside fires involving property of value (e.g., storage, equipment), and unclassified fires.

MicroRNA expression measurement

The protocols used for sample collection and processing have been previously reported [16]. Levels of miRNA expression were measured using an nCounter Human v3 miRNA expression panel (NanoString Technology, Inc., Seattle, WA), a profile of 799 curated and clinically relevant human miRNAs from miRBase v21, in addition to 5 housekeeping genes and 20 assay controls (6 positive, 8 negative, and 6 ligation controls) [22, 23] in four batches analyzed in 2016, 2017, 2018, and 2019. The expression panel accounts for more than 95% of all observed sequencing reads in miRBase 21 [24]. Raw counts from each gene were normalized against background genes. The overall assay performance was assessed through evaluation of positive controls.

Statistical analysis

In order to normalize and remove unwanted variation (batch effects), we adopted the Removing Unwanted Variation-III method (RUV-III), which makes vital use of pseudo-replicates and control genes [25]. In our miRNA design, we purposely measured the same sample at all four time points (2016, 2017, 2018 and 2019), serving as pseudo-replicates. The NanoString platform also includes housekeeping genes and negative and positive control genes on their array. RUV-III (i) takes residuals from the replicate expression measurements and estimates one aspect of the unwanted variation; (ii) takes the results of (i) together with the expression values of the negative controls, and estimates another aspect of the unwanted variation; and (iii) combines the results from (i) and (ii) into an estimate of the unwanted variation and subtracts that from the data. Relative Log Expression (RLE) plots were used to diagnosis batch correction, using the "*ruv*" R package (https://cran.r-project.org/package=ruv). RUV-III corrected miRNAs were then analyzed using the limma software package [26] to determine the association between fireground exposures and miRNA expression at follow-up to expression at baseline. Linear mixed effects models with Empirical Bayes estimators were adopted and adjusted for age, ethnicity, BMI, and batch effects [27]. MiRNAs were considered to be differentially expressed if the p value was less than 0.05 after Bonferroni adjustment [28]. All statistical analyses were performed using R (version 3.4.1).

The exposures of interest were employment duration, measures of chronic fireground exposure (fire-hours and fire-runs) and acute fireground exposure (days since most recent fire exposure). Employment duration was included as a proxy for cumulative firefighter exposures of all types, including but not limited to chemical exposures and shift-work. Fire-runs and fire-hours were considered the fireground exposures of greatest interest, due to their use as measures of cumulative fireground exposure in previous firefighter research [7, 29]. These exposures were treated as continuous variables in our models. Days since most recent fire was included in our models to adjust for potential confounding of acute exposures on the association between chronic fireground exposures and differential miRNA expression. As a novel potential confounder, several definitions were considered for days since most recent fire: continuous log-transformed days, categorized at the median value, categorized at tertile values, and categorized at quartile values. For categorical definitions, the categories representing the longest number of days since fire exposure (more than the median, the third tertile, the fourth quantile) served as the reference value. The definition that explained the most variation of data for each miRNA was selected based on highest Akaike information criteria (AIC), using separate models adjusted for age, BMI and ethnicity [30].

We also hypothesized that firefighters would face increased risk of exposure from structure fires compared to vehicle or other fire types. Previous research has shown that median urinary concentrations of polycyclic aromatic hydrocarbon (PAH) metabolites after a fire incident were greatest among firefighters who performed interior operations at structural fires, such as fire attack and rescue [1] and that mean respirable particles measured at vehicle fires was lower than at structural fires [2, 3]. Therefore, we stratified analyses by fire type (all fires, or structure fires only). For analyses considering all fires, all fire-hours, all fire-runs, and days since most recent fire were included as covariates, in addition to age, BMI and ethnicity. For analyses considering only structure fires, structure fire-hours, structure fire-runs, and days since most recent structure fire were included as covariates, in addition to age, BMI and ethnicity.

The outcome of interest was differential expression of miRNAs. Two sets of miRNA markers were considered: (1) nine a priori markers significant in our previous analysis comparing new recruits to incumbent firefighters [16]; and (2) the full array of miRNAs. The effects of employment duration, fire-hours, fire-runs, and days since most recent fire exposure on differential miRNA expression were first evaluated in separate models adjusted for age, ethnicity, BMI, batch effects and Bonferroni correction (referred to as the univariable models) to compare to values reported in our previous cross-sectional study of incumbent and new recruit firefighters [16]. Second, models containing employment duration, either fire-hours or fire-runs, and days since most recent fire, in addition to age, ethnicity, BMI, batch effects and Bonferroni correction (referred to as the multivariable models) were run. Fire-hours and fire-runs were expected to be highly correlated, so they were examined in separate multivariable models. Differential expression, comparing expression at follow-up to expression at baseline, was presented as log-fold changes (log_2FCs) and fold changes (FCs) with accompanying p values and 95% confidence intervals (95% CIs). Based on Bonferroni correction, p values $< 0.05/n$ (where n is the number of statistical tests performed) were considered statistically significant.

Our previous study presented select cancer associations for the nine a priori miRNAs [16]. To interpret findings from our current analyses of the full array of miRNAs, the select cancer associations of significant full array miRNAs with an absolute FC >1.25 were also evaluated. Given that previous studies have used absolute FC >1.5 to identify potential diagnostic and prognostic miRNAs in cancer samples [31–33], we assumed that 25% might represent a reasonable threshold for increased risk of exposure. When published evidence of an association between miRNA expression and cancer risk could be found, one reference per miRNA was presented. The reference was selected regardless of reported direction of association (increased or decreased FC), instead based on the following additive criteria: (1) the study utilized blood samples, (2) the association was also validated in an independent sample set, (3) the association with the specific cancer was also found in other studies, and (4) the association with the specific cancer was also found using other sample types (e.g., tissue and/or cell lines). In the case that no published studies using blood samples could be found, the criteria were applied to studies using tissue samples.

Results

Study participants and occupational exposures

Of 90 new recruit firefighters offered participation, 89 (99%) consented and were enrolled. Twenty (22%) of the 89 were excluded because they lacked a blood sample collected at baseline or follow-up, 10 (11%) were excluded because they left the fire department before follow-up, 3 (3%) were excluded due to poor RNA yield, and 4 (4%) were excluded because they failed quality control testing for miRNA, leaving 52 individuals (58%) in the study. The participants were all male, mostly white and non-Hispanic (Table 1). At study enrollment, the average participant was 28.2 years of age. The period of follow-up ranged from 20 to 37 months. For all fire types, the average time spent at all fires combined was 27.0 h and the average number of fires was 49.2. The median time between the follow-up blood sampling and most recent fire was 26 days. Employment duration was moderately correlated with time at fires, fire-runs, and days since most recent fire, with correlation coefficients of 0.43, 0.46, and 0.36, respectively. Time at fires was highly correlated with fire runs but not days since most recent fire, with correlation coefficients of 0.86 and 0.21, respectively. Fire runs and days since most recent fire had low correlation (coefficient 0.06).

When restricted to structure fires, the average time spent at all structure fires combined over the follow-up period was 13.2 h, the average number of structure fire-runs was 15.7, and the median time since most recent structure fire was 41.5 days. Employment duration was not highly correlated with time spent at structure fires, structure fire-runs, and days since most recent structure fire (correlations coefficients of 0.19, 0.23, and 0.04, respectively). However, time spent at structure fires was highly correlated with structure fire-runs (correlation coefficient 0.89) as expected, while days since most recent structure fire had low correlation with either structure fire-hours or structure fire-runs (correlation coefficients 0.14 and 0.01, respectively).

Table 1 Characteristics of new recruit firefighters at enrollment, $N = 52$.

	N (%)
Age (years)	
≤29	37 [71]
30–39	11 (21)
≥40	4 (7)
Mean ± SD (range: min–max)	28.2 ± 6.0 (19.1–45.6)
Body mass index (kg/m^2)	
Normal (18.5–24.9)	18 (15)
Overweight (25.0–29.9)	28 [54]
Obese (≥30)	6 (11)
Mean ± SD	26.4 ± 3.7
Race	
White	50 [96]
Other	2 (7)
Ethnicity	
Hispanic	5 (10)
Non-Hispanic	47 [90]
Fire exposure between enrollment and follow-up	
Average employment duration (months) ± SD	26.5 ± 4.3
All fire incident types	
Average cumulative time at fire (hours) ± SD[a]	27.0 ± 8.6
Average cumulative number of fire runs ± SD[a]	49.2 ± 15.0
Days since most recent fire at follow-up, median (IQR)	26 (15.8, 112.5)
Structure fire incidents only	
Average cumulative time at structure fires (hours) ± SD[a]	13.2 ± 6.7
Average cumulative number of structure fire runs ± SD[a]	15.7 ± 7.3
Days since most recent structure fire, median (IQR)	41.5 (18, 141.5)

SD standard deviation, *IQR* interquartile range.

[a]Length of time over which this value accumulated varied by individual (range: 20–37 months).

Fireground exposure and differential miRNA expression

Results are provided separately for all fires and structure fires only. The univariable and multivariable model results restricted to non-structure fires closely followed results of the all fire models, with the same significant miRNAs for each of the models, and the same direction of the coefficients.

Univariable analyses of a priori markers, all fires

In our univariable models (adjusted for age, ethnicity, BMI, batch effects, and multiple comparisons) considering all fire

Table 2 Differential miRNA expression of a priori miRNAs by fire type exposure: separate models adjusted for age, BMI, ethnicity, batch effects.

miRNA	Incumbents vs new recruits[a]			New recruits (follow-up vs baseline measurement)[b]											
				Employment duration			Fire-hours			Fire-runs			Most recent fire[c]		
All fires	FC	95% CI		FC	95% CI		FC	95% CI		FC	95% CI		FC	95% CI	
hsa-miR-1260a	**0.55**	**0.43**	**0.71**	0.96	0.93	1.00	0.98	0.94	1.01	0.95	0.89	1.01	0.86	0.69	1.07
hsa-miR-548h-5p	**0.59**	**0.51**	**0.69**	**1.05**	**1.02**	**1.08**	**1.04**	**1.02**	**1.07**	**1.09**	**1.04**	**1.14**	1.02	0.83	1.26
hsa-miR-145-5p	**0.44**	**0.32**	**0.61**	**0.96**	**0.93**	**0.98**	**0.97**	**0.94**	**0.99**	**0.94**	**0.90**	**0.98**	0.83	0.72	0.96
hsa-miR-4516	**0.56**	**0.48**	**0.65**	0.97	0.95	0.99	0.98	0.96	1.00	0.96	0.93	1.00	0.92	0.82	1.03
hsa-miR-331-3p	**0.60**	**0.52**	**0.70**	0.97	0.94	0.99	0.98	0.96	1.00	0.96	0.92	1.00	0.84	0.70	1.01
hsa-miR-181a-5p	**0.62**	**0.53**	**0.72**	0.99	0.97	1.02	0.99	0.97	1.02	0.99	0.95	1.03	**0.81**	**0.70**	**0.92**
has-miR-5010-3p	**1.59**	**1.41**	**1.81**	**1.13**	**1.10**	**1.16**	**1.10**	**1.08**	**1.31**	**1.20**	**1.15**	**1.26**	**1.44**	**1.14**	**1.82**
hsa-miR-374a-5p	**1.72**	**1.40**	**2.13**	1.03	0.99	1.07	1.03	1.00	1.06	1.05	0.99	1.11	1.09	0.89	1.34
hsa-miR-486-3p	**3.35**	**2.59**	**4.33**	**1.15**	**1.10**	**1.21**	**1.12**	**1.07**	**1.16**	**1.23**	**1.14**	**1.32**	**2.20**	**1.66**	**2.91**
Structure fires															
hsa-miR-1260a	**0.55**	**0.43**	**0.71**	0.96	0.93	1.00	0.94	0.83	1.05	0.94	0.85	1.04	1.00	0.81	1.25
hsa-miR-548h-5p	**0.59**	**0.51**	**0.69**	**1.05**	**1.02**	**1.08**	**1.17**	**1.08**	**1.28**	**1.13**	**1.05**	**1.22**	1.12	0.94	1.35
hsa-miR-145-5p	**0.44**	**0.32**	**0.61**	**0.96**	**0.93**	**0.98**	0.93	0.86	1.01	0.94	0.88	1.00	0.82	0.66	1.00
hsa-miR-4516	**0.56**	**0.48**	**0.65**	0.97	0.95	0.99	0.94	0.89	1.00	0.95	0.90	1.00	0.90	0.77	1.06
hsa-miR-331-3p	**0.60**	**0.52**	**0.70**	0.97	0.94	0.99	0.94	0.87	1.01	0.95	0.89	1.01	**0.83**	**0.70**	**0.99**
hsa-miR-181a-5p	**0.62**	**0.53**	**0.72**	0.99	0.97	1.02	1.01	0.94	1.09	1.00	0.94	1.07	**0.74**	**0.61**	**0.90**
has-miR-5010-3p	**1.59**	**1.41**	**1.81**	**1.13**	**1.10**	**1.16**	**1.35**	**1.24**	**1.48**	**1.30**	**1.20**	**1.39**	1.37	1.07	1.77
hsa-miR-374a-5p	**1.72**	**1.40**	**2.13**	1.03	0.99	1.07	1.07	0.97	1.20	1.07	0.97	1.16	1.10	0.89	1.35
hsa-miR-486-3p	**3.35**	**2.59**	**4.33**	**1.15**	**1.10**	**1.21**	**1.38**	**1.21**	**1.60**	**1.35**	**1.20**	**1.52**	**1.81**	**1.33**	**2.45**

Associations significant after Bonferroni correction are in bolded text.

FC fold-change, *CI* confidence interval.

[a]A priori markers were identified and originally presented in Jeong et al.[16].

[b]Models also adjusted for age, BMI, ethnicity, batch effects, and Bonferroni correction. For employment duration, effect is for a 6-month increase. For fire-hours, effect is for a 10-h increase. For fire-runs, effect is for a 10-fire increase.

[c]The best measure of time since most recent fire exposure (continuous log-days, split at median, split at tertiles, or split at quantiles) was selected for each miRNA by the highest Akaike information criteria (AIC) value. For time since most recent fire exposure, effect is the earliest exposure compared to later exposure(s). hsa-miR-548h-5p and hsa-miR-374a-5p used time split at the median. Hsa-miR-1260a, hsa-miR-145-5p, hsa-miR-4516, hsa-miR-331-3p, hsa-miR-181a-5p, hsa-miR-5010-5p, and hsa-miR-486-3p used time split at quantiles.

incidents, employment duration, fire-hours, and fire-runs were all significantly associated with differential expression of the same four a priori miRNAs (Table 2). These included one miRNA with decreased expression (miR-145-5p) and three with increased expression (miR-548h-5p, miR-5010-3p, and miR-486-3p). Days since most recent fire exposure was associated with three of the nine a priori miRNAs (miR-181a-5p, miR-5010-3p, and miR-486-3p). Levels of these three miRNAs comparing baseline and days since most recent fire categories are shown in Fig. 1.

Univariable analyses of a priori markers, structure fires only

In univariable models (adjusted for age, ethnicity, BMI, batch effects, and multiple comparisons) considering only structure fire incidents, structure fire-hours and structure fire-runs were associated with differential expression of three out of four of the same a priori miRNAs from the analysis of all fire incidents, with miR-145-5p losing statistical significance (Table 2). Days since most recent structure fire were associated with three a priori miRNAs.

Multivariable analyses of a priori markers, all fires

Many of the associations seen in our univariable a priori models lost significance in the multivariable models (Table 3). Employment duration was only significantly associated with miR-5010-3p in the fire-hours and fire-runs models, after adjustment for days since most recent fire exposure, age, BMI, and ethnicity. None of the nine a priori miRNAs were significantly associated with fire-hours or fire-runs after adjustment for Employment duration, days since most recent fire exposure, and age, BMI, and ethnicity. Days since most recent fire exposure was associated

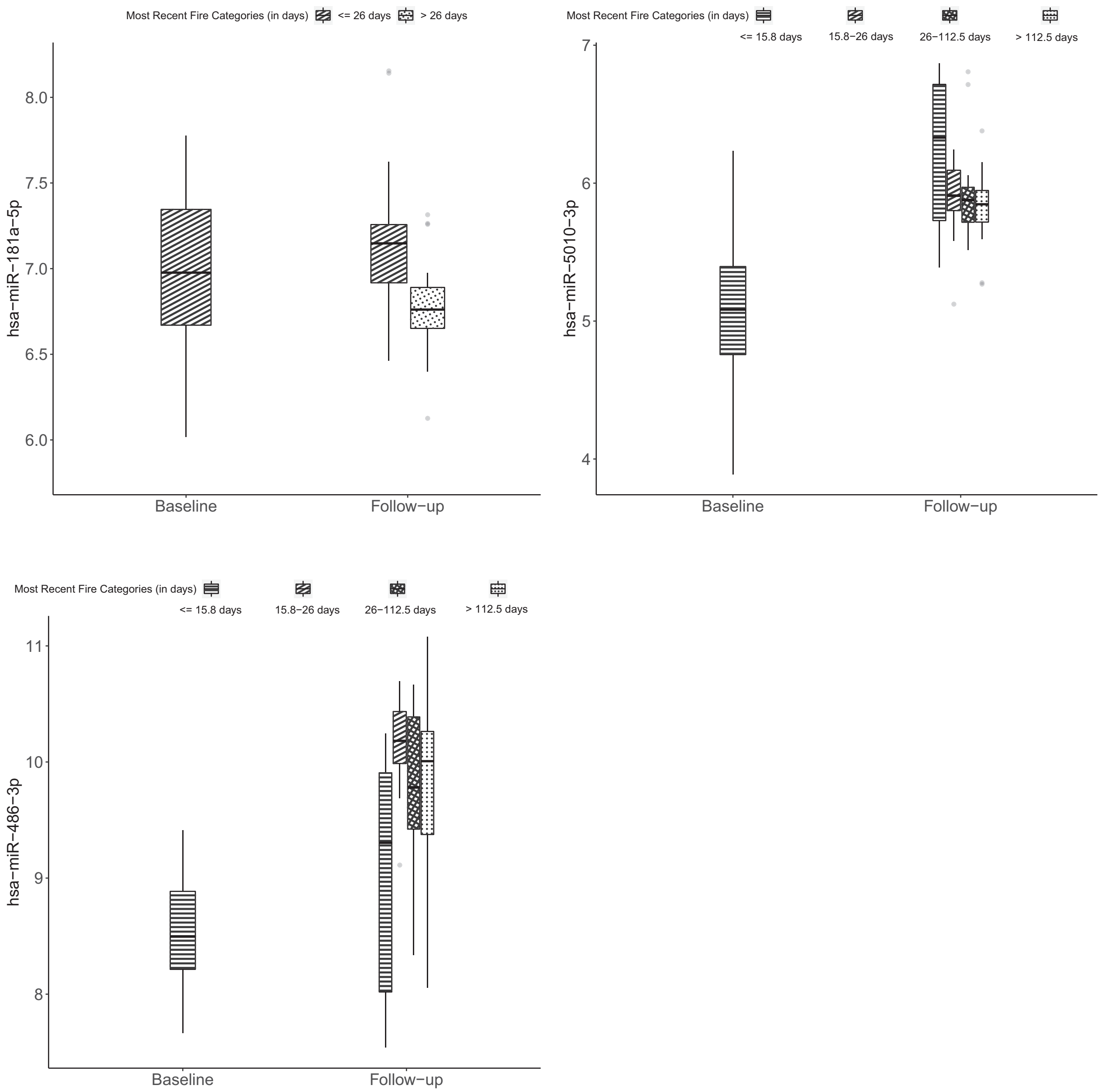

Fig. 1 Expression of microRNAs (miRNAs) among firefighters with fold-changes significantly associated with days between most recent fire and follow-up blood draw. MiRNA expression of miR-181a-5p, miR-5010-3p, and miR-486-3p was measured among 52 new recruit firefighters prior to live-fire training and again ~2 years later using an nCounter Human v3 miRNA expression panel. Within box-plots, the center horizontal line represents the median value and dots represent outliers.

with miR-181a-5p and miR-486-3p in the fire-hours and fire-runs models. R^2 values for the models varied from 0.056 to 0.472.

Multivariable analyses of a priori markers, structure fires only

Most of the associations seen in the univariable analyses restricted to structural fires lost significance in the multivariable models (Table 3). Employment duration in both structure fire-hours and structure fire-runs models was significantly associated with changes in two a priori miRNAs (miR-5010-3p and miR-486-3p). Additionally, employment duration in models adjusted for structure fire-runs (but not structure fire-hours) was found to be associated with expression of miR-1260a. Increasing employment duration was associated with increased expression of miR-5010-3p and miR-486-3p and decreased expression of

Table 3 Multivariable parameter estimates of differential miRNA expression for a priori markers: employment duration, fire exposures and most recent fire exposure[a,b].

miRNA	Model with fire-hours							Model with fire-runs						
	Employment duration		Fire-hours		Most recent fire		R^2	Employment duration		Fire-runs		Most recent fire		R^2
	β	*p* value	β	*p* value	β	*p* value		β	*p* value	β	*p* value	β	*p* value	
All fires														
hsa-miR-1260a	−0.082	0.342	0.055	0.673	−0.061	0.754	0.056	−0.137	0.117	0.085	0.268	−0.096	0.624	0.064
hsa-miR-548h-5p	−0.009	0.892	0.166	0.097	−0.266	0.130	0.182	0.051	0.443	0.037	0.525	−0.287	0.110	0.162
hsa-miR-145-5p	−0.076	0.179	0.041	0.633	−0.105	0.416	0.115	−0.055	0.340	0.003	0.947	−0.103	0.433	0.112
hsa-miR-4516	−0.045	0.312	0.008	0.902	0.014	0.892	0.072	−0.046	0.308	0.006	0.880	0.0116	0.909	0.072
hsa-miR-331-3p	−0.059	0.298	0.088	0.301	−0.210	0.201	0.152	−0.085	0.136	0.076	0.124	−0.235	0.145	0.163
hsa-miR-181a-5p	−0.022	0.673	0.085	0.284	**−0.398**	**0.001**	0.146	−0.005	0.921	0.032	0.483	**−0.406**	**0.001**	0.140
hsa-miR-5010-3p	**0.182**	**0.003**	0.007	0.935	−0.121	0.491	0.449	**0.212**	**<0.001**	−0.025	0.633	−0.115	0.510	0.450
hsa-miR-374a-5p	0.039	0.632	0.011	0.931	−0.011	0.953	0.046	−0.008	0.926	0.050	0.484	−0.034	0.855	0.049
hsa-miR-486-3p	0.080	0.360	−0.077	0.559	**1.015**	**<0.001**	0.463	0.145	0.098	−0.109	0.155	**1.048**	**<0.001**	0.472
Structure fires														
hsa-miR-1260a	−0.178	0.006	0.272	0.122	0.262	0.179	0.108	**−0.207**	**0.004**	0.299	0.078	0.271	0.163	0.113
hsa-miR-548h-5p	0.065	0.177	0.099	0.459	−0.154	0.304	0.181	0.100	0.071	−0.005	0.967	−0.131	0.380	0.174
hsa-miR-145-5p	−0.112	0.010	0.147	0.223	0.043	0.749	0.132	−0.125	0.012	0.159	0.172	0.049	0.713	0.134
hsa-miR-4516	−0.053	0.125	0.015	0.878	0.089	0.403	0.079	−0.061	0.113	0.035	0.701	0.088	0.403	0.080
hsa-miR-331-3p	−0.111	0.008	0.204	0.075	−0.059	0.641	0.140	−0.117	0.014	0.241	0.037	−0.195	0.202	0.158
hsa-miR-181a-5p	−0.020	0.640	0.244	0.027	**−0.666**	**<0.001**	0.188	−0.013	0.776	0.189	0.081	**−0.656**	**<0.001**	0.174
hsa-miR-5010-3p	**0.230**	**<0.001**	−0.052	0.659	−0.261	0.051	0.472	**0.247**	**<0.001**	−0.088	0.440	−0.248	0.061	0.473
hsa-miR-374a-5p	−0.033	0.581	0.192	0.250	0.014	0.941	0.088	−0.051	0.453	0.302	0.075	−0.114	0.610	0.112
hsa-miR-486-3p	**0.197**	**0.005**	−0.392	0.028	0.634	0.020	0.477	**0.254**	**<0.001**	**−0.504**	**0.004**	0.679	0.012	0.496

Models also adjusted for age, BMI, ethnicity, batch effects, and Bonferroni correction. Associations significant after Bonferroni correction are in bolded text.

A priori markers were originally identified in Jeong et al. [16].

[a]For employment duration, effect is for a 6 month increase. For fire-h, effect is for a 10 h increase. For fire-runs, effect is for a 10 fire increase.

[b]Based on the highest Akaike information criteria (AIC), the best measure of time since most recent fire was selected (continuous, split at median value, split at tertile values, or split at quartile values). Hsa-miR-1260a, hsa-miR-145-5p, hsa-miR-4516 and hsa-miR-5010-3p used time split at median value. Hsa-miR-548h-5p used time split at tertiles. Hsa-miR-331-3p, hsa-miR-181a-5p, hsa-miR-374a-5p and hsa-miR-486-3p used time split at quartiles.

miR-1260a. In contrast to analyses of all fires, structure fire-runs, but not structure fire-hours was significantly inversely associated with differential expression of miR-486-3p after adjustment for employment duration and time since most recent structure fire. Time since most recent structure fire was significantly associated with miR-181a-5p. R^2 values varied from 0.056 to 0.496.

Full miRNA array analyses

Restricted to structural fires, nine miRNAs from the full array with absolute fold changes >1.25 were associated with employment duration in multivariable analyses following full adjustment for either fire hours or fire runs, and days since most recent fire exposure, as well as age, BMI, and ethnicity (Table 4). These included eight miRNAs after adjusting for structure fire-hours and most recent structure fire and eight miRNAs after adjusting for structure fire-runs and most recent structure fire, with seven miRNAs in common. The expression of five miRNAs decreased and expression of four miRNAs increased longitudinally. Four of the five miRNAs with decreased expression have tumor suppressive roles in cancer and all miRNAs with increased expression have oncogenic roles in cancer. MiR-422a had decreased expression at follow-up and is reported to have a tumor suppressive role in colorectal cancer [34]. MiRNAs with increased expression (miR-525-3p, miR-548ad-3p and miR-548k) have reported oncogenic roles in hepatocellular, breast, and esophageal cancers, respectively [35–37]. Table 5 displays the $\log_2$FCs and p values for employment duration, time since most recent structure fire, and either fire-hours or fire-runs with miRNAs from the full array that

Table 4 Significant fold-changes of differential miRNA expression for full array markers: employment duration adjusted for chronic fireground exposure (structure fire-hours or structure fire-runs) and time since most recent structure fire[a,b,c].

miRNA	Employment duration			Employment duration			Select cancer association[c]	Proposed role	Reference
	Adjusted for structure fire-hours and most recent structure fire			Adjusted for structure fire-runs and most recent structure fire					
	FC	95% CI		FC	95% CI				
hsa-miR-494-3p	0.60	0.54	0.66	0.65	0.59	0.72	Prostate*	Oncogene	Cai and Peng [39]
hsa-miR-422a	0.74	0.70	0.78	0.77	0.73	0.82	CRC*	Tumor suppressor	Zheng et al. [34]
hsa-miR-26a-5p	0.76	0.70	0.83	0.76	0.69	0.83	HCC*	Tumor suppressor	Tan et al. [53]
hsa-miR-92a-3p	0.78	0.73	0.83	0.79	0.73	0.84	CRC	Tumor suppressor	Slattery et al. [40]
hsa-let-7f-5p	–	–	–	0.80	0.73	0.87	CRC*	Tumor suppressor	Ghanbari et al. [54]
hsa-miR-548a-3p	1.30	1.16	1.46	–	–	–	Prostate*	Oncogene	Nguyen et al. [55]
hsa-miR-556-3p	1.32	1.17	1.48	1.34	1.18	1.53	Osteosarcoma*	Oncogene	Xie et al. [41]
hsa-miR-548ad-3p	1.38	1.21	1.58	1.39	1.20	1.60	Breast	Oncogene	Sugita et al. [36]
hsa-miR-525-3p	1.43	1.29	1.60	1.44	1.28	1.63	HCC	Oncogene	Augello et al. [35]

FC fold-change, *CI* confidence interval, *CRC* colorectal cancer, *HCC* hepatocellular carcinoma.

[a]Effect for employment duration is an increase of 6 months. Models adjusted for employment duration, chronic fire exposure (fire-hours or fire-runs), time since most recent fire (see footnote a), age, BMI, ethnicity, Bonferroni correction, and batch effects.

[b]An absolute FC >1.25 was applied to statistically significant miRNAs associated with length of service presented here.

[c]Cancer association shown was selected based on the following criteria: (1) when possible reported association based on serum samples rather than tissue samples or cell assays, (2) reported association was validated in at least one other dataset, and (3) association reported in multiple types of samples (serum, tissue, cell lines, etc). Select cancer associations based on circulating samples are indicated with an asterisk (*).

Table 5 Parameter estimates of statistically significant miRNAs from full miRNA expression panel associated with employment duration and adjusted for structure fire-hours or structure fire-runs and time since most recent structure fire[a,b,c].

miRNA	Model with structure fire-hours							Model with structure fire-runs						
	Employment duration		Structure fire-hours		Most recent structure fire		R^2	Employment duration		Structure-fire-runs		Most recent structure fire		R^2
	β	*p* value	β	*p* value	β	*p* value		β	*p* value	β	*p* value	β	*p* value	
hsa-miR-494-3p	−0.735	<0.001	−0.392	0.025	−2.229	<0.001	0.778	−0.615	<0.001	−0.504	0.003	−2.247	<0.001	0.794
hsa-miR-422a	−0.439	<0.001	0.297	0.071	−1.321	<0.001	0.795	−0.370	<0.001	0.349	0.003	−1.346	<0.001	0.774
hsa-miR-26a-5p	−0.394	<0.001	0.272	0.120	0.403	<0.001	0.535	−0.403	<0.001	0.263	0.009	0.405	<0.001	0.756
hsa-miR-92a-3p	−0.366	<0.001	0.259	0.005	1.295	<0.001	0.618	−0.345	<0.001	−0.312	0.247	1.329	<0.001	0.611
hsa-let-7f-5p	–	–	–	–	–	–	–	−0.324	<0.001	0.243	0.007	0.388	0.008	0.443
hsa-miR-548a-3p	0.382	<0.001	0.259	0.103	0.398	0.017	0.480	–	–	–	–	–	–	–
hsa-miR-556-3p	0.396	<0.001	0.283	0.022	0.408	<0.001	0.407	0.424	<0.001	0.299	0.077	0.548	<0.001	0.408
hsa-miR-548ad-3p	0.469	<0.001	0.314	0.009	−1.960	0.008	0.426	0.471	<0.001	−0.403	0.002	−1.948	0.009	0.426
hsa-miR-525-3p	0.519	<0.001	−0.325	0.136	0.552	<0.001	0.568	0.529	<0.001	−0.338	0.118	−0.623	<0.001	0.570

Models also adjusted for age, BMI, ethnicity, batch effects, and Bonferroni correction. Associations significant after Bonferroni correction are in bolded text.

β Log_2FC, *FC* fold-change.

[a]For employment duration, effect is for a 6 month increase. For fire-hours, effect is for a 10 h increase. For fire-runs, effect is for a 10 fire increase.

[b]Based on the highest Akaike information criteria (AIC), the best measure of time since most recent fire was selected (continuous, split at median value, split at tertile values, or split at quartile values). Hsa-miR-26a-5p, hsa-miR-548a-3p, hsa-miR-556-3p and hsa-let-7f-5p used time split at the median. Hsa-miR-494-3p, hsa-miR-422a, hsa-miR-92a-3p and hsa-miR-548ad-3p used time split at quantiles. Hsa-miR-525-3p used time split at the median for the model adjusted for structure fire-hours and time split at tertiles for the model adjusted for structure fire-runs.

[c]An absolute FC >1.25 was applied to statistically significant miRNAs associated with length of service presented here.

were found to have be significantly associated with employment duration above the absolute FC of 1.25. We did not observe any significant associations between structure fire-hours or structure fire-runs and differential expression of miRNA, but changes in four miRNAs (three in the structure fire-hours models and three in the structure fire-runs models, with two overlapping) were associated with most recent structure fire exposure. In these adjusted models, R^2 values varied from 0.480 to 0.795.

The results of all other full miRNA analyses are described below. In our univariable models (adjusted for age, ethnicity, BMI, batch effects, and multiple comparisons) for all fires, employment duration, fire-hours and fire-runs were all significantly associated with over 50% of miRNAs in the full array, and time since most recent fire exposure was associated with over 20% of all miRNAs (results not shown). When we considered the full array of miRNAs in multivariable models for all fires, employment duration was associated with four miRNAs (miR-422a, miR-525-3p, miR-548ad-3p and miR-548k) with absolute fold changes >1.25 (Supplementary Table 1). In the multivariable models of all fires, no miRNAs were significantly associated with fire-hours or fire-runs. In our univariable models restricted to structure fires, employment duration, structure fire-hours, and structure fire-hours were each significantly associated with over 50% of all miRNAs in the full array and time since most recent structure fire exposure was associated with over 20% of all miRNAs (results not shown).

Discussion

The results of this study build on our previous comparison of incumbent and new recruit firefighters which identified nine whole blood miRNAs with at least 50% difference in expression between the two groups [16]. Using these nine miRNAs as a priori markers in our current analysis, we found three (miR-1260a, miR-5010-3p and miR-486-3p) in the multivariable models that were significantly associated with employment duration in the new recruits, all of which were in the same direction of effect as in the previous study, albeit of lesser magnitude. The consistency of these findings between the previous and current study suggests that these miRNAs may serve as biomarkers of cumulative effect in firefighters. In addition, nine novel miRNAs out of the full array panel with longitudinal fold changes of at least 25% were also found to be associated with employment duration in multivariable models restricted to structural fires, as well as four miRNAs with longitudinal fold changes of at least 25% associated with employment duration in multivariable models for all fires, with three of the miRNA present in both all fire and structural fire models. Given the overall pattern of reduction in tumor suppressor miRNAs and increase in oncogenic miRNAs, these findings also provide potential mechanisms linking firefighting to increased cancer risk. In relation to cumulative measures of fireground exposure, in the fully adjusted models restricted to structural fires one a priori miRNA was associated with fire-runs, suggesting a potential dose-response relationship with exposure to products of combustion. Furthermore, the statistically significant association between some miRNAs and most recent fire demonstrates the importance of considering both chronic and acute exposures.

A limited number of previously published studies have also evaluated epigenetic modifications among firefighters. In our two previous studies, we compared incumbent to new recruit firefighters for significant differences in miRNAs and DNA methylation using full array panels of markers [16, 18]. For miRNAs, we identified nine markers with a statistically significant >50% difference in expression in incumbent firefighters compared to new recruits. Results from an enrichment analysis of miRNA clusters also identified associations with stem cells, inflammation pathways, carcinomas, Burkitt's lymphoma, and melanoma, among others [16]. Our previous study of DNA methylation at cytosine-guanine dinucleotides (CpG) sites among firefighters identified four with a statistically significant >50% difference in methylation in incumbents compared to new recruits [18]. We were also able to demonstrate that genome-wide methylation could predict with accuracy incumbent and new recruit status as well as employment duration among incumbent firefighters. Another study examined DNA methylation among firefighters at four specific genes and observed decreased methylation at the promoter region of one gene, *DUSP22*, in firefighters compared to non-firefighters, correlated with employment duration but not age [17]. The authors also demonstrated through in vitro tests that decreased *DUSP22* promoter methylation was inducible by low-dose benzo[a]pyrene [17], one of a large number of PAHs formed as a combustion byproduct of organic materials. Certain PAHs, including benzo[a]pyrene, are known or probable carcinogens [38]. Based on these associations, the authors concluded that PAHs, a pervasive fire service exposure [4] may contribute to certain cancer risk through epigenetic mechanisms in firefighters due to chronic occupational exposure.

Surrogate fireground measures have been associated with cancer risk among firefighters in a pooled-cohort analysis of three metropolitan career fire departments in the US, including significantly increased lung cancer and mortality risk associated with fire-hours and similar but non-significant associations with fire-runs as well as a marginally significant association between leukemia mortality and fire-runs but not fire-hours [7]. In our multivariable models of a priori miRNAs, structure fire-runs, but not all fire-runs, were significantly associated with decreased expression of miR-486-3p. The direction of this association for structure fire-runs was unexpected as in this same model miR-486-3p increased significantly with employment duration and also non-significantly with most recent fire. This difference in outcomes between structure fire-runs and all fire-runs may be explained by higher exposures of certain combustion byproducts at structure fires compared to vehicle fires [3]. Previous epidemiologic studies of firefighter cancer that were able to assess for dose-response relationships have typically not differentiated between all fires and structure fires [7]. The biological consequences of the negative association of miR-486-3p with structure fires remains to be determined. Similar to some other miRNAs, miR-486-3p has been shown to manipulate several target genes, in this case acting as an oncogene for some cancers (colon, laryngeal squamous cell cancers) and a tumor suppressors for other cancers (oral squamous cell, lung, cervical cancers) [21, 31].

We identified nine novel miRNAs with at least a 25% fold longitudinal change associated with employment duration in our full array analyses restricted to structural fires. Four of the five miRNAs with decreased expression are reported as being tumor suppressors and the four with increased expression are reported as being oncogenic in various cancers, including colorectal, liver, prostate, breast, and bone cancers [35, 36, 39–41]. Of these, previous studies have found that colorectal and prostate cancers are elevated in firefighters [6, 8, 10, 11]. Given that both decreased expression of tumor suppressive and increased expression of oncogenic miRNAs have been associated with increased risk of cancer diagnosis and prognosis [42], our current findings could help explain the excess of certain cancers in firefighters.

In most of our multivariable analyses, we did not observe significant associations between surrogate measures of fireground exposure and dysregulated miRNA expression. Given the relatively small sample size of our current study, we likely lacked sufficient power to detect additional significant associations. However, the majority of firefighter responses are to medical aid calls [43], and other firefighter occupational factors, such as total call volume, stress, shiftwork, and firehouse exposures like diesel exhaust, might help explain dysregulated miRNA expression and should be evaluated in future analyses. Psychological stress can elicit alterations in human whole blood miRNA [44]. Other studies have observed that exposure to diesel exhaust is associated with epigenetic modifications, including dysregulated miRNA [17, 45]. Additionally, while miRNA expression has not been examined for potential associations with shiftwork to our knowledge, aberrant DNA methylation has been observed in long-term night shiftworkers [46]. Finally, we did not include training fire hours in the exposure metrics, as training fires burn different materials (wood, hay and natural gas) than with the exception of wood are present in fires in the community and training fire hours were evenly distributed across the study participants.

The current study findings are consistent with both chronic and acute contributions of firefighter exposures to longitudinal change in whole blood miRNA. Relative to our prior study comparing whole blood miRNA in incumbent firefighters with an average of 14.1 years of occupational exposure compared to new recruits [16], the fold changes over 20–37 months in some of the a priori miRNA markers of the current study were lesser in magnitude. This suggests that changes in at least some whole blood miRNA may reflect cumulative exposure over multiple years, and that larger and longer-term prospective cohort studies are needed to provide additional information on the slope of the dose-response curve over time. Furthermore, while our study found multiple miRNAs with significant associations with time since most recent fire, additional research is also needed to better understand the nature and timing of these relationships. We were able to find a limited number of other published studies on the relation of blood miRNA to acute exposures. A study of steel plant workers examined the effect of particulate matter on miRNA expression using peripheral blood leukocytes (a component of whole blood) and found that after three days of work, expression of two of three candidate miRNAs were significantly different and also correlated with blood oxidative stress or lead exposure [47]. A controlled study of asthmatics exposed to diesel exhaust collected whole blood samples from participants before and six hours after exposure. This study reported that expression of several miRNAs were associated with acute moderate-dose diesel exhaust exposure [45]. Additionally, some changes in miRNA appear to be reversible with cessation of exposure. A small study reported that while miRNA profile of smokers were significantly different than that of non-smokers, after ~1 month the miRNA profile of individuals who independently decided to quit smoking during the study period resembled that of non-smokers [48]. Another study found that out of 34 miRNA dysregulated by smoking in small airway epithelium, 22 returned to normal within three months of cessation of exposure [49]. In vitro studies have found increases in miRNA expression within hours of exposure [50]. These collective findings indicate the need to consider the contribution of both chronic and acute exposures to epigenetic changes.

To our knowledge, this is the first longitudinal analysis of epigenetic changes in firefighters and the first longitudinal assessment of surrogate fireground exposures associated with changes in miRNA expression. We were able to enroll a cohort of new-recruit firefighters with no previous occupational smoke exposure and collect baseline blood samples prior to live-fire training, allowing us to capture a more accurate assessment of miRNA expression prior to any fireground exposures for comparison to samples collected ~2 years later. Collection of fire response history for each study participant allowed us to examine potential dose-response relationships between surrogate fireground exposures and change in miRNA expression as well as evaluate and adjust chronic surrogate measures of fireground exposure (cumulative fire-hours and fire-runs) for potential confounding by acute fireground exposures (days between follow-up blood draw and most recent fire response).

Despite our study's strengths, there were limitations in regards to both exposure assessment and miRNA measurement. Though we were able to capture administrative information regarding type of fire and our study sample was limited to firefighters within the first two or three years of their career (excluding ranks or responsibilities that require tenure and additional training such as paramedics or engineers), we were unable to account for potential confounding by job assignment (e.g., interior or exterior role at fire). In regards to miRNA measurement, our samples collected at baseline and follow-up were analyzed in four separate batches. To address batch effects, we employed the newer RUV-III correction approach, rather than older methods of batch effect correction such as the ComBat function [51]. Use of methods such as ComBat to remove batch effects in such a situation could lead to biasing (usually deflating) group differences [52]. Finally, the study findings are from one fire department and additional studies in other geographic locations are needed to determine their generalizability.

In conclusion, consistent with previous research, our study provides further evidence that miRNAs may serve as biomarkers of cumulative exposure to firefighters, although the influence of acute exposures and the entirety of firefighter occupational exposures (shiftwork, stress, and other factors in addition to combustion byproducts) requires further investigation. Additionally, we observed significant dose-response relationships between employment duration and dysregulated expression of a priori miRNAs implicated in cancers and cancer pathways. Together, our study provides evidence that alterations of miRNA expression may serve as a mechanism linking firefighter exposures to increased cancer risk.

Acknowledgements We would like to thank the Tucson Fire Department, the Tucson Fire Fighters Association Local 479 of the International Association of Fire Fighters, and students and staff members from the University of Arizona who assisted with sample collection and processing throughout the duration of the study.

Funding US Federal Emergency Management Agency Assistance to Firefighters Grant program, EMW-2014-FP-00200.

Compliance with ethical standards

Conflict of interest The authors declare no competing interest.

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Journal of Exposure Science & Environmental Epidemiology (2021) 31:913–922
https://doi.org/10.1038/s41370-021-00311-x

ARTICLE

Evaluation of fireground exposures using urinary PAH metabolites

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Received: 6 July 2020 / Revised: 10 February 2021 / Accepted: 12 February 2021 / Published online: 2 March 2021

Abstract

Background Firefighters have increased cancer incidence and mortality rates compared to the general population, and are exposed to multiple products of combustion including known and suspected carcinogens.
Objective The study objective was to quantify fire response exposures by role and self-reported exposure risks.
Methods Urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons (PAH-OHs) were measured at baseline and 2–4 h after structural fires and post-fire surveys were collected.
Results Baseline urine samples were collected from 242 firefighters. Of these, 141 responded to at least one of 15 structural fires and provided a post-fire urine. Compared with baseline measurements, the mean fold change of post-fire urinary PAH-OHs increased similarly across roles, including captains (2.05 (95% CI 1.59–2.65)), engineers (2.10 (95% CI 1.47–3.05)), firefighters (2.83 (95% CI 2.14–3.71)), and paramedics (1.84 (95% CI 1.33–2.60)). Interior responses, smoke odor on skin, and lack of recent laundering or changing of hoods were significantly associated with increased post-fire urinary PAH-OHs.
Significance Ambient smoke from the fire represents an exposure hazard for all individuals on the fireground; engineers and paramedics in particular may not be aware of the extent of their exposure. Post-fire surveys identified specific risks associated with increased exposure.

Keywords Workplace exposures · Polycyclic aromatic hydrocarbons · Cancer · Dermal exposure · Inhalation exposure · Vulnerable occupations

Introduction

Firefighters in the United States have been shown to have a higher cancer incidence and mortality rate compared with the general population [1]. During fire suppression, firefighters are exposed to multiple chemicals, including but not limited to known and suspected carcinogens such as benzene, formaldehyde, and certain polycyclic aromatic hydrocarbons (PAHs) [2]. As a result, there is a strong desire in the fire service to better characterize and prevent workplace exposures with the objective of reducing cancer risk.

The largest cohort study of firefighters in the United States to date demonstrated an increased rate in lung cancer (10%), gastrointestinal cancer (30–45%), kidney cancer (29%), and mesothelioma (100%) deaths, with similar increases in cancer incidence, compared to the general population [1]. Further analyses demonstrated a significant association between fire hours and increased lung cancer incidence and mortality, along with fire runs and leukemia mortality [3]. In the Australian Firefighters' Health Study,

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41370-021-00311-x.

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career male firefighters had significantly elevated prostate cancer, melanoma, and kidney cancer incidence compared to the general Australian population, and a significant increase in lymphohematopoietic cancers associated with duration of service [4]. In this same study, prostate cancer and melanoma incidence were increased in part-time paid firefighters compared to the general population, and among male volunteer firefighters prostate cancer was increased compared to the general population and increased testicular cancer was associated with certain measures of increased exposure. Among female firefighters, there was an increase in colorectal cancer with increasing number of structural fire responses. A study of cancer among firefighters in five Nordic countries revealed a significant excess risk of prostate cancer and melanoma among those 30–49 years of age, as well as an increase in nonmelanoma skin cancer, multiple myeloma, lung adenocarcinoma, and mesothelioma among older firefighters [5]. A previous meta-analysis of 32 studies of cancer in the fire service identified an elevated risk for non-Hodgkin's lymphoma, prostate cancer, and testicular cancer [6]. These studies consistently found an association between firefighting and cancer, although the specific cancers with elevated rates varied by study.

Fire department policies have traditionally focused on reducing inhalation exposures. Although use of self-contained breathing apparatus (SCBA) greatly reduces the concentration of contaminants that a firefighter inhales, inhalation exposure to carcinogens continues to occur when firefighters are not wearing a SCBA. We previously demonstrated exposures to products of combustion, including carcinogens, during overhaul when historically SCBA were not worn [7, 8]. Furthermore, adverse respiratory effects during overhaul occurred even when air purifying respirators were used [7, 8]. Although use of a SCBA is recommended during overhaul, compliance is not universal, and exposure to smoke may occur during other phases of firefighting as well. In addition, firefighter gear off-gasses detectable levels of benzene, styrene, toluene, xylenes, and other volatile organic chemicals following fireground use [9, 10], potentially contributing to firefighters' inhalation exposure.

Dermal exposures have been thoroughly documented during firefighting. Wipe samples of skin surfaces collected before and after training fires when firefighters wore their SCBA for all phases including overhaul showed that the neck (protected primarily by Nomex hoods) was the most exposed part of the body [11], and later studies demonstrated high PAH concentrations on the hands of firefighters as well [12]. There is also concern that fireground contaminants can remain in unwashed gear, posing a continuing exposure hazard when reworn [13].

Measurement of urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons (PAH-OHs) concentrations has been used for the assessment of exposure to combustion products in previous studies of firefighters [14, 15]. PAH exposures have been linked to a number of cancers, including skin, lung, bladder, and gastrointestinal cancers [16–18]. Many PAH-related cancers have also been reported at excess rates in firefighters. Among PAHs, known, probable, and possible carcinogens include benzo[*a*]pyrene, dibenz[*a,h*]anthracene, chrysene, benzo[*a*]anthracene, and naphthalene [2, 19, 20]. The current study measured metabolites of naphthalene, fluorene, phenanthrene, and pyrene, the latter three of which are not classifiable as to their carcinogenicity in humans. However, they are measurable in urine after fire exposures and can serve as proxies for the larger mix of PAHs to which firefighters are exposed, including the known carcinogenic PAHs, as well as other products of combustion in smoke and soot. Given their ubiquitous presence in products of combustion, evaluation of PAH metabolites in urine provides a measure of combined inhalation and dermal exposure. As part of a cancer prevention study partnership between the University of Arizona and the Tucson Fire Department (TFD), we set out to evaluate exposure to combustion products through measurement of urinary PAH metabolites in firefighters following structural fires based on their roles in the fire and self-reported activities and exposures.

Materials and methods

Study setting

The study was approved by the University of Arizona Institutional Review Board, Protocol #1509137073, and all subjects provided informed consent. The study included collection of blood, buccal cells, and urine during annual medical surveillance examinations or during new recruit training for both incumbent and new recruit firefighters, and collection of urine after a structural fire. For subjects not able to provide a baseline urine during enrollment, it was collected after the post-fire urine sample. Inclusion criteria included being TFD uniformed personnel and responding to fires as part of their current duties. A survey evaluating firefighter demographics, medical and occupational history, and recent exposures, was collected at baseline. A survey evaluating actions at the fireground and recent exposures was collected at the fire scene during firefighter rehabilitation, and a second post-fire survey focusing on additional activities after the fire was completed after return to the station at the time of urine collection.

Urine collection and analysis

Baseline urine samples were collected throughout the day and transported on ice to University of Arizona laboratory the day of collection. Based on an unpublished pilot study by University of Arizona and TFD evaluating exposure to a training fire with firefighters wearing SCBA at all times within the structure, urinary naphthol metabolite concentrations were found to peak 2–4 h following cessation of exposure. For the current study, post-exposure urine samples were therefore collected 2–4 h post-fire by TFD personnel and transported on ice to University of Arizona laboratory within 24 h. Urine was collected in a 120 mL polypropylene collection cup after providing instructions to the firefighter to wash their hands first, void into the container, and return the resealed collection cup to a research team member for refrigeration until processing. A water control was collected and processed in the same manner as the urine collection for each day of baseline and post-fire collections.

Upon arrival in the laboratory, specific gravity was recorded for each sample using the Atago Refractometer (Model PAL-10S, Cat# 4410, Fisher Scientific). Urine samples were centrifuged at 1900 rpm for 10 min, then 10 mL aliquots of the supernatant were frozen at −20 °C until PAH-OH analysis as previously described [21]. This method was in turn based on a prior publication [22], with slight modifications including the use of urine centrifugation instead of filtration prior to deconjugation. In short, urine was digested with β-Glucuronidase from *Helix pomatia*, and extracted using solid phase extraction. Prior to analysis on the gas chromatography tandem mass spectrometry, samples were derivatized. A surrogate standard mix of the deuterated PAH-OHs containing 1-Hydroxynaphthalene-*d*7, 2-Hydroxyphenanthrene-*d*9, 2-Hydroxyfluorene-*d*9, 1-Hydroxypyrene-*d*9, was added to each sample prior to the extraction.

Detection limits were determined to be 175 ng/L, 100 ng/L, 150 ng/L, and 200 ng/L for each of the naphthols, fluorenols, phenanthrols, and 1-hydroxypyrene, respectively. PAH-OH values were multiplied by a specific gravity factor calculated for each urine sample to correct for renal function and individual hydration levels ($SGF = \frac{1.02-1}{SG-1}$). [23]

Statistical analysis

Non-detectable PAH-OHs were replaced by half the value of their respective detection limit. The PAH-OH concentrations were natural log-transformed to better fit the normal distribution. Univariate and multivariable analyses were performed using a linear mixed-effects model with random intercept to assess mean differences of log-transformed PAH-OHs between baseline and post-fire stratified by job types. The primary outcome was the sum of all PAH-OHs (naphthols, phenanthrols, fluorenols, and 1-hydroxypyrene), and secondary outcomes included the sum of naphthols (1-naphthol and 2-naphthol), sum of phenanthrols (2-phenanthrol, 4-phenanthrol, and 1-phenanthrol + 3-phenanthrol), sum of fluorenols (2-fluorenol, 3-fluorenol, and 9-fluorenol), along with the individual PAH-OHs. Assessment of model fit was performed by the analysis of residuals. All statistical analyses were performed using R version 3.5.3 (https://www.r-project.org) and Stata MP 14.1 (https://www.stata.com/stata14/). Longitudinal analyses were conducted by the R package lme4 [24] and the multilevel mixed-effects linear regression (xtmixed) function in Stata. A two-sided $p < 0.05$ was considered statistically significant.

Firefighters with baseline and post-fire exposure urine samples who took at least one post-fire survey were included in the survey analysis. Univariate regressions were performed to analyze the association between changes in PAH-OH concentrations from baseline to post-fire and each post-exposure survey question. Specifically, the random intercept model was used to control for the serial correlation of repeated intrasubject observations (e.g., multiple measurements of the same subject). The outcomes were the differences of log-transformed PAH-OHs comparing baseline and post-fire urine samples.

Results

Subject consenting and baseline urine collection started on 10/6/2015 and continued through 11/21/2017. Post-fire urine collection started on 2/9/2016 and continued through 12/19/2016. During this interval, 242 firefighters provided a baseline urine and 141 of these firefighters provided at least one post-fire urine. Some firefighters provided post-fire samples from more than one fire event (range 2–6 fires). Of the 141 subjects in the study that provided both a baseline and post-fire urine, 83 provided the baseline urine after the post-fire urine. With the exception of one subject for whom the baseline urine was collected 48 h after the post-fire urine, all other subjects had at least a 14-day interval between baseline and post-fire urines. The absolute value of the time span between the baseline and post-fire urine samples averaged 135 days, with a maximum of 543 days.

Characteristics of the participating firefighters are listed in Table 1. Most firefighters were male non-Hispanic whites and over half were less than 40 years of age. In both baseline and post-fire subject groups, 28–31% had a body mass index (BMI) in the obese range and 5–6% were either occasional or regular smokers. Because the firefighters measured at post-fire ($n = 141$) are a subsample of those recruited at baseline, a bootstrap method was used to evaluate whether there were significant differences between the two groups. Out of 100 bootstrap sample replicates with 141 individuals, more than 95% showed that there were no significant differences of gender, race/ethnicity, age, BMI,

Table 1 Study subjects.

Variable	Baseline *n* (%)	Post-fire *n* (%)
Gender		
Male	234 (96.7)	138 (97.9)
Female	8 (3.3)	3 (2.1)
Race/Ethnicity		
White non-Hispanic	206 (85.1)	115 (81.6)
Hispanic	30 (12.4)	22 (15.6)
African American	4 (1.7)	3 (2.1)
Missing	2 (0.8)	1 (0.7)
Age		
<30	33 (13.6)	26 (18.4)
30–40	89 (36.8)	52 (36.9)
≥40	120 (49.6)	62 (44.0)
BMI		
Normal (18.5–25)	31 (12.8)	17 (12.1)
Overweight (25–30)	143 (59.1)	81 (57.4)
Obese (>30)	68 (28.1)	43 (30.5)
Smoker		
No use	226 (93.4)	134 (95.0)
Occasional	9 (3.7)	5 (3.6)
Regular	6 (2.5)	2 (1.4)
Missing	1 (0.4)	0 (0.0)
Rank		
Captain	66 (26.4)	49 (26.2)
Engineer	42 (16.8)	31 (16.6)
Firefighter	84 (33.6)	74 (39.6)
Paramedic	54 (22.6)	30 (16.0)
Investigator	3 (1.2)	2 (1.1)
Missing		1 (0.5)

smoker, and rank distributions. When comparing smokers ($n = 15$, including nine occasional and six regular smokers) to nonsmokers ($n = 226$) at baseline, none of the quantified PAH-OHs were significantly different between the two groups (data not shown).

During the study period, 15 fires were studied (Supplemental Table 1). These fires were predominantly residential, including eleven house fires and one apartment fire. Additionally, three commercial fires were studied, including one church, one business, and one school fire. The duration for each fire, measured as the total time that firefighters were on the scene, ranged between 13 and 120 min. In this study, we used the terms offensive and defensive to refer to the overall fire attack strategy, whereas interior and exterior related to the location of individuals during a fire. Twelve of the fires were fought offensively (i.e. fire attack from inside the structure), two of them started as an offensive response and then switched to defensive (i.e. fire attack from the outside of the structure) and one was purely a defensive response. In the offensive fire attacks, firefighters as well as captains operated inside or outside of the burning structure, or both.

The concentrations of the sum of urinary PAH-OHs at baseline and post-fire are presented in Table 2 and Fig. 1, categorized by role at the fire. For each PAH-OH, the percent of urine samples with concentrations below the LOD for baseline and post-fire, respectively, varied as follows: 1-naphthol (29%, 3%), 2-naphthol (2%, 0%), 2-fluorenol (83%, 44%), 3-fluorenol (82%, 48%), 9-fluorenol (83%, 44%), 2-phenanthrol (81%, 38%), 4-phenanthrol (97%, 72%), 1-phenanthrol and 3-phenanthrol (52%, 14%) and 1-hydroxypyrene (84%, 51%). Urine specific gravity increased significantly from baseline to post-fire, with means (and 95% confidence intervals) of 1.016 (1.015–1.017) and 1.021 (1.020–1.022), respectively, indicating the firefighters were more dehydrated post-fire. While the statistical analysis was conducted on log-transformed data, the plots were created using the raw data for easier interpretation. All groups (firefighter, captain, engineer, paramedic), with the exception of fire investigators, had a significantly greater ($p < 0.05$) concentration of the sum of urinary PAH-OHs post-fire compared to baseline. The results of multivariable models adjusting for baseline age, BMI, and smoking yielded similar results (data not shown). All groups except the investigators also had significant increases in the sum of naphthols, sum of fluorenols, and sum of phenanthrols comparing baseline and post-fire. Results for individual PAH-OHs are included in the supplementary material (Supplemental Table 2). The sum of urinary PAH-OHs post-fire for each role at individual fires is presented in the supplementary material (Supplemental Fig. 1). Mean post-fire PAH-OH concentrations varied significantly for each fire ranging between 13.196 and 52.422 ng/L.

Evaluation of post-fire survey responses in relation to urinary PAH-OH concentrations is listed in Table 3 for the sum of all PAH-OHs, sum of naphthols, sum of fluorenols, sum of phenanthrols, and 1-hydroxypyrene. Results for individual PAH-OHs are provided in the supplementary material (Supplemental Table 3). Survey response variables associated with the sum of all PAH-OHs in post-fire urines included fire type (commercial vs. residential), interior vs. exterior fire response, duration of interior exposure, smoke odor on the skin, and not having laundered or changed one's hood within the last month. These same variables were also significantly associated with one or more other urinary PAH-OH markers (sum of naphthols, sum of fluorenols, sum of phenanthrols, and individual PAH-OHs). A number of other survey response variables were not significantly associated with sum of all PAH-OHs but were associated with one or more other PAH-OH markers. These included fire attack minutes, overhaul or salvage minutes, wearing one's SCBA for over 60% of the time during fire attack or ventilation, and having a dirty hood before the response. Finally, neither total duration of the fire response

Table 2 Urinary PAH-OHs (ng/L) at baseline and post-fire by role in fire responses.

	FC (95% CI)	Baseline *n*	Post-fire *n*	Baseline median (IQR)	Post-fire median (IQR)
Sum of PAH-OHs[a]					
Captain	**2.06 (1.61–2.64)**[‡]	66	49	6855 (4216, 11,701)	12,170 (7640, 29,845)
Engineer	**2.08 (1.46–3.02)**[‡]	39	31	7858 (5402, 11,245)	16,305 (9330, 25,528)
Firefighter	**2.80 (2.13–3.66)**[‡]	82	74	7380 (4441, 13,744)	21,343 (11,392, 36,415)
Paramedic	**1.84 (1.33–2.59)**[‡]	52	30	8434 (6156, 15,039)	16,935 (9832, 29,200)
Investigator	1.64 (1.26–2.13)	3	2	4875, 5128, 6935[b]	9595, 11,360[b]
Sum of naphthols					
Captain	**2.03 (1.53–2.71)**[‡]	66	49	6254 (3528, 10,332)	10,265 (6020, 24,205)
Engineer	**2.05 (1.43–3.01)**[‡]	39	31	6200 (4436, 9950)	14,800 (7828, 21,640)
Firefighter	**2.91 (2.14–3.94)**[‡]	82	74	5949 (3636, 12,588)	18,963 (9581, 34,916)
Paramedic	**2.00 (1.32–3.10)**[†]	52	30	7959 (4945, 11,223)	13,795 (8435, 25,325)
Investigator	1.70 (1.19–4.08)	3	2	2505, 4088, 5945[b]	8530, 10,720[b]
Sum of fluorenols					
Captain	**1.88 (1.44–2.44)**[‡]	66	49	<LOD (<LOD, <LOD)	315 (<LOD, 895)
Engineer	**2.44 (1.59–3.73)**[‡]	39	31	<LOD (<LOD, 190)	495 (<LOD, 858)
Firefighter	**3.69 (2.86–4.77)**[‡]	82	74	<LOD (<LOD, 284)	760 (401, 1510)
Paramedic	**2.49 (1.66–3.76)**[‡]	52	30	<LOD (<LOD, 419)	798 (346, 1469)
Investigator	1.14 (0.29–3.89)	3	2	<LOD, <LOD, 575[b]	<LOD, 400[b]
Sum of phenanthrols					
Captain	**2.44 (1.83–3.24)**[‡]	66	49	<LOD (<LOD, 570)	890 (455, 1610)
Engineer	**2.60 (1.71–3.94)**[‡]	39	31	385 (<LOD, 662)	1055 (548, 1720)
Firefighter	**3.39 (2.62–4.39)**[‡]	82	73	378 (<LOD, 662)	1180 (805, 2498)
Paramedic	**2.96 (2.13–4.14)**[‡]	52	30	<LOD (<LOD, 665)	880 (730, 2390)
Investigator	1.93 (0.35–3.54)	3	2	225, 790, 1470[b]	390, 565[b]
1-Hydroxypyrene					
Captain	**1.83 (1.33–2.49)**[‡]	66	49	<LOD (<LOD, <LOD)	<LOD (<LOD, 685)
Engineer	1.58 (1.00–2.51)	39	31	<LOD (<LOD, <LOD)	<LOD (<LOD, 551)
Firefighter	**2.04 (1.54–2.70)**[‡]	82	73	<LOD (<LOD, <LOD)	243 (<LOD, 882)
Paramedic	**2.34 (1.57–3.53)**[‡]	52	30	<LOD (<LOD, <LOD)	<LOD (<LOD, 811)
Investigator	–	3	2	<LOD, 325, 615[b]	<LOD, <LOD

FC fold change, *CI* confidence interval.

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

[a]Includes all naphthols, phenanthrols, fluorenols and 1-hxdroxypyrene.

[b]Actual values.

Bolded values are statistically significant.

nor having dirty turnout gear before the response were significantly associated with any of the PAH-OH markers.

Discussion

Our study demonstrated significant increases in the sum of all urinary PAH-OHs following fireground operations in four main groups: firefighters, captains, engineers, and paramedics. These results also highlight the understudied exposure of engineers and paramedics to combustion emissions while providing nonentry support at fire incidents. Though fire investigators were not found to have a significant increase, only two post-fire urine samples were available for analysis.

The baseline urinary PAH-OH concentrations found in our study are comparable to those of the general population whereas the post-exposure values are less than those seen in the most highly exposed workers. Compared to urinary PAH-OH levels of participants 18–65 years of age in the 2015–2016 National Health and Nutrition Examination Survey (NHANES) multiplied by 1.48 to transform their creatinine corrected values to our specific gravity corrected values [25], the median values for the sum of naphthols in our study (Table 2) were slightly lower than in NHANES (median 12,530 ng/L, IQR 6451–23,918 ng/L). While the

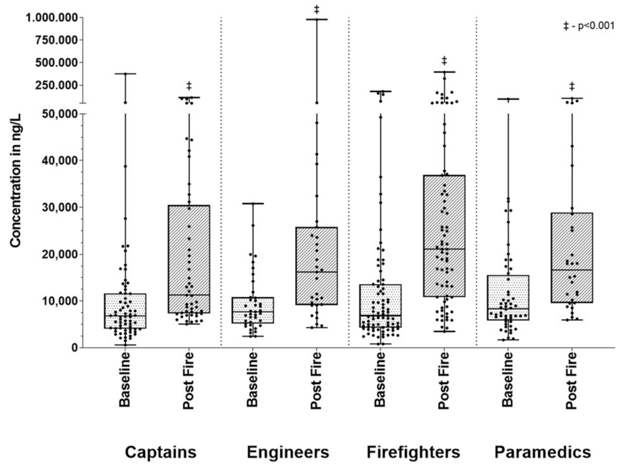

Fig. 1 Baseline and post-fire sum of PAH-OHs (including 1-OH-Pyrene) by role in the fire. ‡ - $p < 0.001$.

sum of phenanthrols were very similar in both studies (NHANES median 357 ng/L, IQR 233–572 ng/L), the median concentrations for the sum of fluorenols and 1-hydroxypyrene were below the detection limit in our study and 357 and 193 ng/L, respectively in the NHANES study. For the sum of all PAH-OHs, the median values and IQR in our study were slightly lower than NHANES (median 13,535 ng/L, IQR 7438–26,106 ng/L), but the analytical method used in our study differed from NHANES and contained two compounds, 9-Fluorenol and 4-Phenanthrol, that were not included in NHANES. The mean post-fire urinary PAH-OHs in our study were at lower concentrations than those reported for coke over workers (2-naphthol 100,000–150,000 ng/L after transformation of the data using a factor of 1.48 to convert creatinine normalized result to specific gravity normalized results as described above) [26]. It should also be noted that coke oven workers are exposed to PAHs for longer durations than firefighters.

Our study results are consistent with other recent studies of firefighter entry teams [14, 15]. Ottawa firefighters responding to fires in the community showed urinary PAH-OH increases from baseline to post-fire of 2.9–5.3 fold depending on the PAH-OH group [14]. This was of similar magnitude to the firefighters in our study, increasing between 3.1 and 5.1 fold for the sum of naphthols, sum of fluorenols and sum of phenanthrols. Although the Ottawa study did not break out the study results by fire response roles, our study found lesser fold increases in the other fire response roles (captains, engineers, paramedics, and investigators) than in firefighters. Differences in study methods included collection of urine over an 18-h period post-fire in the Ottawa study, as compared with our 2–4-h post-fire sampling period. A study of controlled residential fires measured urinary PAH metabolites at 3-h post-fire, similar to our study, although they used a standardized house fire model which was much more consistent both in materials burned and size of the structure across fires than the community responses measured in our study [15]. Nevertheless, their fold increases of 2.4–6.6 based on PAH metabolite group were similar to the 3.1–5.1 fold increased seen in ours. Interestingly their smallest fold increase was for fluorenols, while this group showed the largest fold increase in our study, which could potentially be due to differences in the relative amounts of materials being burned and/or fire and smoke conditions. The controlled residential fire study also found increased urinary PAH-OHs in interior as compared to transitional fire attack (although statistically significant only for the fluorenols), generally consistent with our findings based on self-reported interior v. exterior fire response activities with statistically significant increases for all PAH-OH groups except 1-hydroxypyrene.

The marked variability in post-fire urinary PAH-OH concentrations observed in our study is likely due to differences in exposures based on distinct job tasks within roles at a fire, the complex and evolving nature of each individual fire and differences in use of respiratory protection. Entry/fire attack or ventilation teams are made up of two firefighters and a captain serving as team lead. At times the captain sets up the fireground/tactical operations as the firefighters make the initial entry before the captain joins the firefighters inside the burning structure or on the roof. In addition, we have identified instances where captains removed their respirators to facilitate radio communication while outside of the burning structure but still in a smoky area [27]. This increased exposure may explain why captains had the greatest exposure in 6 of the 15 fires evaluated, as shown in Fig. 1. Other captains may also have roles that require them to stay exterior to the fire. The engineers (also known as driver-operators) work the vehicle pump panel and carry out other outside support activities. The paramedics do not engage directly in firefighting activities but work outside the immediate vicinity of the fire to set up a rehabilitation station for the other fireground personnel. In fires where an engineer or a paramedic had the highest exposure, it is likely that the smoke plume moved over their location after their initial set-up. Although the number of fire cause investigators in the study was limited, they were the group most likely to have measureable 1-hydroxypyrene in their baseline urine samples, and we were not able to exclude the possibility that these levels were from prior fire responses, given the relatively longer elimination half-live of pyrene metabolites compared to the other PAH-OH measured. From an inhalation perspective, the use of respiratory protection while outside of a burning structure varied greatly among study participants. While positive-pressure SCBAs should provide adequate protection against inhalation exposures [28, 29], reduced use of SCBA is common during nonentry fireground activities, particularly

Table 3 Multilevel mixed-effects linear regression modeling of urinary PAH-OH fold changes post-fire based on survey responses.

		Sum of PAH-OHs[a]	Sum of naphthols	Sum of phenanthrols	Sum of fluorenols	1-Hydroxypyrene
Variable	*n* (total)	FC (95% CI)	FC (95% CI)	FC (95% CI)	FC (95% CI)	FC (95% CI)
Fire and response type						
Commercial fire (Ref: Residential)	19 (180)	**0.646 (0.421, 0.992)***	**0.569 (0.356, 0.909)***	0.836 (0.513, 1.363)	0.949 (0.568, 1.584)	1.142 (0.645, 2.020)
Interior response (Ref: Exterior response)	71 (178)	**1.469 (1.068, 2.021)***	**1.509 (1.058, 2.153)***	**1.481 (1.053, 2.083)***	**1.631 (1.156, 2.302)**†	1.216 (0.820, 1.802)
Duration (minutes)						
Total duration of fire response	180 (180)	1.002 (0.999, 1.006)	1.002 (0.999, 1.006)	1.003 (0.999, 1.006)	0.999 (0.995, 1.003)	1.001 (0.997, 1.006)
Interior response	105 (105)	**1.010 (1.002, 1.019)***	**1.011 (1.002, 1.020)***	**1.014 (1.006, 1.023)**†	**1.010 (1.001, 1.019)***	1.005 (0.994, 1.017)
Fire attack	88 (88)	1.004 (0.995, 1.014)	1.004 (0.994, 1.015)	**1.011 (1.000, 1.022)***	1.010 (0.999, 1.020)	1.007 (0.995, 1.019)
Overhaul/Salvage	56 (56)	0.999 (0.992, 1.006)	0.999 (0.991, 1.006)	1.000 (0.994, 1.006)	0.998 (0.992, 1.004)	**1.008 (1.001, 1.015)***
Percent time on air						
Fire attack 61–100% (Ref: 0–60%)	81 (88)	0.527 (0.217, 1.280)	0.393 (0.151, 1.020)	1.122 (0.433, 2.907)	1.030 (0.400, 2.656)	0.851 (0.329, 2.203)
Overhaul/Salvage 61–100% (Ref: 0–60%)	48 (56)	0.949 (0.400, 2.253)	1.004 (0.375, 2.684)	1.001 (0.481, 2.082)	1.094 (0.493, 2.430)	0.821 (0.336, 2.004)
Ventilation 61–100% (Ref: 0–60%)	21 (23)	0.668 (0.254, 1.756)	0.730 (0.266, 2.002)	0.250 (0.052, 1.202)	1.163 (0.352, 3.839)	1.561 (0.193, 12.64)
Rehab 61–100% (Ref: 0–60%)	3 (25)	1.792 (0.695, 4.620)	1.709 (0.572, 5.105)	1.454 (0.361, 5.859)	0.865 (0.269, 2.779)	0.766 (0.141, 4.166)
Odor, soot, and gear status						
Smoke odor on skin (Ref: No)	43 (180)	**1.461 (1.032, 2.066)***	1.442 (0.980, 2.123)	1.299 (0.885, 1.906)	1.060 (0.712, 1.577)	1.026 (0.659, 1.597)
Black mucus in nose, mouth or throat (Ref: No)	20 (46)	1.010 (0.471, 2.168)	0.982 (0.433, 2.228)	1.420 (0.920, 2.192)	1.135 (0.501, 2.568)	0.614 (0.290, 1.298)
Washed with water or wipe on scene (Ref: No)	30 (46)	**2.095 (1.021, 4.299)***	2.226 (0.998, 4.963)	**2.073 (1.883, 2.283)**†	2.227 (0.965, 5.141)	0.639 (0.288, 1.419)
Turnout gear dirty (soot) before response (Ref: No)	83 (180)	1.013 (0.732, 1.403)	1.073 (0.748, 1.542)	0.781 (0.554, 1.100)	0.948 (0.667, 1.348)	1.025 (0.693, 1.516)
Hood dirty (soot) before response (Ref: No)	27 (171)	1.437 (0.903, 2.285)	1.357 (0.808, 2.279)	1.416 (0.870, 2.306)	**1.688 (1.040, 2.738)***	1.546 (0.892, 2.677)
Laundered turnout gear >1 month (Ref: <1 mo)	117 (180)	1.172 (0.863, 1.592)	1.238 (0.882, 1.738)	0.966 (0.690, 1.352)	0.977 (0.690, 1.384)	0.771 (0.525, 1.131)
Laundered/changed hood >1 month (Ref: <1 mo)	92 (180)	**1.468 (1.108, 1.945)**†	**1.581 (1.160, 2.155)**†	1.234 (0.898, 1.697)	1.067 (0.767, 1.486)	0.774 (0.536, 1.119)

FC fold change, *CI* confidence interval.

*$p < 0.05$; †$p < 0.01$.

[a]Includes all naphthols, phenanthrols, fluorenols, and 1-hxdroxypyrene.

Bolded values are statistically significant.

during overhaul and activities where the equipment might interfere with mobility and field visibility [30, 31]. Dermal exposure is known to be an important source of PAH absorption as well, as PAH metabolites have been detected in urine of firefighters using SCBA during fire suppression events [11]. The firefighter's role during the fire event is linked to PAH dermal concentration, with tasks such as fire attack and search correlating to higher contamination on the skin [12, 14]. Both inhalation and dermal exposures need to be considered when planning exposure reduction interventions.

The survey data revealed information which could be used to inform a firefighter job exposure matrix. Response to residential fires had increased exposures compared to commercial fires, although there was a great deal of variability within each group and the number of commercial fires was limited to three. A study of controlled experimental fires found a significant increase in median concentration of urinary PAH-OH metabolites from pre- to post- exposure on firefighters assigned to attack and search roles [15]. In our study, interior response was associated with a substantial increase in concentration of urinary PAH-OHs. This was also supported by the significant association of the number of minutes of interior response with the increase in concentration of urinary PAH-OHs from baseline to post-fire. Contrary to our expectations, overall duration (minutes) of fire response, and minutes of overhaul/salvage were not associated with increase in urinary PAH-OHs. These combined study findings suggest that more detailed exposure records are needed for epidemiologic studies of firefighters, and that the use of cumulative fire hours or fire runs as proxy measures of exposure may need to be refined to include information on interior and exterior responses.

The use of PPE while conducting certain duties was assessed using the surveys to identify its contribution to decreasing exposure. A significant decrease in urinary 2-naphthol and 1- and 3- phenanthrol were observed in individuals who had their SCBA on greater than 60 percent of the time during fire attack and ventilation, respectively, in comparison to those individuals reporting SCBA use 60 percent or less of the time. These results are consistent with other research findings that increased use of SCBA while at a fire scene can decrease exposure to products of combustion [11, 28]. Our ability to evaluate the effectiveness of SCBA use during incident command, pump operation, rapid intervention crew, or emergency medical services was limited due to the small number of individuals wearing their SCBA during these activities.

The survey data also identified that some self-reported exposures were associated with increased urinary PAH-OHs, including smoke odor on the skin. While these results are not surprising, we are not aware that they have been previously studied. A finding contrary to our expectations was that cleaning of the skin with water or a wipe while still on scene was associated with a significant increase in the sum of PAH-OHs. This finding could potentially be explained by a greater use of wipes when exposures had been higher, such as having visible soot on the skin. Dermal decontamination with wipes has been shown to reduce the amount of skin contamination [12, 21], and we previously found that 'wash-down' of turnout gear prior to doffing after a fire response, in combination with other fireground interventions, was associated with a 36% reduction in post-fire urinary PAH-OHs [27]. The current practice among many fire departments of hood exchange after a fire is validated by the finding within our study that a longer time interval without cleaning is associated with increased urinary PAH-OHs. In addition, routinely laundered hoods were previously found to have an 81 percent average lower concentration of PAHs compared to unlaundered hoods [32]. There was no significant increase in urinary PAH-OHs associated with wearing turnout gear that had not been recently laundered, although cleaning of turnout gear after each response is considered a best practice [13].

The results of this study affirm the need for fireground exposure reduction interventions for firefighters. Previous studies have investigated various post-fire interventions conducted at the fire scene to reduce dermal and inhalation exposure. Interventions include decontamination of PPE with soap and water along with bagging of gear to reduce exposure from off gassing contaminants, along with cleaning of skin as soon as possible with wipes to reduce dermal absorption [9, 12, 21, 33]. Resources already available to departments recommend the use of these and additional interventions, such as showering and changing of clothes as soon as possible after a fire, having two sets of turnout gear, and diesel capture and removal systems, along with strategies on how to best communicate these interventions for the greatest chance of implementation [13, 34]. The results of this current fireground exposure study were used by the TFD to plan specific exposure reduction interventions, the results of which were previously reported [27].

Limitations of our study include exposure monitoring limited to PAH metabolites, as many other toxic chemicals are present in fire smoke. While this limited scope of exposure monitoring does not affect the study findings, the differential exposures identified may not be generalizable to chemical exposures beyond PAHs. The timing of urine collection at 2–4 h post-exposure was chosen both to maximize the urinary concentrations of 1- and 2- naphthol, as the combined naphthols had the highest concentration of the measured PAH metabolite groups, and because collection at this time period was acceptable by the fire service. However, measurement at this time period likely underestimates post-fire concentrations of PAH-OHs with a longer elimination half-life. The baseline urine was not collected immediately before the fireground response as this was not possible given the unpredictable timing of the fires and the rapid firefighter response to the fires. The urinary PAH-OH concentrations may have been influenced by exposures outside of the fireground, as we did not have any restriction on diet or smoking, both of which can contribute PAH exposures. Beyond smoke from fires, diesel exhaust is also a source of PAH exposure [35], which is associated with acute inflammatory effects [36] and lung and esophageal cancer [37]. Diesel exhaust continues to be an inhalation hazard for firefighters at incident scenes, and also in fire stations, where emissions from the truck bay may infiltrate the living quarters through open doors, cracks in the building, and due to poor ventilation and differences in air pressure between the bays and the living areas [38–40]. We were not able to differentiate between fire smoke and diesel exhaust exposure at the fire scene. Finally, the study was limited to exposure monitoring and the toxicity of the combined exposures was not evaluated.

In conclusion, our study results showed that all fire service personnel at a fire scene are at risk for exposure to products of combustion. Characteristics of the fire, firefighter activities at the scene and self-reported exposures were all significantly associated with urinary sum of all PAH-OHs measurements. Specifically, residential fires, interior responses including duration of interior response, smoke odor on skin, and lack of recent laundering or changing of hoods were significantly associated with increased post-fire urinary sum of PAH-OHs. Fire departments should continue to implement measures to reduce dermal and respiratory exposures.

Funding This study was funded by the Federal Emergency Management Agency (FEMA), grant number EMW-2014-FP-00200. We would like to thank the Tucson Fire Department, the Tucson Fire Fighters Association Local 479 of the International Association of Fire Fighters, and students and staff members from the University of Arizona who assisted with sample collection and processing throughout the duration of the study.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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International Journal of Hygiene and Environmental Health xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Firefighters' and instructors' absorption of PAHs and benzene during training exercises

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ARTICLE INFO

Keywords:
Breath
Urine
Biomarker
Occupational exposure
Fire
Combustion products

ABSTRACT

Introduction: Training fires may constitute a major portion of some firefighters' occupational exposures to smoke. However, the magnitude and composition of those exposures are not well understood and may vary by the type of training scenario and fuels.

Objectives: To understand how structure fire training contributes to firefighters' and instructors' select chemical exposures, we conducted biological monitoring during exercises involving combustion of pallet and straw and oriented strand board (OSB) or the use of simulated smoke.

Methods: Urine was analyzed for metabolites of polycyclic aromatic hydrocarbons (PAHs) and breath was analyzed for volatile organic compounds (VOCs) including benzene.

Results: Median concentrations of nearly all PAH metabolites in urine increased from pre-to 3-hr post-training for each scenario and were highest for OSB, followed by pallet and straw, and then simulated smoke. For instructors who supervised three trainings per day, median concentrations increased at each collection. A single day of OSB exercises led to a 30-fold increase in 1-hydroxypyrene for instructors, culminating in a median end-of-shift concentration 3.5-fold greater than median levels measured from firefighters in a previous controlled-residential fire study. Breath concentrations of benzene increased 2 to 7-fold immediately after the training exercises (with the exception of simulated smoke training). Exposures were highest for the OSB scenario and instructors accumulated PAHs with repeated daily exercises.

Conclusions: Dermal absorption likely contributed to the biological levels as the respiratory route was well protected. Training academies should consider exposure risks as well as instructional objectives when selecting training exercises.

1. Introduction

Studies suggest that firefighters have increased risk for numerous types of cancer (Daniels et al., 2014, 2015; Glass et al., 2014; Pukkala et al., 2009; Tsai et al., 2015) and the International Agency for Research on Cancer (IARC) classified occupational exposure as a firefighter to be possibly carcinogenic to humans (Group 2B) (IARC, 2010). Firefighters' exposure to chemical carcinogens during emergency fire responses may contribute to this increased risk (Daniels et al., 2015). Firefighters could also be exposed to chemical carcinogens during training fires. A recent study found a dose-response relationship between estimated exposures from training fires and cancer incidence at a fire training college in Australia (Glass et al., 2016). The high exposure group at the fire training college had increased risk of all cancers, testicular cancer, and

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https://doi.org/10.1016/j.ijheh.2019.06.006
Received 1 April 2019; Received in revised form 4 June 2019; Accepted 19 June 2019

melanoma compared to the general population.

Training fires may constitute a major portion of some firefighters' occupational exposures to smoke. Many fire departments require routine live-fire training for their firefighters to maintain and build proficiencies and certifications. Training institutes often utilize firefighters and officers from surrounding communities, or employ dedicated personnel, to serve as instructors. Instructors often oversee 3–5 live instructional fires per day over a combined period of several weeks or even months. This could add up to as many or more live-fire exposures (albeit in a controlled setting) than what firefighters in busy fire departments experience.

Fuels used for fire training vary, but typically follow recommendations from the National Fire Protection Association (NFPA) standard NFPA 1403 *Standard on Live Fire Training Evolutions* in an attempt to control the risk involved with this type of training (NFPA, 2018). Such training scenarios will often include Class A materials such as pallet and straw, which tend to produce light grey smoke for obscuring visibility, as well as elevated temperatures. In recent years, many training institutes have also begun to use engineered wood products, such as oriented strand board (OSB) in addition to the pallet and straw to generate products of combustion that more closely replicate those encountered in residential structure fires (e.g., flames "rolling" across the ceiling, darker smoke and higher temperatures) (Horn et al., 2011). Some fire training institutes have begun using simulation technologies to produce training environments with no live fire. These systems typically use theatrical smoke or pepper fog for visual obscuration; they may also incorporate propane burners or an electronic display of fire glow. While simulated smoke exercises are assumed to be less hazardous than live-fire training, chemical hazards like insoluble aerosols and formaldehyde have been measured at concentrations above or just below occupational exposure limits during these exercises (NIOSH, 2013). The relative risk of these varying approaches has not been studied in an integrated manner to allow direct comparison between fire training environments.

A relatively small number of studies have investigated firefighters' exposures during various types of live-fire training exercises, including those that used firewood, particle chipboard, plywood, OSB, diesel fuel, and heating oil as fuel sources (Feunekes et al., 1997; Kirk and Logan, 2015; Laitinen et al., 2010; Moen and Ovrebo, 1997; Stec et al., 2018). These studies generally show that firefighters can be exposed to single-ring and polycyclic aromatic hydrocarbons (PAHs) during training fires, leading to contamination of protective clothing and skin, as well as potential for biological uptake of benzene and pyrene. However, the accumulation of toxicants from repeated training exercises, especially among instructors, has not been fully characterized.

In a recent companion paper (Fent et al., In Press-a), we reported airborne contamination levels measured during firefighting exercises that used pallet and straw alone or in concert with OSB as fuel for the fires or used simulated smoke. Generally, the magnitude of contaminants measured in air were highest for the OSB exercises, followed by pallet and straw and then simulated smoke exercises. Although the participants wore self-contained breathing apparatus (SCBA) prior to entering the structure, as is typically the case for firefighters, some biological absorption could still take place via inhalation before donning respirators while outside of the structure. Dermal absorption may also be responsible for the biological absorption of toxicants. A number of firefighter exposure studies have documented absorption of toxicants despite the consistent use of SCBA, suggesting that the dermal route contributes substantially to the dose (Fent et al., 2014; Fent et al., In Press-b; Keir et al., 2017).

Building on our previous work evaluating airborne contamination (Fent et al., In Press-a), we assessed both firefighters' and instructors' exposures to PAHs and volatile organic compounds (VOCs) by collecting biological specimens over a five-day period of training exercises involving a) pallet and straw, b) OSB, and c) simulated smoke. This study design provides the opportunity to investigate the biological accumulation of hazardous substances in instructors over a typical workday involving routine training exercises with broad applicability in the U.S. fire service and abroad. By following the same methodology as in the previous controlled residential fires project (Fent et al., 2018; Fent et al., In Press-b), we are also able to compare findings between exercises involving training fuels and those involving furnishings typical of a residential home.

2. Methods

2.1. Participants

This study was approved by the Institutional Review Boards at the University of Illinois and the National Institute for Occupational Safety and Health (NIOSH). All participants were required to be active members of a fire department and/or fire training organization and have completed a medical evaluation consistent with National Fire Protection Association (NFPA) 1582 in the past 12 months. Firefighters with any known cardiovascular disease, who used tobacco, were younger than 18 or older than 55 years of age, had gastrointestinal complications, or pregnant were excluded from the study. All firefighters were fit tested for the SCBA mask which they used for this study within the past 12 months. Participants were also requested to avoid eating char-grilled or smoked foods 24-hr before and during each study day and were provided a standardized meal 1 h prior to reporting for pre-firefighting data collection. Twenty-four firefighters (22 male, 2 female) and ten fire instructors (9 male, 1 female) participated in the study.

2.2. Study design

Horn et al. (2019) provides a detailed description of the study design. Briefly, two sets of five instructors (designated alpha and bravo) worked alternating days (three study days in five calendar days each). On each study day, the instructors led three training exercises with a different crew of four firefighters involved in each daily exercise (Table 1). The training exercises took ~10 min to complete with ~3 h between each daily exercise. The firefighters had about 46 h between the previous training exercise and the following pre-firefighting data collection, while the instructors had about 40 h between the last training exercise of the day and the next pre-firefighting data collection.

For all three training scenarios, the firefighters had the same objective to suppress a two-room fire and to locate and rescue two simulated occupants of the structure. The three scenarios differed primarily by fuel package and type or orientation of the structure as described below:

- **Pallet and straw scenario** – Fires were ignited using three pine wood pallets and one bale of straw in two separate bedrooms in a single story concrete training structure. All pallets used in the study were new and had not been used for shipping or handling any materials that could potentially contaminate the wood. The structure was laid out similar to a mid-20th century single family dwelling (Supplemental Materials, Fig. S1). In all scenarios, flaming combustion was contained within the burners in the two bedrooms (did not spread to the structure or other rooms) and smoke filled the remaining rooms of the training structure, to the point of limiting visibility at crawling level. As is common in live-fire training, firefighters responded when smoke conditions reached limited visibility, which resulted in suppressing the fires when flaming combustion was still being supported by the pallets in each room. In each case, the pallets were not completely consumed prior to suppression.
- **OSB scenario** – Fires ignited in burners using two pallets and one bale of straw along with OSB in each of two separate bedrooms in a T-shaped metal shipping container based prop (Supplemental Materials, Fig. S2). Two different types of OSB were used, identified

Table 1
Training schedule and participant roles.

Participant	Description	Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Instructors	5 instructors, 2 assigned as stokers, and 3 assigned as officers, ~3-hr transpired between each daily exercise	Alpha	3 simulated smoke exercises	Day off	3 pallet and straw exercises	Day off	3 OSB exercises	Day off
		Bravo	Day off	3 OSB exercises	Day off	3 pallet and straw exercises	Day off	3 simulated smoke exercises
Firefighters	3 crews, 4 firefighters per crew, 2 firefighters assigned to attack and 2 firefighters assigned to search and rescue	Alpha	1 simulated smoke exercise per crew	Day off	1 pallet and straw exercise per crew	Day off	1 OSB exercise per crew	Day off
		Bravo	Day off	1 OSB exercise per crew	Day off	1 pallet and straw exercise per crew	Day off	1 simulated smoke exercise per crew

in the paper as alpha OSB (used for the alpha groups) and bravo OSB (used for the bravo groups). Each type of OSB contained the same Engineered Wood Association APA rating for 7/16″ thickness (panel grade 24/16, exposure 1). One and half sheet of the 7/16″ alpha OSB were placed along the ceiling to provide adequate fuel supply for the training fires. Because of supply limitations, we only had access to 1/4″ sheets of the bravo OSB sheathing. One sheet of this OSB was cut in half and stacked together and then two sheets were also stacked together and placed along the ceiling. This effectively produced one and half sheets of bravo OSB with a similar thickness and orientation to the alpha OSB fuel package. According to their safety data sheets (SDS), both OSB sheathing contained phenol formaldehyde adhesive and polymeric methylene bisphenyl diisocyanate (pMDI) adhesive, but the exact volume percentage of each is unknown. The primary difference between the SDSs for the two types of OSB was that bravo OSB reported $< 0.01\%$ of free formaldehyde, while alpha OSB reported $< 0.1\%$ of free formaldehyde. Flaming combustion was isolated to the burners in each fire room and the OSB sheets along the ceiling of the rooms, while smoke migrated to the other rooms of the training structure, again banking down to the floor. For each scenario, firefighters suppressed the fires while pallet and OSB materials were still undergoing combustion as is typical in live-fire training, so these materials were not completely consumed in any trial.

- **Simulated smoke scenario** – An electronic means of simulating a fire that also incorporated simulated smoke generation (Attack Digital Fire System, Bullex; Albany, NY) was utilized in a building constructed from metal shipping containers to have an identical layout to a mid-20th century single family dwelling (Supplemental Materials, Fig. S1). Smoke was allowed to collect throughout the structure and bank down to limit visibility, similar to the conditions common in live-fire scenarios.

The order in which the training fire environments were introduced was mirrored for the alpha and bravo groups (Table 1). Each crew was composed of two firefighters assigned to fire attack who advanced the fire hose from an engine and suppressed all active fires, and two firefighters assigned to search and rescue who performed forcible entry and then searched for and rescued two simulated victims (75 kg manikins). During each scenario, two instructors acted as stokers to light the fires and control ventilation for fire and smoke development, two instructors assigned as company officers supervised the attack teams, and the remaining instructor was the officer in charge of the search and rescue operations. The firefighters and instructors maintained these roles throughout the study.

Both the firefighters and instructors were required to wear SCBA while inside the structures during the firefighting simulation. Instructors assigned as stokers donned their SCBA masks prior to ignition, while instructors assigned as company officers and the firefighters generally donned their SCBA masks just before entry. A few donned their SCBA masks as soon as they exited the apparatus, although this was left up to the individual firefighter. Both the instructors and firefighters spent similar amounts of time inside the structures during smoky conditions (~10 min).

After each exercise, the firefighters and instructors doffed their turnout gear in a large open bay with ample ventilation and then promptly entered an adjacent climate-controlled transport container for specimen collection. Investigators performed surface sampling and wet-soap decontamination of the turnout gear as previously described (Fent et al., 2017). The firefighters' turnout gear was decontaminated after each exercise and the instructors' turnout gear was decontaminated at the end of each training day. Field decontamination was done because it is considered a best practice (if laundering cannot be done) and to reduce the potential for turnout gear to act as another source of exposure with subsequent use. The firefighters and instructors were also provided with cleansing wipes to use for decontaminating their skin, which all firefighters and most instructors used during rehab (within the first 10 min following each training exercise).

2.3. Urine sampling and analysis

Firefighters provided spot urine samples pre-firefighting and 3-hr post-firefighting for all training exercises (n = 24 firefighters per scenario). Previous work has indicated that 3-hr post exposure may represent peak excretion of many PAH biomarkers (Fent et al., 2014; Fent et al., In Press-b). We collected urine from instructors before the first crew's training exercise (pre-firefighting), right after the second crew's training exercise (~3 h after first scenario), and 3-hr after the last crew's training exercise (~9 h after first scenario) (n = 10 instructors per scenario). The last sample collected from instructors each day represented the end-of-shift sample.

Urine samples (144 from firefighters and 90 from instructors) were shipped to the CDC National Center for Environmental Health to be analyzed for mono-hydroxylated PAH metabolites (OH-PAHs). Briefly, after enzymatic hydrolysis of conjugated OH-PAHs in urine (100 μL), the target OH-PAHs were quantified by online solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry. Limits of detection (LODs) ranged from 8 to 90 ng/L, depending on the analyte (Wang et al., 2017).

Creatinine was measured using a Vitros Autoanalyzer (Johnson & Johnson, New Brunswick, NJ). Cotinine, a metabolite of nicotine, was measured using the Immulite® 2000 immunoassay system (Siemens Corp., Washington, DC). Cotinine concentrations were used to confirm current non-tobacco use status of the participants and to quantify possible exposure to environmental tobacco smoke (ETS), another source of PAH exposure (Suwan-ampai et al., 2009). The vast majority of urine samples (96%) had cotinine levels consistent with non-tobacco use status and no ETS exposure (< 10 ng/mL).

2.4. Exhaled breath sampling and analysis

Exhaled breath samples were collected from firefighters before and immediately after each scenario. Previous research has suggested that peak VOC breath concentrations occur right after firefighting (Fent et al., 2014; Fent et al., In Press-b). For instructors, breath samples were collected before the first crew's exercise and immediately after both the second and third crew's exercise. For the simulated smoke scenario, only two instructors and two firefighter per crew (n = 4 and 12, respectively) were sampled because we expected minimal VOC exposure during this scenario. All participating firefighters (n = 24 firefighters per scenario) and instructors (n = 10 instructors per scenario) were sampled for the other scenarios.

Breath samples were collected within 3–4 min after doffing SCBA. Participants were instructed to take a deep breath in and then forcefully exhale their entire breath into the Bio-VOC™ sampler (Markes International, Inc., Cincinnati, OH), which serves to collect the final 129- mL of breath. The collected air was pushed through Markes thermal desorption tubes (Carbograph 2TD/1TD dual bed tubes). The thermal desorption tubes were capped and stored at −20 °C until shipment to the U.S. Environmental Protection Agency analytical laboratory.

The method used to analyze the breath samples for benzene, toluene, ethylbenzene, and styrene is described in detail elsewhere (Geer Wallace et al., 2017). Method detection limits ranged from 0.14 ng/tube (styrene) to 1.1 ng/tube (ethylbenzene). The ng on tube was converted to ng/L by dividing by the total breath volume collected (129 mL) and results are reported as parts per billion by volume (ppbv).

2.5. Data analysis

We used instrumental readings for the OH-PAH metabolite results < LOD (3.7%). The OH-PAH concentrations were normalized by creatinine. To simplify the analyses, the individual OH-PAH concentrations of each parent compound were summed together to create the following variables: hydroxyphenanthrenes, hydroxynaphthalenes, and hydroxyfluorenes. In addition, some analyses were performed on the sum of all OH-PAH concentrations (ΣOH-PAHs).

Benzene, toluene, ethylbenzene, and styrene were non-detectable in 21, 8.1, 69, and 44% of the breath samples. We estimated breath concentrations < LOD using ordered imputations and Q-Q plots as described in Pleil et al. (Pleil, 2016a, b). This method relies on plotting the natural log of the compound concentrations (minus non-detects) versus the Z-scores to obtain a linear best fit equation. This equation is then used to impute values for the samples with concentrations < LOD by plugging the corresponding calculated Z-scores into the obtained equation.

All statistical analyses were performed in R version 3.4.3. Quartiles were used to summarize data. Since data were skewed, values were log-transformed for all statistical analyses. The Shapiro-Wilk test for normality was performed on the log-transformed data and it was determined that data did not violate the normality assumption. The difference between pre and post measurements were calculated and, since each individual participated in multiple scenarios, mixed linear models were used to control for repeated measures in testing whether the differences were different than 0 as well as to compare differences between groups.

3. Results

3.1. Urinary excretion of PAHs after training exercises

Figs. 1 and 2 provide a comparison of the hydroxyphenanthrenes and 1-hydroxypyrene results in urine over time between firefighters and instructors for the three types of scenarios, with further stratification between the two types of OSB. The Supplemental Materials provide the hydroxynaphthalenes and hydroxyfluorenes results (figs. S3–S4), as well as summary statistics for all the biomarkers that were measured (table S1). Firefighters had a significant increase in OH-PAH concentrations 3-hr after training for all scenarios ($p \leq 0.001$). Furthermore, instructors' OH-PAH concentrations increased steadily throughout each training day and by the end of the shift were significantly greater than the pre-training levels for all scenarios ($p \leq 0.001$). The relative magnitude of these increases generally followed the pattern: bravo OSB > alpha OSB > pallet and straw > simulated smoke. For firefighters undergoing the bravo OSB training scenarios, hydroxyphenanthrenes had the largest pre-to 3-hr post-training urine concentration increase on a percentage basis (median +1074%) while hydroxynaphthalenes had the largest increase on a unit basis (median +32.7 μg/g). For instructors in the bravo OSB training scenarios, 1-hydroxypyrene showed the largest pre-to end-of-shift percentage increase in concentrations (median +2860%) and hydroxynaphthalenes had the largest unit increase (median +34.3 μg/g).

We compared the pre-to 3-hr post training change in OH-PAHs for firefighters to the pre 1st exercise to post 2nd exercise change in OH-PAHs for instructors, as the timing of these biological samples were similar. Although instructors completed two training exercises between their urine collection sessions, exposures from their second training exercise was unlikely to contribute to their post 2nd exercise urine concentrations because of the timing of the collections (Fent et al., 2014). The change in urine concentrations did not differ significantly between firefighters and instructors (for all scenarios combined) except for 1-hydroxypyrene (firefighters +103%, instructors +46%, $p = 0.015$) and hydroxyphenanthrenes (firefighters +234%, instructors +188%, $p = 0.047$). Stratifying by type of scenario, only hydroxyphenanthrenes during the bravo OSB scenario differed significantly ($p = 0.026$) between firefighters (+1074%) and instructors (+316%). Importantly, we found no differences between firefighters

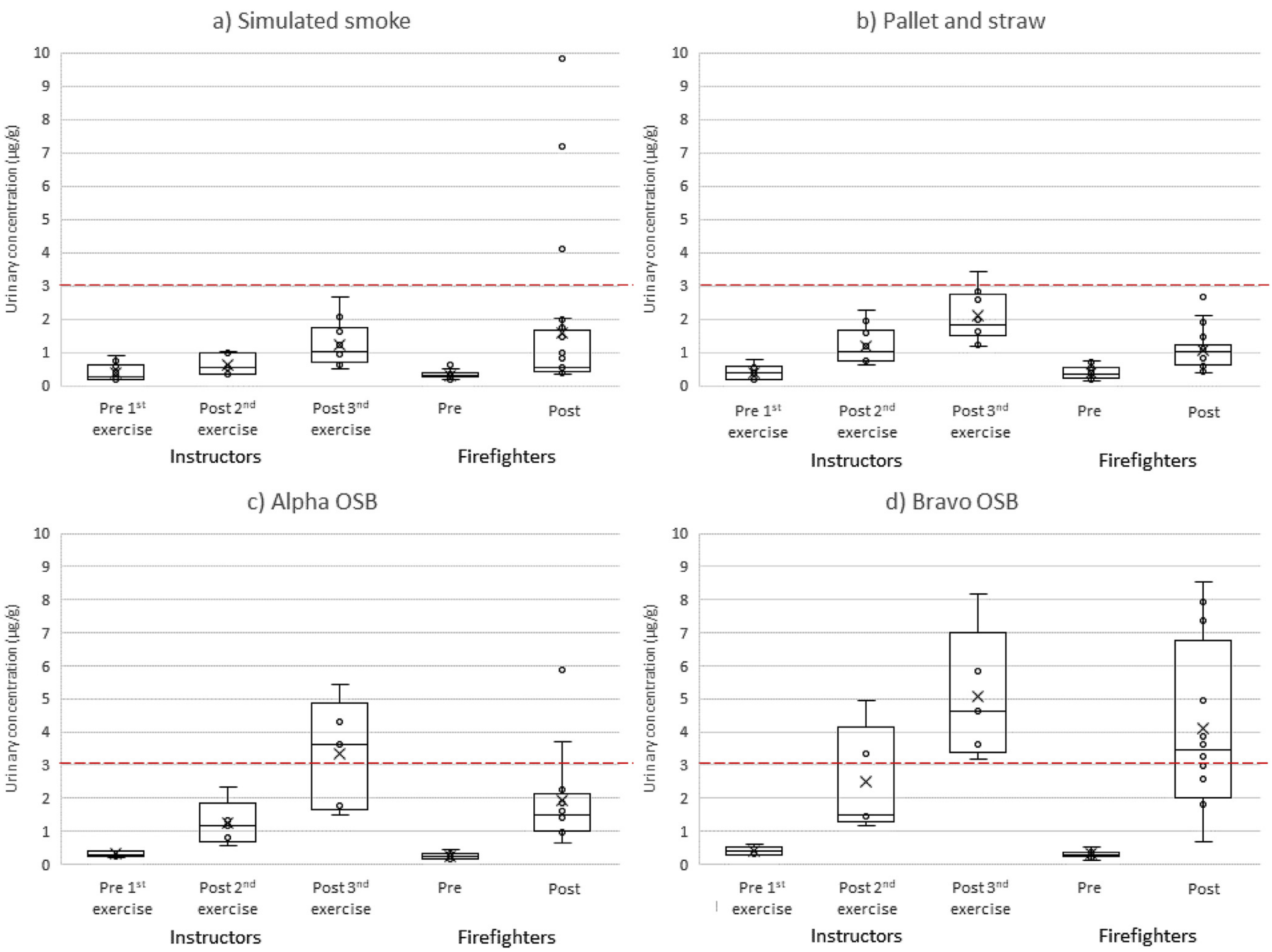

Fig. 1. Urinary concentrations of **hydroxyphenanthrenes** by participant type and collection period for training scenarios using a) simulated smoke, b) pallet and straw, c) alpha OSB, and d) bravo OSB. The lower quartile, median, and upper quartile are shown with the box and whiskers (excluding outliers 1.5 times greater or less than the upper and lower quartiles). The mean of the distribution is shown by X. A red dashed line representing the median 3-hr post-firefighting concentration (3.1 µg/g) measured from attack and search firefighters during our residential fire study (Fent et al., In Press-b) is provided for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and instructors for the change in ΣOH-PAHs. In a related paper, we found that instructors had lower air concentrations of total PAHs than firefighters, but instructors had longer duration exposures, which could explain the similar magnitude of absorption in comparison to firefighters (Fent et al., In Press-a).

To further explore the impact of repeated exposures on OH-PAH concentrations, we compared instructors' pre-to end-of-shift change in concentrations (for all scenarios combined) to firefighters' pre-to 3-hr post-training change in concentrations (for all scenarios combined). Differences were statistically significant for 1-hydroxypyrene (firefighters +103%, instructors +397%, $p < 0.001$) and hydroxyphenanthrenes (firefighters +234%, instructors +480%, $p = 0.046$).These findings suggest cumulative exposures to PAHs in the instructors from overseeing multiple training exercises in a day.

The simulated smoke exercises are not expected to have produced PAHs because no combustion took place, which is supported by our previously published air sampling results (Fent et al., In Press-a). To further investigate why the participants experienced biological uptake of PAHs during these exercises, we compared the urinary concentrations of the alpha (started with simulated smoke) and bravo (ended with simulated smoke) participants to each other (Supplemental Materials, table S2) to determine whether the order of the scenarios had any effect. We found significantly greater ($p < 0.001$) pre-to 3-hr post-training increases in the ΣOH-PAHs during the simulated smoke scenario for the bravo firefighters (+538%) than the alpha firefighters (+48%). Similarly, the bravo instructors had significantly greater ($p = 0.023$) pre-to end-of-shift increases (+248%) in the ΣOH-PAHs than the alpha instructors (+89%). As is commonly the case, the hydroxynaphthalenes were the dominant species in the ΣOH-PAHs. These results suggest another source of PAHs was present during the simulated smoke training that was more abundant during the bravo exercises. However, it is unclear what the source of this contamination was and why this effect was more pronounced in firefighters than instructors.

We also explored the effect of job assignment by comparing the change in urine concentrations of OH-PAHs between the fire attack and search positions for the firefighters and between stoker and company officer positions for the instructors (data not shown). The changes in ΣOH-PAHs were similar between these comparison groups, with p-values > 0.4 for stoker vs. Officer instructors and p-values > 0.13 for attack vs. search firefighters.

Table 2 provides a comparison between the U.S. general non-smoking adult population urine concentrations of individual OH-PAHs to the firefighters' median 3-hr post-training concentrations and instructors' end-of-shift concentrations. These time points represent the peak excretion identified within the constraints of this study. It is important to note that the firefighters and instructors started each study day with many of the OH-PAH concentrations above (≤2-fold) general

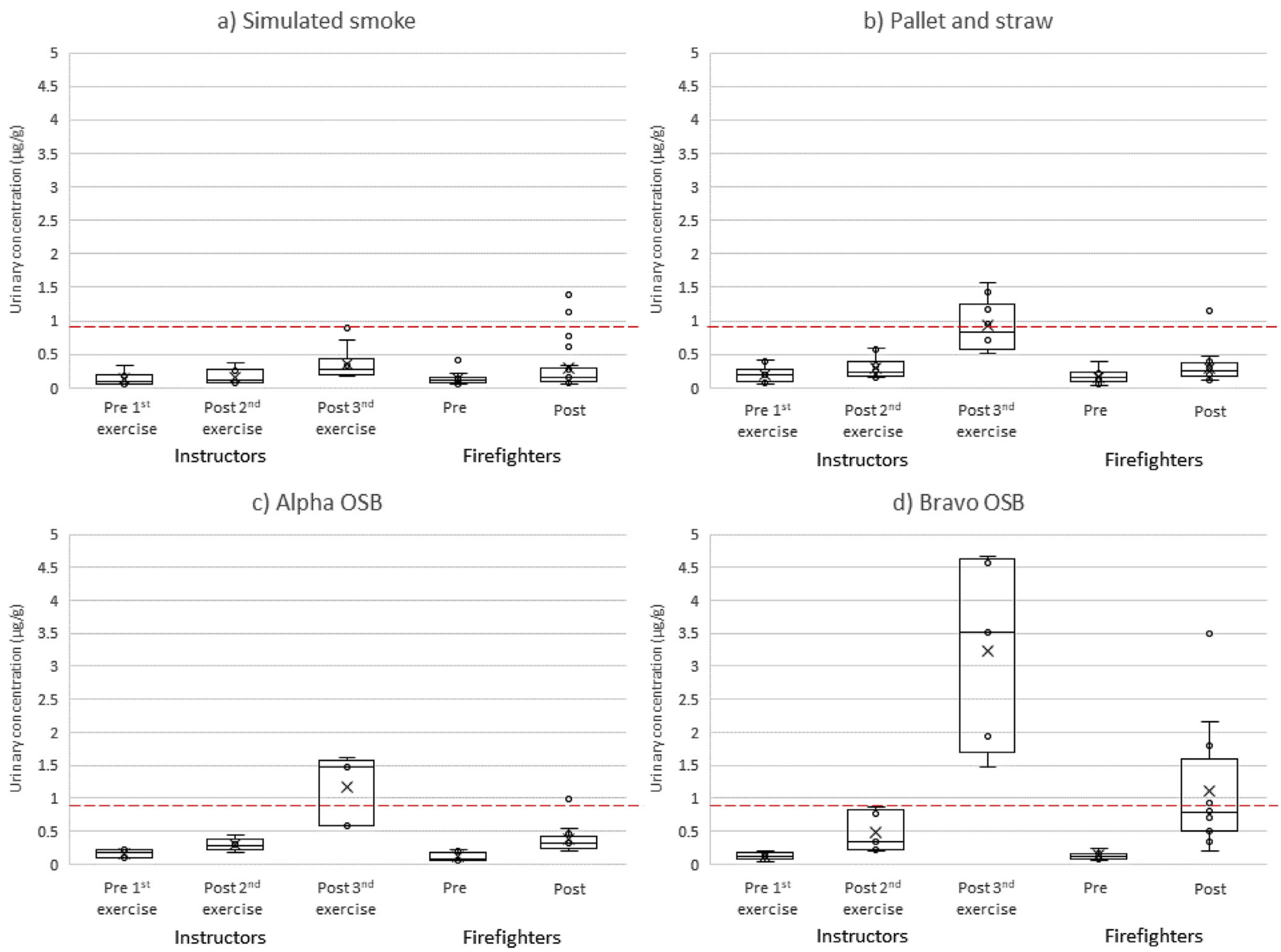

Fig. 2. Urinary concentrations of **1-hydroxypyrene** by participant type and collection period for training scenarios using a) simulated smoke, b) pallet and straw, c) alpha OSB, and d) bravo OSB. The lower quartile, median, and upper quartile are shown with the box and whiskers (excluding outliers 1.5 times greater or less than the upper and lower quartiles). The mean of the distribution is shown by X. A red dashed line representing the median 6-hr post-firefighting concentration (0.81 µg/g) measured from attack and search firefighters during our residential fire study (Fent et al., In Press-b) is provided for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

population medians. Regardless of the scenario, the firefighters' and instructors "peak" concentrations of individual OH-PAHs were significantly higher ($p < 0.05$) than their pre-training concentrations (with the exception of 1-hydroxynaphthalene measured during the simulated smoke scenario). In addition, many of the firefighters' and instructors' peak urine concentrations after live-fire scenarios (OSB and pallet and straw) were greater than the respective 95th percentiles of the general population.

3.2. Exhaled breath concentrations of VOCs after training

Table 3 provides the percent change in exhaled breath concentrations of VOCs for firefighters and instructors by training scenario, and Fig. 3 provides the specific results for benzene. In general, exhaled breath concentrations increased from the pre-training levels for all scenarios except the simulated smoke exercises, which had mixed results (although most VOC concentrations declined). For firefighters, the change in breath concentrations during bravo OSB training scenario was significantly greater than the alpha OSB training scenario for all analytes ($p < 0.05$). A similar pattern was found for instructors when comparing the change in breath concentrations of benzene (pre 1st exercise to post 2nd exercise) by the two types of OSB; however, the difference was not statistically significant ($p = 0.161$). The change in breath concentrations for instructors and firefighters generally did not differ (for all scenarios combined), with one exception for styrene (instructor pre 1st exercise to post 3rd exercise change +599% vs. Firefighter pre to post change +79%, $p < 0.001$). As with the OH-PAHs, job assignment for firefighters and instructors did not affect the change in exhaled breath concentrations ($p > 0.05$ for all VOCs and scenario combinations).

4. Discussion

This study improves our understanding of firefighters' and instructors' exposures during training exercises commonly performed at training institutes in the United States and many other countries around the world. The most important results of this study are: 1) firefighters and instructors are exposed to combustion byproducts even when wearing SCBA throughout the training exercise, and 2) firefighters and instructors undergoing training exercises involving OSB experienced higher exposures than pallet and straw (alone) as the fuel source. Furthermore, there is strong evidence of instructors' increasing cumulative exposure to PAHs with repeated training exercises. This is an important finding because instructors commonly oversee numerous live-fire training exercises during a single day and such activity may be repeated many days over the course of a year.

For the OSB training exercise, two types of OSB were used. It is not possible to be certain of the relative proportion of different adhesives in

Table 2
Comparison of urinary OH-PAH metabolite concentrations measured from ***firefighters*** 3 h after each training exercise and measured from ***instructors*** at the end of each shift to the non-smoking adult general population (μg/g creatinine).

OH-PAH biomarker	NHANES 2011–2012 data for 20–49 year old non-smokers[a]		Firefighters' median 3-hr post-firefighting concentrations by scenario				Instructors' median end-of-shift concentrations by scenario			
	Median	95th percentile	Simulated smoke (n = 24)	Pallet and straw (n = 24)	Alpha OSB (n = 12)	Bravo OSB (n = 12)	Simulated smoke (n = 10)	Pallet and straw (n = 10)	Alpha OSB (n = 5)	Bravo OSB (n = 5)
1-Hydroxynaphthalene	1.0	6.2	2.2	3.6	8.6	**21**	3.3	6.8	**17**	**22**
2-Hydroxynaphthalene	3.7	16.4	8.4	7.5	12	**20**	13	14	**18**	**17**
1-Hydroxyphenanthrene	0.12	0.47	0.23	0.38	0.49	**1.3**	0.32	0.72	**1.4**	**1.5**
2-Hydroxyphenanthrene and 3-hydroxyphenanthrene[b]	0.13	0.48	0.33	0.55	0.93	**2.3**	0.67	1.2	**2.2**	**3.0**
1-Hydroxypyrene	0.10	0.33	0.15	0.26	0.32	0.78	0.27	0.84	**1.5**	**3.5**
2-Hydroxyfluorene	0.18	0.64	0.45	0.55	0.96	**1.5**	0.88	1.3	**1.5**	**2.2**
3-Hydroxyfluorene	0.07	0.25	0.18	0.19	0.29	**0.45**	0.21	0.58	**0.68**	**0.92**

[a] Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018, Volume Two (NCEH, 2018).

[b] 2-Hydroxyphenanthrene and 3-hydroxyphenanthrene were reported separately for 2011–2012 NHANES data. Thus, 2013–2014 NHANES summary statistics are provided for 2-hydroxyphenanthrene and 3-hydroxyphenanthrene (combined) for general population 20 years and older (which may include smokers). Values that are bolded were higher than the median concentrations measured from attack and search firefighters in our residential fire study (Fent et al., In Press-b).

the two OSB products as this is proprietary information. Median area air concentrations of methyl isocyanate, phenyl isocyanate, and MDI during the bravo OSB exercises were higher than the alpha OSB exercises, although the differences were not statistically significant (Fent et al., 2019a). This could suggest that the bravo OSB (with < 0.01% free formaldehyde) contained higher amounts of pMDI adhesives than the alpha OSB (with < 0.1% free formaldehyde). Differences in the types and amounts of adhesives used in the alpha and bravo OSB could have influenced the production of PAHs during combustion. PAH emissions could be further affected by the slightly different amounts of OSB used (i.e., 1.5 sheets of 7/16″ OSB for alpha vs. effectively 1.5 sheets of 1/2" OSB for bravo). However, the OSB sheathing was not fully consumed in the fires, so the exposure was not limited by the mass of fuel in any of the OSB scenarios. Other factors such as ventilation and temperature could also affect the production of combustion byproducts; although these factors were standardized to the extent possible among the different scenarios.

PAH and benzene exposures from the bravo OSB exercises (based on urine OH-PAH and breath VOC concentrations) were consistently higher than the alpha OSB exercises, which may be expected if the bravo OSB contained higher quantities of adhesives. Previously, we showed that the bravo OSB produced significantly ($p < 0.01$) higher personal air concentrations of total PAHs and benzene than the alpha OSB. If OSB is to be used for live fire training burns, OSB with the least amount of synthetic adhesives should be selected when possible. However, this information is not always readily available from the suppliers.

Prior to this study, we investigated firefighters' exposures during controlled residential fires involving household furnishings using the same methodology. We hypothesized that the firefighters and instructors would have lower PAH and benzene exposures from training fires than residential fires involving a variety of typical synthetic materials including foams, plastics, and textiles. On a per training fire basis, exposures were generally below what was measured from attack and search firefighters during the residential fire study (who conducted similar firefighting tasks and timeframes as in the current study) for all training scenarios other than the bravo OSB exercises. For example, firefighters and instructors had higher median urinary concentrations of hydroxynaphthalenes and hydroxyfluorenes ~3-hr after the bravo OSB exercises than reported during the same time period in the residential fire study (see Figs. S3 and S4 in Supplemental Materials).

It is important to consider the pre-to post-firefighting unit changes for breath results when comparing the training and residential fire studies because background levels on the breath tubes varied. For example, the 611% increase in exhaled breath concentrations of benzene for firefighters after the bravo OSB exercises is due to a unit increase of 18 ppbv, which is less than the median increase found for attack and search firefighters in the residential fire study (+24 ppbv). Interestingly, we found very little, if any, increase in breath concentrations of toluene, ethylbenzene, and styrene in the residential fire study (e.g., < 0.45 ppb increase for attack and search firefighters). However, for the present training fire study, we generally found marked increases in these VOCs in breath for all scenarios except for the simulated smoke exercises (Table 3). While median personal air concentrations of these VOCs were higher for the training fires (OSB and pallet and straw) than the residential fires (Fent et al., 2019a), the levels were at least an order of magnitude below applicable occupational exposure limits (ACGIH, 2018; NIOSH, 2010). Personal air concentrations of benzene for both the training fires (other than simulated smoke) and residential fires, on the other hand, were well above the NIOSH short-term exposure limit (1 ppm) (National Institute for Occupational Safety and Health, 2010), and may present a greater concern for toxicity, especially with repeated exposures.

Whereas the firefighters only participated in one training exercise per day, the instructors supervised three exercises per day. For both of the OSB scenarios, instructors' end-of-shift median urine concentrations

Table 3
Median change in exhaled breath concentrations during training scenarios.

Scenario	Participant	Comparison	n	Benzene		Toluene		Ethyl benzene		Styrene	
				ppbv	%	ppbv	%	ppbv	%	ppbv	%
Simulated smoke	Firefighters	Pre to post	12	−0.6	−15	−0.0	+0	+0.0	+172	−0.1	−28
	Instructors	Pre 1st to post 2nd exercise	4	−2.1	−38	−1.0	−45	−0.2	−44	−1.0	−65
		Pre 1st to post 3rd exercise	4	+1.4	+29	+1.3	+51	−0.1	−17	+2.9	+176
Pallet and straw	Firefighters	Pre to post	24	**+2.8**	**+99**	**+0.4**	**+55**	**+0.1**	**+553**	**+0.1**	**+99**
	Instructors	Pre 1st to post 2nd exercise	10	+0.9	+46	+0.2	+34	**+0.1**	**+773**	+0.2	+259
		Pre 1st to post 3rd exercise	10	+0.6	+20	+0.3	+40	**+0.2**	**+1134**	**+1.0**	**+1831**
Alpha OSB	Firefighters	Pre to post	12	**+6.6**	**+195**	**+1.2**	**+105**	+0.1	+63	−0.0	−1
	Instructors	Pre 1st to post 2nd exercise	5	+3.6	+149	+0.7	+73	+0.1	+25	+0.2	+25
		Pre 1st to post 3rd exercise	5	+3.0	+109	**+1.0**	**+63**	+0.0	+4	**+3.8**	**+572**
Bravo OSB	Firefighters	Pre to post	12	**+18.0**	**+611**	**+3.1**	**+292**	**+0.4**	**+669**	**+0.8**	**+460**
	Instructors	Pre 1st to post 2nd exercise	5	**+11.0**	**+327**	**+1.4**	**+162**	+0.2	+244	+0.2	+51
		Pre 1st to post 3rd exercise	5	+5.9	+165	**+1.2**	**+125**	**+0.2**	**+281**	**+1.9**	**+550**

Bolded values represent statistical significance at $p < 0.05$.

of all OH-PAHs were above the concentrations measured 3-hr after firefighting from attack and search firefighters in the residential fire study. In particular, 1-hydroxypyrene was 3.5-fold greater (Fig. 2), which is 35-times higher than the general population median. Of the PAH urine metabolites examined in this study, 1-hydroxypryene correlates most closely with the higher molecular weight PAHs (≥4 rings), many of which tend to be excreted in feces. Of these higher molecular weight PAHs, benzo[a]pyrene is a known human carcinogen widely considered the most toxic PAH (IARC, 2010). Our previous work shows that the composition of airborne PAHs were consistent across the OSB and pallet and straw scenarios, with benzo[a]pyrene constituting ~1% of the mixture. In contrast, naphthalene was the most abundant PAH measured in air, constituting 66–68% of the mixture (Fent et al., 2019a).

For all scenarios, firefighters' and instructors' OH-PAH urine concentrations (reported in Table 2) were greater than the medians for

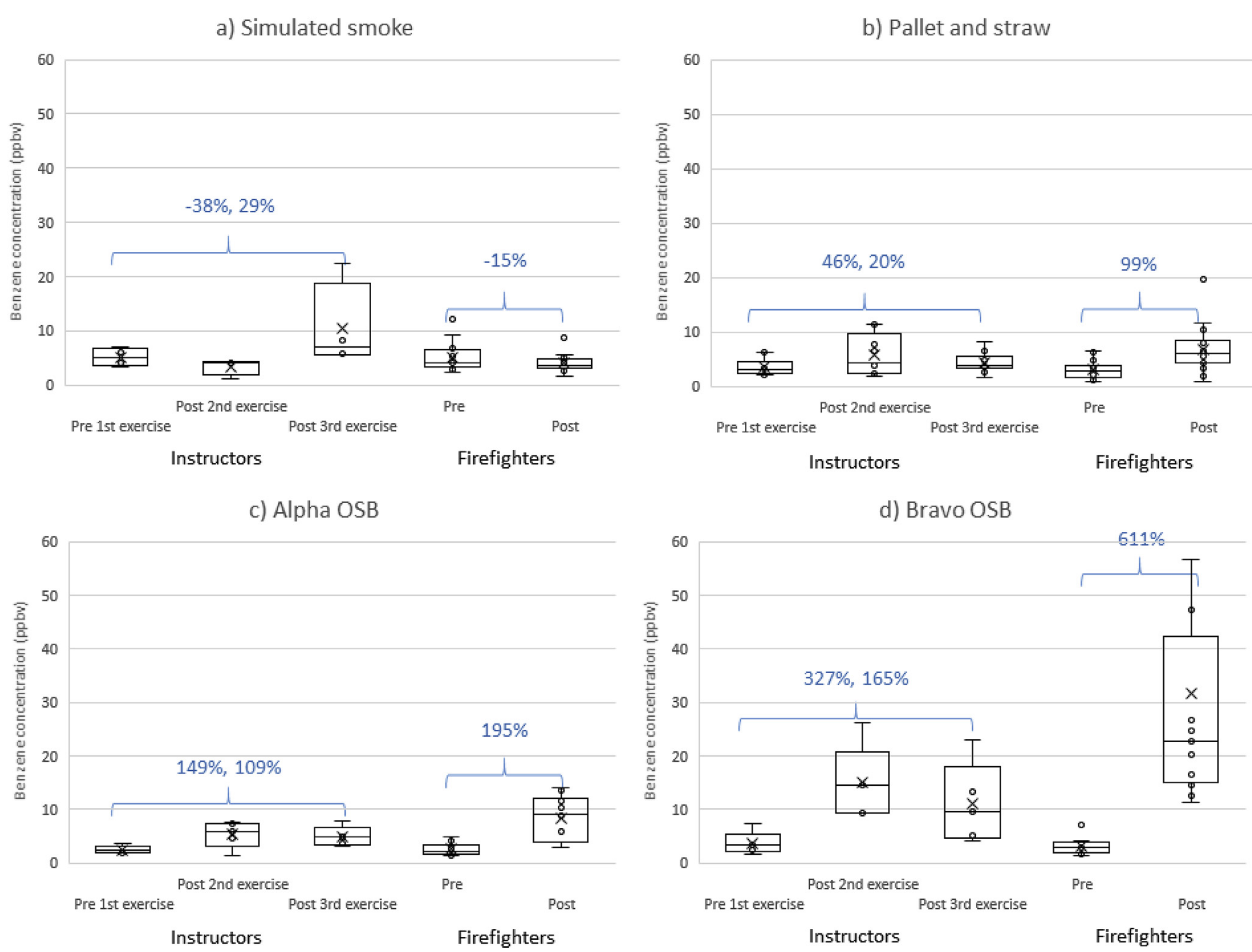

Fig. 3. Exhaled breath concentrations of **benzene** by participant type and collection period for training scenarios using a) simulated smoke, b) pallet and straw, c) alpha OSB, and d) bravo OSB. The percent change from pre-training levels are provided for instructors and firefighters.

non-smoking adults in the general population. Nearly all of the OH-PAHs measured after the OSB scenarios (and a few metabolites during the pallet and straw scenario) exceeded the 95th percentiles of the general population. The median end-of-shift concentration of 1-hydroxypryene measured from instructors after the bravo OSB scenario (3.5 μg/g) is within the range of average concentrations in gas workers (0.3–7.7 μg/g) and road pavers (1.2–3.5 μg/g), who are among the more exposed worker populations (Huang et al., 2004). Maximal urinary excretion of 1-hydroxypyrene following ingestion of PAHs has been estimated at 5.5 h (Li et al., 2012), and we previously found maximal excretion 6 h after firefighting (Fent et al., 2019b); thus, our study design may not have captured the peak urinary concentration of this metabolite. It is also important to note that PAHs and VOCs only represent a portion of the combustion products that are produced during fires, so caution should be exercised when interpreting the concentrations of only these compounds in firefighters relative to other populations. In addition, the firefighters and most of the instructors in this study used cleansing wipes post-training and all showered within an hour of completing their exercises each day. We have shown previously that commercial cleansing wipes can remove a median of 54% of PAH contamination from skin (Fent et al., 2017). Without these measures, we expect that exposures would have been even greater.

One unexpected finding from this study was the increase in ΣOH-PAHs during the simulated smoke exercises. Upon closer examination, we found statistically significant differences between the alpha and bravo groups ($p \leq 0.002$), where the bravo firefighters and instructors experienced higher post-training increases in urine OH-PAHs. Efforts were taken to minimize the firefighters' and instructors' exposures from peripheral sources at the training institute. For example, no live-fire training was permitted on the IFSI campus during the week other than the training required for the study. All turnout gear had been laundered before the start of the study, and field decontamination (using water, dish soap, and scrubbing) was used to clean the gear during the study. Although this type of decontamination has been shown to be effective (removing a median of 85% of PAHs on the outer shell) (Fent et al., 2017), some residual contamination will remain on the turnout gear. Moreover, field decontamination does little for contaminants on the inner liner of the gear that can directly contact firefighters' skin.

Because the alpha participants performed simulated smoke training first, any PAHs on their turnout gear (post-laundering) should have been low, although, laundering may not remove 100% of PAH contaminants (Mayer et al., 2018). The bravo participants, however, performed simulated smoke training last. As such, their gear would have received contamination from the OSB and pallet and straw scenarios performed 4 and 2 days prior, respectively. Thus, PAHs not removed via field decontamination could have been available for biological uptake upon subsequent use of the turnout gear as suggested by Stec et al. (2018). Any residual naphthalene could off-gas and be inhaled by the participants when not wearing SCBA. In addition to contaminated turnout gear, other sources of PAH exposure at the training institute (e.g., contaminants deposited in turnout gear storage area) cannot be ruled out. It is also important to note that the sample sizes (statistical power) were small, especially for the instructors (where $n = 5$ for each comparison group). Further research on how contaminated gear contributes to firefighters' exposure to PAHs is warranted.

5. Conclusions

Biological monitoring can be affected by a number of factors, such as physiological makeup and metabolism of workers, work-rate intensity and duration, and PPE use and maintenance. Thus, it is prudent to be cautious when comparing results between studies. Overall, this study suggests that live-fire training may expose firefighters and instructors to hazardous chemicals. Their dose will depend on the number of training fires and type of fuel package. Instructors' PAH exposures may be higher from repeated training fires than responding to a single emergency residential fire. Likewise, training fires will result in the uptake of benzene and other VOCs. Contamination on turnout gear may also contribute to the biological uptake of PAHs upon subsequent use. Exposures from training fires over time could increase firefighters' and instructors' risk of developing certain types of cancer. Efforts should be taken to reduce these exposures, including donning SCBA before approaching the structure, cleaning skin as quickly as possible (preferably immediately after exiting the structure), laundering turnout gear after live-fire training (or field decontamination if laundering cannot be done), showering as soon as possible following training, and selecting training fuels to provide realistic training while limiting unnecessary exposures for firefighters and instructors.

Declarations of interest

None.

Conflicts of interest

The authors declare that they have no competing financial interest in relation to the work described.

Acknowledgements

The authors would like to thank all the individuals who assisted in collecting data for this study, including Kenneth Sparks and Kelsey Babik at NIOSH; Yuesong Wang, Nikki Pittman, and Debra Trinidad at NCEH; Jacob DeBlois and Margaret Morrisey at Skidmore College; and Richard Kesler, Sean Burke, Sue Blevins, as well as the field staff and support team at the Illinois Fire Service Institute. We also thank Jen Roberts at NIOSH for her help in conditioning the breath tubes for collection. We are especially grateful to the firefighters and instructors who participated in this study. This study was funded through a U.S. Department of Homeland Security, Assistance to Firefighters Grant (EMW-2014-FP-00590). The U.S. Environmental Protection Agency (EPA) analyzed the breath samples for the study but were not involved in funding the main project. The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of NIOSH, Centers for Disease Control and Prevention, or EPA. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.06.006.

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Fent, K. et al, *Understanding airborne contaminants produced by different fuel packages during training fires, JOEH (2019)*:

"Air concentrations for the majority of chemicals of interest were highest for the Bravo OSB scenarios followed by Alpha OSB, pallet, and straw, and then simulated smoke." Pg 10

"...our overall findings suggest that burning OSB releases more airborne toxicants than pallet and straw or simulated smoke." Pg 10

"Efforts should be taken to minimize the use of OSB during training fires where appropriate, particularly when possible to meet training objectives without the use of this material." Pg 10

Fent, K. et al, *Firefighters' and instructors' absorption of PAHs and benzene during training exercises, IJHEH (2019):*

"Median concentrations of nearly all PAH metabolites... were highest for OSB, followed by pallet and straw, and then simulated smoke." Pg 1

"A single day of OSB exercises led to a 30-fold increase in 1-hydroxypyrene for instructors, culminating in a median end-of-shift concentration 3.5-fold greater than median levels measured from firefighters in a previous controlled-residential fire study." Pg 1

"Exposures were highest for the OSB scenario and instructors accumulated PAHs with repeated daily exercises." Pg 1

"Generally, the magnitude of contaminants measured in air were highest for the OSB exercises, followed by pallet and straw and then simulated smoke exercises." Pg 2

"The most important results of this study are... firefighters and instructors undergoing training exercises involving OSB experienced higher exposures than pallet and straw (alone) as the fuel source." Pg 6

Some have asserted that Fig 2 from ***Firefighters' and Instructors' Absorption of PAHs and Benzene During Training Exercises (2019) IJHEH**,* indicates that OSB and pallet and straw exposures are similar*.*

This is incorrect as Fig 2 clearly shows the average instructors post 3rd exercise concentration of 1-hydroxypyrene in urine is 33% higher with OSB Alpha than pallets and straw. Furthermore Fig 1 in the same paper shows the average instructors post 3rd exercise concentration of hydroxyphenanthrenes in urine is 57% higher with OSB Alpha than pallets and straw **AND** the data in the supporting information shows that the average instructors post 3rd exercise concentration of total PAH-OHs is 82% higher with OSB Alpha than pallets and straw.

Data in this paper also indicates that fire fighters exhaled breath benzene after one exposure increases by 195% after one OSB Alpha exposure while it increases by 99% after one pallet and straw exposure. These are bio-markers of exposure to chemical carcinogens and the higher exposures quantified with OSB Alpha are significant.

Laitinen, J. et al, *Fire fighting trainers' exposure to carcinogenic agents in smoke diving simulators, Toxicology Letters (2010):*

"The highest excretion of 1-pyrenol... and hydrogen cyanide...
were measured during the burning of conifer plywood and chipboard,
and the lowest when pure pine and spruce wood... was burned." Pg 61

"When conifer plywood was replaced with pure spruce and
pine wood, hydrogen cyanide emission decreased even more, by
almost 95%." Pg 62

"We focused on the concentration of carcinogenic benzene, of which the
burning of conifer plywood emitted the highest concentrations." Pg 62

"The concentration of benzene emissions from the burning of pure spruce
and pine wood, on the other hand, was 20% of that emitted from the
burning of conifer plywood." Pg 62

"The excretion of muconic acid showed that the burning of
conifer plywood caused greater exposure to benzene for the trainers than
the burning of pure spruce and pine wood or propane." Pg 62

"Exposure to polycyclic aromatic hydrocarbons was measured by
following fire fighters' urinary 1-pyrenol excretions in time...
The highest exposure levels were measured when conifer plywood
was burned, the second highest being recorded when chipboard was burned." Pg 63

"The highest excretion of 1-pyrenol and muconic acid and emis-
sion of benzene and hydrogen cyanide were measured during the
burning of conifer plywood and chipboard, and the lowest when
pure pine and spruce wood was burned." Pg 64

"As a result of these findings, we suggest glueless wood or gas
as the safest burning material..." Pg 64

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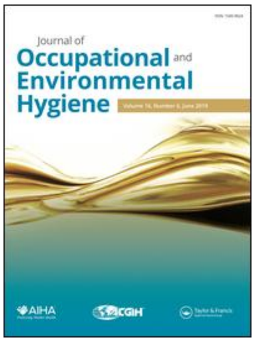

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ISSN: 1545-9624 (Print) 1545-9632 (Online) Journal homepage: https://oeh.tandfonline.com/loi/uoeh20

Understanding airborne contaminants produced by different fuel packages during training fires

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JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
https://doi.org/10.1080/15459624.2019.1617870

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Understanding airborne contaminants produced by different fuel packages during training fires

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ABSTRACT

Fire training may expose firefighters and instructors to hazardous airborne chemicals that vary by the training fuel. We conducted area and personal air sampling during three instructional scenarios per day involving the burning of two types (designated as alpha and bravo) of oriented strand board (OSB), pallet and straw, or the use of simulated smoke, over a period of 5 days. Twenty-four firefighters and ten instructors participated. Firefighters participated in each scenario once (separated by about 48 hr) and instructors supervised three training exercise per scenarios (completed in 1 day). Personal air samples were analyzed for polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and hydrogen cyanide during live-fire scenarios (excluding simulated smoke). Area air samples were analyzed for acid gases, aldehydes, isocyanates, and VOCs for all scenarios. For the live-fire scenarios, median personal air concentrations of benzene and PAHs exceeded applicable short-term exposure limits and were higher among firefighters than instructors. When comparing results by type of fuel, personal air concentrations of benzene and PAHs were higher for bravo OSB compared to other fuels. Median area air concentrations of aldehydes and isocyanates were also highest during the bravo OSB scenario, while pallet and straw produced the highest median concentrations of certain VOCs and acid gases. These results suggest usage of self-contained breathing apparatus (SCBA) by both instructors and firefighters is essential during training fires to reduce potential inhalation exposure. Efforts should be taken to clean skin and clothing as soon as possible after live-fire training to limit dermal absorption as well.

KEYWORDS
Firefighters; HCN; isocyanates; PAHs; particulate; VOCs

Introduction

Firefighters are occupationally exposed to a number of airborne pollutants and contaminants during emergency fire responses, including polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), polychlorinated biphenyls (PCBs), dioxins, plasticizers, flame retardants, hydrogen cyanide (HCN), hydrogen chloride, and other respirable particulates.[1,2] Some of these compounds may also be produced during live-fire training, and may contribute substantially to firefighters' exposure over their career, depending in part on the relative amount of time spent in training vs. emergency responses. Occupational exposure during training may also depend on the fuel package used in training, as the pyrolysis of OSB is different than the pyrolysis of pallet and straw.

A meta-analysis conducted in 2006 indicated that firefighters have increased risk of testicular, multiple myeloma, non-Hodgkins lymphoma, and prostate cancer.[3] Following this meta-analysis, Daniels et al.[4] conducted a retrospective study of 30,000 firefighters and found increased mortality and incidence risk for

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cancers of the esophagus, intestine, lung, kidney, and oral cavity, as well as mesothelioma. Daniels et al.[4] also found a dose-response relationship between fire-runs and leukemia and fire hours and lung cancer.[5] While a number of risk factors increase cancer risks, firefighters' inhalation exposure to toxic combustion products like PAHs and benzene are thought to play an important role.

Many fire departments require live-fire training for their members in order to maintain competency and certifications. Often, firefighters and officers serve as instructors. Training fires may account for a large portion of firefighters and instructors' total occupational exposure to airborne contaminants, particularly for instructors who may see three to five live fires per day over a period of several weeks or even months. These exposures may increase their risk of cancer, cardiovascular disease, and other chronic diseases. A recent study of fire instructors in Australia found a dose-response relationship between estimated training exposures and cancer incidence.[6]

Fuels used for fire training varies, but often follows recommendations from National Fire Protection Association (NFPA) 1403 *Standard on Live Fire Training Evolutions* in an attempt to control the risk involved with live fires.[7] Such training scenarios will often utilize fuels like pallets and straw, which tend to produce light grey smoke for obscuring visibility. Some training institutes will also use engineered wood products such as oriented strand board (OSB) in addition to pallet and straw to produce fire conditions that more closely replicate residential structure fires (e.g., darker smoke and higher temperatures).[8] Other fire training programs have begun using simulation technologies like theatrical smoke or pepper fog to produce training environments, removing the live-fire scenarios altogether. While some dangerous airborne contaminants like PAHs and VOCs are expected to be low during simulated smoke exercises, chemical hazards like insoluble aerosols and formaldehyde have been measured at concentrations above or just below occupational exposure limits during these exercises.[9]

A number of studies have investigated firefighters' exposures during various types of live-fire training exercises, including those that used firewood, particle chipboard, plywood, and heating oil as fuel sources.[10–12] These studies generally show that firefighters can be exposed to high airborne concentrations of aromatic hydrocarbons (e.g., benzene) and PAHs during training fires. However, the potential exposure from airborne toxicants during repeated training fires has not been fully characterized, and is of particular interest for instructors who may encounter several repeated exposures over a given year.

The primary goal of this study was to gain a better understanding of the concentrations of airborne contaminants (i.e., PAHs, VOCs, acid gases, isocyanates, aldehydes, and HCN) produced during training scenarios. Over a period of several days, firefighters and instructors conducted training scenarios involving pallet and straw, OSB, and simulated smoke. Personal air samples were collected from firefighters and instructors during scenarios involving two different types of OSB and pallet and straw. Area air measurements were collected inside the structure during active fire, as well as downwind from the fire and in the background before the fire was started for all scenarios.

This study design allowed us to investigate the hazardous airborne substances instructors and firefighters are exposed to during routine training scenarios with broad applicability in the U.S. fire service. By following the same methodology, we were also able to compare airborne contaminants from this study involving training fuels with our previous study where we examined controlled residential fires containing modern furnishings.[13]

Methods

Study population

This study was performed at the University of Illinois Fire Service Institute (IFSI), with collaboration from the National Institute for Occupational Safety and Health (NIOSH) and Underwriters Laboratories (UL) Firefighter Safety Research Institute (FSRI), and was approved by Institutional Review Boards at NIOSH and the University of Illinois. Individuals with any known cardiovascular disease, gastrointestinal complications, who were pregnant, used tobacco, or were younger than 18 or older than 55 years of age were excluded from the study. All firefighters were required to have completed a medical evaluation consistent with NFPA 1582 and a self-contained breathing apparatus (SCBA) fit-test in the past 12 months. All firefighters were also required to wear their SCBA prior to entering the structure. Twenty-four firefighters (22 male, 2 female) from nine states across the United States participated in this study. Ten fire instructors (9 male, 1 female) also participated.

Study design

The study design is described in detail elsewhere.[14] Briefly, two sets of five instructors (designated Alpha and Bravo) worked alternating days (3 study days in 5 calendar days each). The study used a repeated measures design in which firefighters participated in training scenarios involving three different fuel packages and enclosures commonly used to simulate single-family residential fires. Three crews of four firefighters and five instructors were assigned to Alpha Group (Days 1, 3, and 5) and three additional crews of four firefighters and five instructors were assigned to Bravo Group (Days 2, 4, and 6). On each study day, each crew participated in one training scenario and the instructors supervised three training scenarios. The training scenarios took about 10 min to complete with 3 hr between each scenario. Each firefighter had approximately 48 hr between training scenarios and each instructor had about 40 hr between his/her last scenario of the day and the next scenario.

For all three training scenarios, the firefighters had the same objective—to suppress a two-room fire and rescue two simulated occupants of the structure. The three scenarios differed primarily by fuel package and type or orientation of the structure is described as follows.

- **Pallet and straw scenario**—Fires were ignited using three pine wooden pallets and one bale of straw in two separate bedrooms in a single-story concrete training structure. All pallets used in the study were new and had not been used for shipping or handling any materials that could potentially contaminate the wood. The structure was laid out similar to a mid-20th century single family dwelling (Supplemental Materials, Figure S1).
- **OSB scenario**—Fires ignited in burners using two pallets and one bale of straw along with OSB in each of two separate bedrooms in a T-shaped metal shipping container-based prop (Supplemental Materials, Figure S2). Two different types of OSB were used, identified in the paper as alpha OSB (used for the alpha groups) and bravo OSB (used for the bravo groups). Each type of OSB contained the same Engineered Wood Association APA rating for 7/16" thickness (panel grade 24/16, exposure 1). One-and-a-half sheet of the 7/16" alpha OSB were placed along the ceiling to provide adequate fuel supply for the training fires. Because of supply limitations, we only had access to 1/4" sheets of the Bravo OSB sheathing. One sheet of this OSB was cut in half and stacked together and then two sheets were also stacked together and placed along the ceiling. This effectively produced one-and-a-half sheets of Bravo OSB with a similar thickness and orientation to the alpha OSB fuel package. According to their safety data sheets (SDS), both OSB sheathing contained phenol formaldehyde adhesive and polymeric methylene bisphenyl diisocyanate (pMDI) adhesive, but the exact volume percentage of each is unknown. The primary difference between the SDSs for the two types of OSB was that Bravo OSB reported <0.01% of free formaldehyde, while alpha OSB reported <0.1% of free formaldehyde.
- **Simulated smoke scenario**—An electronic means of simulating a fire that also incorporated glycol-based simulated smoke generation (Attack Digital Fire System, Bullex; Albany, NY) was utilized in a building constructed from metal shipping containers to have an identical layout to a mid-20th century single family dwelling (Supplemental Materials, Figure S1)

The order in which the training fire scenarios were introduced was staggered. Alpha firefighters and instructors started with the simulated smoke scenario, then pallet and straw, and ended with the OSB scenario. Bravo firefighters and instructors began with the OSB scenario, followed by pallet and straw, and then simulated smoke.

Each crew was composed of two firefighters assigned to fire attack, who advanced the fire hose from an engine and suppressed all active fires, and two firefighters assigned to search and rescue, who performed forcible entry and then searched for and rescued two simulated trapped occupants (75 kg manikins). During each scenario, two instructors were assigned as stokers or fire starters (ignited the fuel packages and controlled ventilation for fire and smoke development) and three instructors were assigned as company officers (two supervised the attack team and one supervised the search and rescue team). Both the firefighters and instructors were required to wear a full complement of NFPA compliant personal protective equipment (PPE), including SCBA while inside the structures during the training scenarios. Instructors assigned as stokers donned their SCBA masks prior to ignition, while instructors assigned as company officers and the firefighters generally donned their SCBA masks just before entry. Some firefighters went "on-air" as soon as they exited the fire truck/engine (upon arrival at the scene), while others went "on-air" just

Table 1. Summary of area air sampling methods.

Sampling performed[A]	Scenario[B]	n	Duration of scenario (min)	Sampling time during scenario (min)[C]	Method
Acid gases: hydrogen bromide, hydrogen fluoride, hydrogen chloride, phosphoric acid	Pallet and straw	6	26–30	23–30	Silica gel tube (Supelco ORBO 53), 500 mL/min, analyzed by ion chromatography (NIOSH method 7903)
	Alpha OSB	3	25–28	12–26	
	Bravo OSB	3	25–31	7–33	
Aldehydes: acetaldehyde, acrolein, formaldehyde	Pallet and straw	6	26–30	24–31	XAD-2 tube (SKC 226-117), 200 mL/min, analyzed by GC/NPD (OSHA method 52)
	Alpha OSB	3	25–28	16–29	
	Bravo OSB	3	25–31	15–41	
	Simulated smoke	6	22–31	20–32	
Isocyanates: methyl isocyanate, methylene diphenyl diisocyanate (MDI), phenyl isocyanate	Alpha OSB	3	25–28	9–25	Asset denuder sampler (Supleco EZ4), 200 mL/min, analyzed by LC/MS/MS (ISO method 17734)
	Bravo OSB	3	25–31	12–45	
VOCs: 64 compounds	Pallet and straw	4	26–30	∼15	6 L evacuated canister with 15-min regulator and fritted pre-filter, analyzed by GC/MS (EPA method TO-15)
	Downwind	4		∼15	
	Alpha OSB	2	25–28	∼15	
	Downwind	2		∼15	
	Bravo OSB	2	25–31	∼15	
	Downwind	2		∼15	
	Simulated smoke	4	22–31	∼15	
Respirable particles (downwind only)	Pallet and straw	6	26–30	22–28	Aluminum cyclone (SKC 225-01-02), tared PVC, 2.5 L/min, analyzed gravimetrically, 50% cut-point of 4 μm
	Alpha OSB	2	25–28	25–29	
	Bravo OSB	3	25–31	21–25	

GC/NPD = gas chromatography/nitrogen phosphorous detector; LC/MS/MS = liquid chromatography/tandem mass spectrometry; GC/MS = gas chromatography/mass spectrometry; VOCs = volatile organic compounds; MDI = methylene diphenyl diisocyanate.
[A]Area air samples were also collected for PAHs, BTEX, and HCN during the simulated smoke exercises by placing samplers inside the training structure using the same methodology as for personal air sampling (n = 6 for each analyte).
[B]OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw.
[C]Occasionally, sampling time was less than the duration of the scenario because of pump faults due to extreme conditions. Sampling times higher than the scenario duration were due to a delay in turning off the sampling pumps.

prior to entering the structure. Individuals chose when to don SCBA based on their own FD policies around SCBA use. The buildings' windows and doors were opened during or shortly after fire suppression efforts to ventilate the structures as is common in coordinated firefighter training scenarios (simulating best practice on the fire ground).

After each scenario, the firefighters and instructors doffed their turnout gear in an empty gear and materials storage bay ∼60–70 m west of the burn structures, which in most cases was upwind from the prevailing wind direction, and then promptly entered an adjacent climate-controlled transport container for additional sample and specimen collections that are reported in a companion paper.[15]

Personal air sampling

Personal air samples were collected for PAHs, HCN, and benzene, toluene, ethyl benzene, and xylenes (BTEX) using NIOSH methods 5528, 6010, and 1501, respectively.[16] The sampling pumps were stored in pockets or straps on the outer shell of the turnout jackets, and sampling media were positioned near the collar of the jackets. Flow rates were set at 1 L/min for the PAH samplers and 200 mL/min for the HCN and BTEX samplers. At least two firefighters and three instructors were sampled during the live-fire scenarios (i.e., alpha OSB, bravo OSB, and pallet and straw). Personal air samples were not collected during simulated smoke scenarios because concentrations were expected to be low. Instead, area air samples for PAHs, HCN, and VOCs were collected inside the simulated smoke structure. Median sampling times for each analyte ranged from 9–12 min for firefighters and 25–30 min for instructors (Supplemental Materials, Table S1).

Area air sampling

Table 1 provides a summary of the area air sampling methods for each of the training scenarios. Tygon tubing (Saint-Gobain, Malvern, PA) was wrapped in insulation and inserted into the pallet and straw and OSB structures (Figures S1 and S2) at a height of ∼0.9 m to approximate crouching or crawling height. Areas were chosen that would be most representative of the location where firefighters were working during a large portion of the response. The tubing was attached to the inlet of the sampling media on the outside of the structures with outlet of the media being connected to sampling pumps. Use of tubing to collect air from the structure was done to protect the sampling media from hot gases. After each scenario, the tubing was rinsed with soap and water and dried with compressed air, visually removing loose particulate. However, no testing was done to determine the efficiency of cleaning. New Tygon tubing was used for each training day. For the simulated smoke scenarios—where thermal hazards did not exist—sampling trains were positioned inside the training structure also with media at ~0.9 m height. For all scenarios, the sampling pumps were started with ignition (or start of smoke generation) and stopped as soon as possible after completion of the scenario (once instructors left the scene). Afterward, sampling media were capped and stored in a -20 °C freezer prior to shipment to the laboratory.

In addition to the substrate-based sampling, we also performed whole-gas sampling to measure VOCs. Prior to sampling, a 15-min regulator was attached to an evacuated canister (6 L stainless steel). The regulator contained a 2 m piece of copper tubing with a fritted pre-filter at the end. For live-fire scenarios, this tubing was wrapped in insulation and inserted into the structures at a height of ~0.9 m, while the canisters remained outside the structures. For the simulated smoke scenarios, the canisters and tubing were placed inside the structure (with the sample inlet at ~0.9 m height). Once the fire was ignited (or smoke machine started), the regulator was opened to permit air to be collected over a ~15 min period. After this duration, the remaining pressure was recorded and the regulator was closed.

VOC samples and respirable particles were also collected downwind of the training scenarios to provide an estimate of airborne exposure potential for support personnel not directly involved in the firefighting activities. The downwind samples were ~7 m from the structures (similar to distance of incident command) and at a height of 1 m. Their downwind position was contingent on the prevailing wind direction (according to windsock) and placed in locations without nearby obstructions. No other weather conditions were monitored. In addition, VOC and respirable particle samples were collected inside the training structures before igniting fires to estimate background levels.

Data analysis

Descriptive statistics and other data analyses were carried out using SAS software (version 9.4, SAS Institute, Cary, NC). Pump faults due to overloading of sampling media with particulate were common for the area air samples (VOCs, respirable particles, aldehydes, isocyanates, and acid gases) collected during the fire period and for the personal air samples (PAHs, HCN, and BTEX). The time the pumps ran from ignition (area air) or arrival at the structure (personal air) until the end of the scenario (or when the pumps faulted) was used to calculate the volume of air collected in determining the time-weighted averaged air concentrations. Personal air samples that did not run for at least 3 min of the response were excluded because they may not accurately represent the average concentrations during the response. Three min was chosen as the cut-off because it took approximately 2 min for the firefighters to force open the prop and enter the structure, and thus would only include approximately 1 min of operation inside the structure where concentrations are expected to be the highest. In total, five PAHs, five HCN, and five BTEX personal air samples were excluded due to a sampling time of less than three min.

Total PAHs were calculated by summing the 15 quantified PAHs. Zero was used for non-detectable concentrations in this summation. Minimum detectable concentrations were calculated for non-detectable measurements by dividing the limits of detection by the volume of air collected. A Kruskal-Wallis test was used to test whether personal air concentrations varied by type of participant (instructor vs. firefighter). Further analyses using the Kruskal–Wallis test were completed to compare differences in personal air concentrations among pallet and straw, Alpha OSB, and Bravo OSB scenarios, as well as differences in area air concentrations among these different scenarios. Supplementary box-plots were created with lower quartile, median, and upper quartile indicated with the box and whiskers extending to the minimum and maximum of the distribution.

Results

Personal air concentrations for HCN, total PAHs, and VOCs

Table 2 provides a summary of the personal air concentrations grouped by type of participant (instructor vs. firefighter) and fuel package (pallet and straw, Alpha OSB, Bravo OSB) for HCN, total PAHs, and benzene. OSB scenarios (Alpha and Bravo) included two pallets and one bale of straw, while the pallet and straw scenarios consisted of three pallets and one bale of straw. As is typical of live-fire training, the entire fuel package was not consumed on any of the

Table 2. Summary of personal air concentrations by type of participant and fuel package.

Analytes	Type of participant	Type of Fuel Package/Job Assignment[B]	N	ND (%)	Median	Range	P-value firefighter vs. instructor	P-value alpha OSB vs. bravo OSB
HCN (ppm)	Instructor	Pallet and straw	28	0	0.608	0.0913–2.31	<0.01	
	Firefighter		19	0	2.240	0.691–6.96		
	Instructor	Alpha OSB	12	0	0.376	0.154–1.760	0.06	0.57
	Firefighter		9	0	0.830	0.137–2.02		
	Instructor	Bravo OSB	11	0	0.457	0.270–0.882	0.02	
	Firefighter		6	0	0.889	0.645–1.29		
Residential Fire Study HCN (ppm)[A]	N/A	Attack	13	0	33.5	4.10–100	N/A	N/A
	N/A	Search	17	29	0.085	<0.060–106	N/A	
Total PAHs (mg/m^3)	Instructor	Pallet and straw	17	0	2.78	1.23–6.89	0.02	
	Firefighter		9	0	3.39	2.27–18.10		
	Instructor	Alpha OSB	9	0	4.44	1.77– 9.21	0.07	<0.01
	Firefighter		5	0	8.33	4.95–29.9		
	Instructor	Bravo OSB	9	0	14.2	3.21–19.9	<0.01	
	Firefighter		6	0	34.0	22.2–56.4		
Residential Fire Study total PAHs (mg/m^3)[A]	N/A	Attack	19	0	23.8	7.46–78.2	N/A	N/A
	N/A	Search	16	0	17.8	9.77–43.8	N/A	
Benzene (ppm)	Instructor	Pallet and straw	28	0	3.00	1.09–7.10	<0.01	
	Firefighter		20	0	4.18	2.33–11.9		
	Instructor	Alpha OSB	12	0	4.01	0.470–12.1	0.02	<0.01
	Firefighter		11	0	7.30	2.93–25.6		
	Instructor	Bravo OSB	12	0	9.09	5.25–26.2	<0.01	
	Firefighter		10	0	31.7	18.1–54.9		
Residential Fire Study benzene (ppm)[A]	N/A	Attack	17	0	40.3	12.4–322	N/A	N/A
	N/A	Search	22	0	37.9	12.0–306		

[A]Results from Fent et al.[13] were provided for comparison.
[B]We stratified by fuel package in the current study and job assignment in the previous study. OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw.
[C]Most protective short-term occupational exposure limit for: HCN NIOSH STEL (4.700 ppm), Total PAHs ACGIH excursion limit for coal-tar pitch volatiles (1 mg/m^3), and Benzene NIOSH STEL (1.000 ppm).

scenarios, so slight differences in pre-fire fuel package weights are not expected to influence fire behavior. Note that firefighters and instructors wore SCBA while inside the structure during the trainings and were protected from inhaling these substances such that these values represent potential exposures available to those operating in these conditions, not necessarily the direct exposures. Nearly all personal air HCN concentrations were below the NIOSH STEL (4.70 ppm),[17] regardless of type of participant or fuel package. In contrast, median concentrations of benzene exceeded the STEL (1.00 ppm)[17] for both instructors and firefighters for all three fuel packages used in the live-fire scenarios. Similarly, total PAH levels exceeded the ACGIH® excursion limit for coal-tar pitch volatiles (1.00 mg/m^3)[18] for both instructors and firefighters for all three live-fire scenarios. Of the 15 PAHs analyzed in this study, naphthalene was responsible for 66–68% of the total PAH concentration depending on the fuel package (Supplemental materials, Table S2).

Personal air sampling during combustion of Bravo OSB measured higher concentrations of total PAHs and benzene compared to Alpha OSB. Median personal air concentrations of total PAHs and benzene were lower for pallet and straw compared to both types of OSB. Interestingly, firefighters training in a fire with pallet and straw as the fuel package had the highest median HCN air concentrations (although still below the NIOSH STEL).

After stratifying by type of participant, firefighters generally had higher personal air concentrations than instructors for HCN, total PAHs, and benzene. Benzene concentrations were higher for firefighters compared to instructors for all fuel packages. Total PAH concentrations for firefighters were higher than for instructors in the Bravo OSB scenarios.

Supplementary figures are provided that compare styrene, ethylbenzene and toluene (Figures S3–S5) concentrations by fuel package and type of firefighter. Results were similar to PAHs and benzene as firefighters responding to the Bravo OSB scenarios had the highest levels, but all concentrations were below each compounds' respective STEL. Area air samples of PAHs, HCN, and benzene taken during simulated smoke scenarios (instead of personal air samples) were low or near the minimum detectable concentration (< 0.0021 mg/m^3).

Area air concentrations for acid gases, aldehydes, isocyanates, and VOCs

Table 3 provides a summary of area air concentrations of acid gases inside the structure by fuel package. All acid gas concentrations were below the minimum detectable concentrations (<0.175 mg/m^3) for the simulated smoke scenarios. Hydrogen bromide and phosphoric acid concentrations were below the minimum detectable concentrations for all scenarios (< 0.826 and <0.551 mg/m^3, respectively). Hydrogen chloride and hydrogen fluoride concentrations were highest during pallet and straw scenarios compared to alpha OSB and bravo OSB, with median concentrations above the ACGIH ceiling limit (2.00 mg/m^3).[18] The hydrogen chloride and hydrogen fluoride air concentrations were similar between the Alpha OSB and Bravo OSB scenarios.

Table 4 summarizes the area air concentrations of aldehydes and isocyanates inside the structure by type of training scenario, along with the most conservative applicable exposure limits. Almost all aldehyde air concentrations measured during simulated smoke

Table 3. Area air concentrations of acid gases inside structure by type of fuel package.

Acid gases[A]	Type of fuel package[B]	n	ND (%)	Median	Range	P-value Pallet and Straw vs. Alpha OSB vs. Bravo OSB	Most protective short-term occupational exposure limit[C]
Hydrogen Fluoride (mg/m^3)	Pallet and Straw	6	0	3.84	2.97–4.72	0.01	ACGIH C: 2.00 (mg/m^3)
	Alpha OSB	3	0	1.03	0.766–1.06		
	Bravo OSB	3	0	1.93	0.500–2.31		
Hydrogen Chloride (mg/m^3)	Pallet and Straw	6	0	8.74	7.15–12.6	0.04	ACGIH C: 2.00 (mg/m^3)
	Alpha OSB	3	0	4.60	3.93–9.10		
	Bravo OSB	3	33	1.26	<0.550–6.260		

[A]Hydrogen bromide (<0.826 mg/m^3) and Phosphoric acid (<0.551 mg/m^3) were non-detect for all samples.
[B]Simulated smoke acid gas results (<0.175 mg/m^3) were non-detect for all samples. OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw.
[C]Based on review of short-term exposure limits (STELs) or ceiling limits (C) as listed with NIOSH Recommended Exposure Limits, Occupational Safety and Health Administrations (OSHA) Permissible Exposure Limits, and or ACGIH Threshold Limit Values (TLVs)®. If no STEL or C exists, ACGIH excursion limits (5x the TLV) are provided.

Table 4. Area air concentrations of aldehyde and isocyanates inside structure by type of fuel package.

Aldehydes	Type of fuel package[A]	n	ND (%)	Median	Range	P-value pallet and straw vs. Alpha OSB vs. Bravo OSB	Most protective short-term occupational exposure limit[B]
Acetaldehyde (mg/m^3)	Pallet and Straw	6	0	79.3	51.5–135	0.03	ACGIH C: 45.0 mg/m^3
	Alpha OSB	3	0	60.7	48.0–77.6		
	Bravo OSB	3	0	291	180–419		
	Simulated smoke	6	83	<0.154	<0.137–0.620		
Acrolein (mg/m^3)	Pallet and Straw	6	0	5.38	3.53–7.24	0.03	ACGIH C: 0.230 mg/m^3
	Alpha OSB	3	0	4.85	3.60–4.97		
	Bravo OSB	3	0	60.6	10.5–71.6		
	Simulated smoke	6	100	<0.497	<0.458–0.732		
Formaldehyde (mg/m^3)	Pallet and Straw	6	0	4.61	2.89–5.59	0.04	NIOSH C: 0.123 mg/m^3
	Alpha OSB	3	0	4.45	3.77–6.52		
	Bravo OSB	3	0	35.2	13.1–36.7		
	Simulated smoke	6	100	<0.133	<0.122–0.195		
Isocyanates	**Type of fuel package**	**n**	**ND (%)**	**Median**	**Range**	**P-value pallet and straw vs. Alpha OSB vs. Bravo OSB**	**Most protective short-term occupational exposure limit**
Methyl Isocyanate (µg/m^3)	Alpha OSB	3	0	20.5	11.8–52.7	0.83	ACGIH EL: 230 µg/m^3
	Bravo OSB	3	0	35.0	10.9–166		
MDI (µg/m^3)	Alpha OSB	3	100	<0.051	<0.041–0.113	0.51	NIOSH C: 200 µg/m^3
	Bravo OSB	3	0	0.273	0.031–0.831		
Phenyl Isocyanate (µg/m^3)	Alpha OSB	3	33	<0.034	<0.015–0.041	0.83	NA
	Bravo OSB	3	0	0.033	0.019–0.120		

[A]OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw.
[B]Based on review of short-term exposure limits (STELs) or ceiling limits (C) as listed with NIOSH Recommended Exposure Limits, Occupational Safety and Health Administrations (OSHA) Permissible Exposure Limits, and or ACGIH Threshold Limit Values (TLVs). If no STEL or C exists, ACGIH excursion limits (EL, 5x the TLV) are provided.

Table 5. VOCs air concentrations by location and type of fuel package.

VOCs	Fuel Type[B]	Location	n	ND (%)	Median	Range
Benzene[A] (ppm)	Pallet and Straw	Downwind	4	0	0.0033	0.000980–0.013
		Background	2	100	<0.000354	<0.000354
		Inside Structure	4	0	1.30	0.900–1.40
	Bravo OSB	Background	1	100	<0.000354	<0.000354
		Downwind	2	0	0.0665	0.041–0.092
		Inside Structure	2	0	2.57	0.049–5.10
	Alpha OSB	Background	1	0	0.00059	0.00059
		Downwind	2	0	0.0139	0.0098–0.018
		Inside Structure	2	0	0.0139	0.420–4.200
	Simulated Smoke	Background	2	50	0.000477	<0.000354–0.000600
		Inside Structure	4	50	0.000877	<0.000354–0.0021

[A]Benzene NIOSH STEL: 1.00 ppm
[B]OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw.

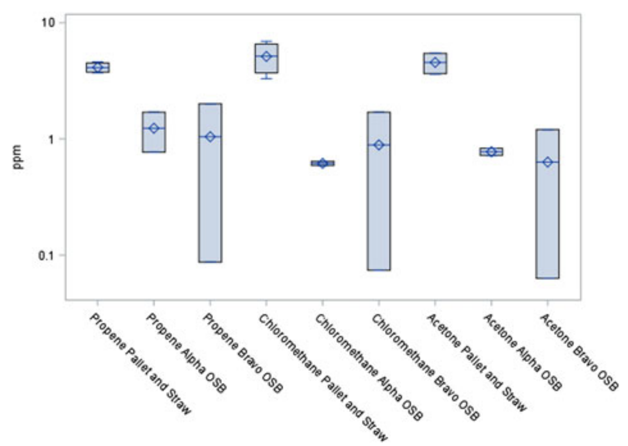

Figure 1. VOCs (ppm) area air concentrations inside structure by type of fuel package: pallet and straw (n = 4), Alpha OSB (n = 2), and Bravo OSB (n = 2). OSB scenarios also included two pallets and one bale of straw. Pallet and straw scenarios included three pallets and one bale of straw. The box and whiskers provide the minimum, 25th percentile, median, 75th percentile, and maximum values.

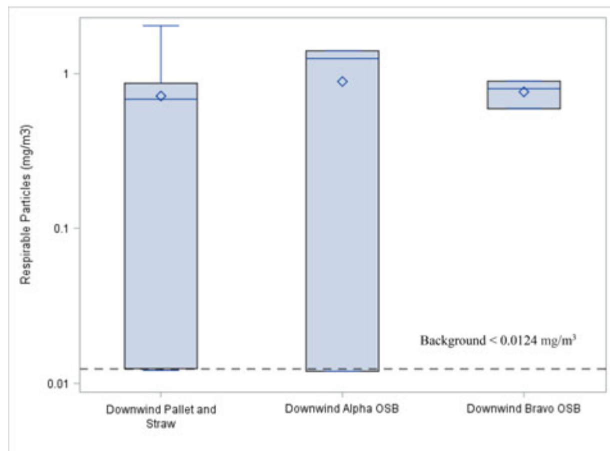

Figure 2. Downwind area air concentrations (mg/m^3) of respirable particles by type of fuel package. All background samples were non-detect. The box and whiskers provide the minimum, 25th percentile, median, 75th percentile, and maximum values.

scenarios were below detection. Interestingly, the Bravo OSB scenarios produced median air concentrations of formaldehyde, acrolein, and acetaldehyde above applicable ceiling limits that were also 4.8–12-fold higher than what was measured during the alpha OSB scenarios. Area air samples for isocyanates were only taken during the Bravo and Alpha OSB scenarios because the OSB panels were expected to contain MDI-based glues. Bravo OSB had higher median concentrations of all measured isocyanates than Alpha OSB. All concentrations of isocyanates were below their respective exposure limits (ceiling and excursion limits).

Median area air concentrations of three of the most abundant VOCs by type of fuel package are presented in Figure 1. Median concentrations of propene, chloromethane, and acetone were highest when pallet and straw were the fuel package. The VOC concentrations inside the structure were near background concentrations during the simulated smoke exercises (Supplemental Materials, Table S3). Because benzene was the most abundant VOC relative to its STEL, Table 5 provides additional information on benzene concentrations inside structure, downwind, and in the background (inside structures). Median benzene concentrations downwind were above background for all the live-fire scenarios. Benzene was highest when Bravo OSB was the fuel package, with median area air concentrations 4.8-fold higher than those measured during the alpha OSB scenario.

Figure 2 compares downwind area air concentrations of respirable particles by type of fuel package. Background concentrations of respirable particles (inside structures) were below or near detection limits. Downwind concentrations were highly variable, but median values were well above background for all live-fire scenarios. Alpha OSB had the highest median downwind concentration of 1.33 mg/m^3.

Discussion

This study provides a characterization of airborne concentrations of several chemicals during fire training scenarios commonly used in the fire service. Personal air samples were collected to allow comparisons between firefighters and instructors and between training scenarios and fuel packages. We also collected area air samples for multiple contaminants inside and downwind of the structure. The latter provides important information for personnel on the training ground who are not directly involved in the response and seldom wear SCBA.

The personal air sampling results indicate that airborne contaminants during live-fire scenarios can exceed applicable short-term occupational exposure limits, and depend largely on the participant's training ground position as well as the fuel package utilized. Table 2 compares these results to our previous residential fire study,[13] where we examined differences in airborne contaminants by fire ground job assignment and burned typical residential furnishings. Personal air concentrations of HCN in the current study were much lower (maximum = 6.96 ppm) than the residential fire study (maximum = 106 ppm) and a study by Jankovic et al.[2] (maximum = 23.0 ppm) that examined 22 fires, including 15 residential, 6 training, and 1 car fire.

The personal air concentrations of benzene measured from firefighters during the Bravo OSB scenario (median = 31.7 ppm) were similar to the residential fire study (attack firefighters median = 40.3 ppm; search firefighters median = 37.9 ppm).[13] Meanwhile, the firefighters' personal air concentrations of benzene for the other fuel packages (maximum levels ranging from 7.10–25.6 ppm) were within the ranges reported by Jankovic et al. (maximum = 22.0 ppm).[2]

We found a similar trend when examining personal air concentrations of total PAHs, whereby firefighters' concentrations during bravo OSB exercises (median = 34.0 mg/m^3) were similar to the attack firefighters (median = 23.8 mg/m^3) and search firefighters (median = 17.8 mg/m^3) in the residential fire study.[13] Personal air concentrations of total PAHs for the other fuel packages (range in medians: 2.78–8.33 mg/m^3), however, were lower than the residential fire study, but within the ranges reported previously for particleboard training fires (0.430–2.70 mg/m^3).[12]

When we stratified by type of participant, firefighters had higher personal air concentrations of every compound compared to instructors, regardless of type of fuel package. However, the instructors' sampling times were longer than firefighters' (∼25 min vs. ∼10 min), and included periods of relatively low exposure during job assignments like ignition and cleanup. These important differences in assigned activities may be the primary reason for the observed differences in personal air concentrations by participant type. Another factor that could affect these results is that firefighters completing search and rescue and fire attack jobs are typically closer to the source of the fires than the instructors, although instructors are often oriented a bit higher in the compartment. While SCBA protects firefighters from airborne contaminants, previous results suggest airborne chemicals can still be absorbed through the skin during firefighting.[19,20] Thus, efforts should be taken to reduce personal air concentrations (and the overall burden) when feasible.

According to our area air sampling results, the pallet and straw scenario produced the highest concentrations of hydrogen fluoride and hydrogen chloride of all the scenarios, with median levels above applicable ceiling limits. Hydrogen bromide and phosphoric acid were not detected in any of the scenarios. Area air samples from the residential fire study found levels of hydrogen chloride (median = 7.33 mg/m^3) that were similar to those found when pallet and straw was burned (median = 8.74 mg/m^3). Hydrogen bromide (median = 6.78 mg/m^3) results were higher in the residential fire study than those reported here, while hydrogen fluoride concentrations were lower in the residential fire study (median < 0.190 mg/m^3). The source of these halogens is unknown, especially for pallets (pinewood) and straw, but it is possible that the fuel packages were contaminated with chlorinated or fluorinated compounds from unknown treatments. Pallets used in this study were not used to transport any material between the time they were constructed and delivered to IFSI specifically for this study.

Our area air sampling results show that aldehyde concentrations were highest for the Bravo OSB exercises. Among the aldehydes assessed in this study, acetaldehyde was the most abundant and had the highest median concentration at 291 mg/m^3 during the bravo OSB scenario (exceeding its ceiling limit). Although less abundant than acetaldehyde, median concentrations of formaldehyde and acrolein were above their applicable ceiling limit for all live-fire scenarios. In another study examining aldehyde levels during emergency structure fire responses, maximum concentrations of formaldehyde (9.83 mg/m^3), acrolein

(7.34 mg/m^3), and acetaldehyde (14.6 mg/m^3)[2] were lower than the levels reported here.

Isocyanate concentrations were also highest during the bravo OSB scenarios. To our knowledge, this is the first study to quantify airborne isocyanates during training fires. Diisocyanates (e.g., MDI) are known respiratory sensitizers and exposures should be controlled to the lowest feasible levels.[21] We were not able to identify exact proportions of different adhesives in the two OSB products as this is proprietary information, but these results suggest that bravo OSB (with <0.01% free, unbounded formaldehyde) may have contained higher amounts of MDI-based adhesives than the alpha OSB (with <0.1% free, unbounded formaldehyde), as area samples during alpha OSB scenarios were non-detect for MDI. Combustion of the MDI-based adhesives could have also contributed to higher airborne concentrations of the other isocyanates and aldehydes.

Median area air concentrations of respirable particles downwind from the training structures were highest for the alpha OSB scenario, but median downwind concentrations for all live-fire scenarios were well above background (>12.7 μg/m^3). Benzene concentrations downwind of the live-fire scenarios were also above background and highest for bravo OSB (0.0665 ppm). These results are similar to the residential fire study where median benzene concentrations downwind of the structure were 0.210 ppm. These results corroborate previous findings indicating that support personnel in the fire ground can be exposed to combustion byproducts, especially when they are downwind of the structure.

Air concentrations for the majority of chemicals of interest were highest for the Bravo OSB scenarios followed by Alpha OSB, pallet, and straw, and then simulated smoke. The notable exceptions to this trend were with some of the VOCs and acid gases. While personal air concentrations of styrene, benzene, ethylbenzene, and toluene followed this trend (Supplemental Materials), some area air concentrations of other VOCs and acid gases did not. Specifically, area air concentrations of propene, chloromethane, acetone, hydrogen chloride, and hydrogen fluoride were highest for the pallet and straw scenarios. Despite these results, our overall findings suggest that burning OSB releases more airborne toxicants than pallet and straw or simulated smoke.

When comparing personal air concentrations to area air concentrations of benzene, we uncovered marked differences. Median personal air concentrations of benzene were 2–10 times higher than area air concentrations of benzene. Benzene is heavier than air (vapor density = 2.7), and may have partitioned to the lower part of the structure where the firefighters were crawling or crouching during the training.[13] Moreover, firefighters were closer to the source of contamination compared to the area air samples (located near an exterior wall). It is also possible that some of the benzene and other vapors condensed in the copper tubing leading to the evacuated canisters. However, the tubing was wrapped in insulation to minimize this effect. Regardless of the cause, the area air concentrations may not accurately represent the levels encountered by the firefighters and instructors inside the training structures.

Other limitations of this study include the high frequency of sampling pump faults and variability in training and environmental conditions that could influence the measured air concentrations. To address these limitations, personal and area air samples that did not run for at least three min of the training exercise were excluded. No testing was done to determine the efficiency of our process for cleaning tubing after each scenario. However, soap and water removed most of the loose particulate, and sampling tubing was replaced each day. Another limitation to this study is the low sample size for area air samples. However, we designed our study to ensure repeatable fuel loads and conditions over multiple days to permit comparisons between scenarios and fuel packages.

Conclusions

This study suggests firefighters and instructors operate in high concentrations of airborne contaminants during training fires that can potentially result in systemic exposures. Maximum area and personal air concentrations during the fire period of the OSB and pallet and straw scenarios were above applicable short-term occupational exposure limits for many of the measured compounds, including PAHs, benzene, acrolein, formaldehyde, and hydrogen chloride. Formaldehyde concentrations of this magnitude are noteworthy, particularly during bravo OSB scenarios where concentrations were over 280 times higher than the NIOSH ceiling limit. Efforts should be taken to minimize the use of OSB during training fires where appropriate, particularly when possible to meet training objectives without the use of this material. Area air concentrations inside the structure during the simulated smoke exercises were well below applicable exposure limits, and so, this type of training scenario would likely expose firefighters to the least amount of chemicals analyzed in this study. Chemical concentrations downwind of the training structures were above background but an order of magnitude below applicable exposure limits. When possible, efforts should be taken to position the fire apparatus and command post upwind from the burning structure. Regardless of the scenario, firefighters and instructors should wear SCBA throughout the entire training response to protect their airways, including donning SCBA before entering the structure or areas where any level of visible smoke is present (including light haze). Dermal absorption of some of the contaminants is also possible during live-fire training, and so, efforts should be taken to wear all NFPA-compliant PPE during exercises, while also cleaning skin and clothing as soon as possible post-fire. If OSB is to be used, it is suggested that training institutes should attempt to purchase OSB with the least amount of synthetic adhesives.

Acknowledgments

This was a collaborative project that could not have been completed without the help from several people. We thank Melissa Seaton, Adrienne Eastlake, and Myles O'Mara for their help in collecting the area air samples, Kelsey Babik for performing the personal air monitoring, and Kenneth Sparks for preparing and maintaining our sampling equipment along with support staff from the Illinois Fire Service Institute, in particular Chief Sean Burke who ran incident command for each of these scenarios. Most of all, we thank the firefighters and instructors for participating in this study. Participants were compensated up to $599 to participate in this study. This study was approved by the Institutional Review Boards at NIOSH and the University of Illinois.

Funding

This study was funded through a U.S. Department of Homeland Security, Assistance to Firefighters Grant (EMW-2014-FP-00590). This project was also made possible through a partnership with the CDC Foundation. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of NIOSH. Mention of any company or product does not constitute endorsement by NIOSH.

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March 30, 2022

The Honorable Paul G. Pinsky
Chairman
Education, Health and Environmental Affairs Committee
2 West Miller Senate Office Building
11 Bladen Street
Annapolis, Maryland 21401

Dear Chairman Pinsky and Committee Members:

I am writing to communicate our position of support for House Bill 1381- Fire Fighting Training-Oriented Strand Board (OSB)-Prohibition.

As you know, there are tremendous concerns regarding various chemicals produced during live fire training in the Firefighter Training Environment. Many of these chemicals have been found to be carcinogens with health hazards to humans and animals. The Maryland Fire and Rescue Institute at the University of Maryland recognized the potential problems associated with the production of hazardous/harmful chemicals in the fire training environment many years ago.

As the State's comprehensive training and education system for all EMS/Fire/Rescue programs we train on average more than 35,000 Maryland emergency responders a year. We are happy to report that this product is not used at any of our seven statewide training facilities. In fact, we have moved to a healthier and environmentally safer alternative during live fire training.

I want to thank you for continued support of the EMS/Fire/Rescue Service. I hope you find this information helpful. If you have any questions or require additional information, please feel free to contact me.

Sincerely,

Michael E. Cox, Jr.

Michael E. Cox, Jr., MS, CFO, EFO, NRP
Executive Director

MEC/mec

CC: Mr. F. Patrick Marlatt, Deputy Director
Mr. Stephan Cox, Operations Section Chief
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HB 1381 Environment – Fire–Fighting Training – Oriented Strand Board – Prohibition

My name is Robert Phillips and I am the Legislative Committee Chairman for the Maryland State Firefighter's Association (MSFA).

I wish to present testimony in favor of **House Bill 1381: Environment – Fire–Fighting Training – Oriented Strand Board – Prohibition**

According to a 2019 study published in the International Journal of Hygiene and Environmental Health titled "Firefighters' and instructors' absorption of PAHs and benzene during training exercises" (Fent, Toennis, et al.), "training fires may constitute a major portion of some firefighters' occupational exposures to smokes." The study found that instructors who were carrying out 3 trainings per day had a 3.5-fold greater concentration of these carcinogens in their systems compared to the firefighters that took part in a residential fire study.

The MSFA fully supports this bill. We feel that anything that can be done to protect our firefighters and training staff should be done. During an emergency we have to do the best we can to protect ourselves given the situation. When training we can determine the risk ahead of time and should do so. The elimination of OSB is a step in the right direction to protect our firefighters and training staff.

I thank the committee for their time on this important issue and ask that you FAVORABLY support House Bill 1381.

I will gladly answer any questions you may have