Environmental Science & Technology

Article

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Elevated Exposures to Polycyclic Aromatic Hydrocarbons and Other Organic Mutagens in Ottawa Firefighters Participating in Emergency, On-Shift Fire Suppression

Jennifer L. A. Keir,[†] Umme S. Akhtar,[†] David M. J. Matschke,[‡] Tracy L. Kirkham,[§] Hing Man Chan,[†] Pierre Ayotte,[⊥] Paul A. White,*[,†,∥] and Jules M. Blais*[,†]

[†]Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario K1N 6N5, Canada

[‡]Ottawa Fire Services, 1445 Carling Avenue, Ottawa, Ontario K1Z 7L9, Canada

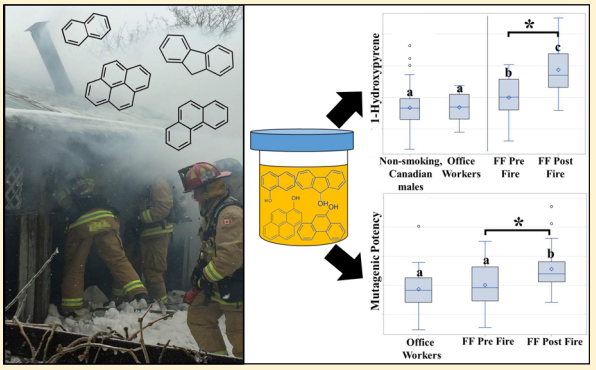
[§]Dalla Lana School of Public Health, University of Toronto, 155 College Street, Toronto, Ontario M5T 3M7, Canada

[∥]Environmental Health Science and Research Bureau, Health Canada, 50 Colombine Driveway, Ottawa, Ontario K1A 0K9, Canada

[⊥]Centre de toxicologie du Québec, Institut national de santé publique du Québec and Université Laval, 945 Avenue Wolfe, Québec City, Québec G1V 5B3, Canada

Supporting Information

ABSTRACT: Occupational exposures to combustion emissions were examined in Ottawa Fire Service (OFS) firefighters. Paired urine and dermal wipe samples (i.e., pre- and post-event) as well as personal air samples and fire event questionnaires were collected from 27 male OFS firefighters. A total of 18 OFS office workers were used as additional controls. Exposures to polycyclic aromatic hydrocarbons (PAHs) and other organic mutagens were assessed by quantification of urinary PAH metabolite levels, levels of PAHs in dermal wipes and personal air samples, and urinary mutagenicity using the Salmonella mutagenicity assay (Ames test). Urinary Clara Cell 16 (CC16) and 15-isoprostane F_{2t} (8-iso-$PGF_{2\alpha}$) levels were used to assess lung injury and overall oxidative stress, respectively. The results showed significant 2.9- to 5.3-fold increases in average post-event levels of urinary PAH metabolites, depending on the PAH metabolite ($p < 0.0001$). Average post-event levels of urinary mutagenicity showed a significant, event-related 4.3-fold increase ($p < 0.0001$). Urinary CC16 and 8-iso-$PGF_{2\alpha}$ did not increase. PAH concentrations in personal air and on skin accounted for 54% of the variation in fold changes of urinary PAH metabolites ($p < 0.002$). The results indicate that emergency, on-shift fire suppression is associated with significantly elevated exposures to combustion emissions.



INTRODUCTION

Compared to the general population, firefighters experience increased risk of injuries and chronic diseases including kidney, ureter, and pancreatic cancers, respiratory diseases, and heritable genetic effects.[1−3] In fact, in 2007 the International Agency for Research on Cancer (IARC) declared occupational exposures from firefighting as possibly carcinogenic to humans.[4] Exposures to carcinogens during firefighting can occur via contact with combustion byproducts from diesel and automobile exhaust, from other motorized equipment, and from live or smoldering fires. Many hazardous compounds have been detected at municipal structural fires including formaldehyde, benzene, benzyl chloride, freon, acetic acid, and polycyclic aromatic hydrocarbons (PAHs).[5] PAHs are of concern due to their ubiquitous formation during the incomplete combustion of organic matter and their mutagenic, carcinogenic, and teratogenic properties.[6]

PAH exposure can be assessed in a number of different ways including urinary monitoring, personal air monitoring, and dermal wipe sampling. Urinary monitoring is a convenient, noninvasive PAH exposure assessment technique that involves quantification of the concentrations of specific PAH metabolites that are reported to be indicative of combustion emission exposure.[7−9] Numerous studies have employed urinary concentrations of hydroxylated PAH metabolites to assess occupational exposures to combustion emissions and PAHs in facilities or environments associated with charcoal production, road paving, rubber manufacturing, production of coke and refined metals, cooking, and wildland firefighting.[10−15] Interestingly, firefighters can be engaged in a variety of

Received: June 6, 2017
Revised: September 15, 2017
Accepted: September 17, 2017

DOI: 10.1021/acs.est.7b02850
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

occupational activities that can contribute to vastly different combustion emission exposure levels (e.g., active fire suppression, overhaul, vertical ventilation, etc.).[16,17]

Because combustion emissions are complex mixtures, it can be useful to employ exposure biomarkers that do not require a priori information regarding the composition of the mixture and/or the identity of the putative contaminants. For example, a bioassay such as the Ames−Salmonella reverse mutation assay (Ames test) can be used to provide a comprehensive assessment of overall mutagen exposure by monitoring the mutagenic activity of deconjugated metabolites in urine extracts. The technique has been employed to assess urinary mutagenicity in subjects exposed to complex combustion emissions of coal and biomass fuels, selected industrial chemicals, and airborne particulate matter at urban and nonurban locations.[18−20] More specifically, studies have assessed urinary mutagenicity in benzidine-exposed factory workers in India, wood smoke-exposed charcoal workers in Brazil, and wood-smoke-exposed individuals using traditional Native American saunas. These studies showed positive correlations between exposure metrics (e.g., duration) and urinary mutagenicity.[10,21,22]

In addition to urinary monitoring of chemical metabolites and mutagenicity, urinary biomarkers of physiological condition have also been used for occupational monitoring (e.g., isoprostanes and Clara Cell secretory protein (CC16)). Isoprostanes are products of free-radical fatty acid peroxidation that can be generated and excreted in the urine as 15-isoprostane F2t (i.e., 8-iso-$PGF_{2\alpha}$). 8-Iso-$PGF_{2\alpha}$ has been shown to be a reliable biomarker of in vivo lipid peroxidation and oxidative stress in smokers, individuals with systemic sclerosis disorder, and Olympians exposed to air pollution during the Beijing Olympics.[23−26] Moreover, Wang et al. showed that co-exposure to PAHs and toxic metals contributed to dramatic increases in oxidative stress, demonstrated by increases in urinary 8-iso-$PGF_{2\alpha}$.[27] Previous studies investigating pulmonary injury from environmental exposures have shown increases in the urinary levels of CC16 in rats exposed to O_3, as well as in elite swimmers exposed to chloramine; however, no significant changes in CC16 were observed in individuals exposed to second-hand tobacco smoke or wood smoke.[28−31] Interestingly, significant increases in serum CC16 have been shown in firefighters examined after fire suppression, suggesting lung injury resulting from occupational exposure.[32,33]

Several studies have used analyses of skin and gear wipes, air, or urine to examine firefighters' toxicant exposures. Analyses of personal protective equipment (PPE) and skin wipes after live fires found multiple PAHs, including the known carcinogen benzo(*a*)pyrene, on at least one piece of an individual's equipment. Benzofluoroanthenes (i.e., benzo[*bjk*]fluoranthene) were detected on 65% of skin wipe samples.[34,35] At training fires, Fent et al. noted that carcinogenic and probably carcinogenic PAHs represent 0.8−5.7% of PAHs found in personal air samples; moreover, significant post-training increases in PAHs in neck-wipe samples.[17] Similarly, Britz-McKibbin et al. examined exposures associated with firefighter training activities and found significant increases in urinary PAH metabolites and dermal PAH concentrations.[36] Few studies have examined the impact of on-shift fire suppression on firefighters' exposures to combustion emissions and PAHs. Caux et al. examined urinary PAH metabolite changes after on-shift fire suppression and found significant increases in urinary 1-hydroxypyrene. However, reference samples used for that study, which are supposed to indicate baseline metabolite levels, were collected from the same individuals several days after the fire event.[37] This is an unfavorable practice because daily variations in urinary metabolite levels related to, for example, changes in air quality or diet, can lead to baseline values that are poorly matched with post-exposure values.

To the best of our knowledge, no published study has rigorously examined occupational, on-shift exposures of municipal firefighters' to PAHs and other organic mutagens. Ottawa firefighters attended 715 fires in 2015 alone; thus, there is ample opportunity to conduct on-shift exposure assessments to PAH-contaminated combustion emissions.[38] This study employed analyses of paired urine samples, collected before and after fire suppression events, to examine exposures associated with on-shift fire suppression. Spot urine samples were also collected from Ottawa Fire Services (OFS) office workers (i.e., an unexposed control group employed by the same institution). Urinary PAH metabolite and mutagenicity analyses were employed to assess event-related exposures to PAHs and other organic mutagens, and measurements of PAHs in personal air and dermal-wipe samples provide an indication of external exposures and environmental contamination. The results provide an improved understanding of municipal firefighters' occupational exposures to PAHs and other organic mutagens, and this information can be used to design and implement policies and procedures to reduce exposures and the associated health hazards.

MATERIALS AND METHODS

Research ethics approval was obtained from the University of Ottawa Research Ethics Board (i.e., H07-14-01B) and Health Canada's Research Ethics Board (i.e., REB 2014-0035). All subjects signed informed consent forms provided in their language of choice (i.e., French or English).

To maximize the likelihood of sample collection, OFS stations that historically had the highest frequency of firefighting calls were selected. Eligible participants (i.e., both firefighters and office-worker controls) were never smokers who do not live with smokers and who agreed not to consume charbroiled foods or be exposed to non-occupational combustion sources for the duration of enrollment. Each participant completed a detailed questionnaire about their personal habits, overall health, and the nature of their employment (i.e., duration, secondary employment, etc.); each was given an ID code for anonymity and that code was used to label all samples. Firefighter participants were recruited for study blocks of 5 consecutive 24 h shifts typically spanning 12 days. An overview of the sample collection strategy is illustrated in Figure S1. At the beginning of each shift, pre-exposure samples were collected, including urine and wipe samples of skin, under-gear clothing, and PPE. In the event of a fire, firefighters activated a personal air sampling pump immediately following deployment, and the pump operated until the fire suppression activity was complete. Post-exposure samples were collected as soon as possible after return to the station and included matching wipe samples (i.e., skin, under-gear clothing, and PPE) and an integrated 18 h urine sample. The 18 h post-fire sampling period was chosen as it encompasses two times the half-life for many of the PAHs investigated. More specifically, Brzeźnicki, Jakubowski, and Czerski (1997) showed the half-life of urinary 1-OHP to be 6.0−9.0 h for inhalation, 4.4−12 h for ingestion, and 11.5−15 h

DOI: 10.1021/acs.est.7b02850
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

for dermal contact.[39] The urinary elimination half-life of fluorene is also in this range (i.e., 4.1−8.2 h) as is phenanthrene (i.e., 3.5−5.1) and naphthalene (i.e., 2.5−4.3).[40,41] Furthermore, the 18 h post-fire sampling period was pragmatic for compliance and sample volume; a questionnaire detailing the fire suppression event was also completed (e.g., role in fire suppression, type and scale of fire, etc.). All samples were stored in a locked −20 °C freezer located at the fire station until collection by university research staff. Urine and wipe samples were also collected from the control group of OFS office personnel, as were air samples from three office locations. Once initiated, the study continued until samples from 30 events were collected.

Urine Samples. Pre-shift spot urine samples were collected using 120 mL polypropylene containers. Post-exposure 18 h integrated urine samples (i.e., all urine for 18 h) were collected using 500 mL high-density polyethylene (HDPE) containers. If the end of the 18 h sample collection period fell after the 24 h shift was completed, a kit was provided for continued post-shift sample collection. Subjects stored the urine samples in a domestic freezer until return to the fire station at the start of the next shift. To create integrated 18 h post-fire urine samples, the collected urine was pooled in 2 L HDPE beakers. To prevent extraneous contamination, HDPE beakers were soaked in 10% nitric acid for 24 h followed by 5 rinses with Milli-Q water (EMD Millipore, Etobicoke, ON, Canada) and 3 rinses with deionized water. Both pre- and post-exposure urine samples were aliquoted into 15 mL presterilized polypropylene tubes and stored at −20 °C until analysis. Samples for biomarker analyses were stored at −80 °C to prevent degradation of CC16 and 8-iso-$PGF_{2\alpha}$.

Urine aliquots were shipped on dry ice to the ISO/IEC 17025- and ISO/IEC 17043-accredited Human Toxicology Laboratory of the National Institute of Public Health of Quebec (INSPQ) (Quebec City, Quebec, Canada) for analysis of urinary PAH metabolites via gas chromatography−tandem mass spectrometry as previously described.[42] Briefly, urinary metabolites were deconjugated using β-glucuronidase in 5 mL of a pH 5.0 sodium acetate buffer solution, extracted twice with hexane, and derivatized with *N*-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). Samples were spiked with 25 μL of a recovery standard solution (1-methoxyfluorene 50 μg/L in benzene) prior to injection. The same 19 urinary PAH metabolites measured for the Canadian Health Measures Survey (CHMS), a national biomonitoring program in Canada, were determined and expressed as μg/g creatinine.[42] Table S2 provides a list of the PAH metabolites analyzed as well as their parent PAHs. Samples below the detection limit were assigned a value equal to the detection limit divided by the square root of 2. Samples were thawed and aliquoted in batches, with each batch including a blank of Milli-Q water (EMD Millipore, Etobicoke, Ontario, Canada). Method blanks were run for each analysis batch (N = 14); detectable levels were subtracted (0−0.004 μg/L) from each value in the respective batch. Metabolites were grouped by their parent PAH (i.e., pyrene is metabolized to 1-hydroxypyrene; naphthalene to 1-hydroxynaphthalene or 2-hydroxynaphthalene; fluorene to 2-hydroxyfluorene, 3-hydroxyfluorene, or 9-hydroxyfluorene; and phenanthrene to 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, or 9-hydroxyphenanthrene), and the results expressed as the sum of the metabolites for each PAH. Urinary creatinine concentration was used to correct for urinary dilution. Urinary mutagenicity was measured using the Ames−Salmonella reverse mutation assay (i.e., the Ames test) with *Salmonella typhimurium* strain YG1041 (kindly provided Dr. Takehiko Nohmi, NIHS, Tokyo, Japan) in the presence of an exogenous metabolic activation mixture containing Aroclor-induced rat liver S9 (i.e., Molecular Toxicology Inc., Boone, NC).[43] In preparation for the Ames test, samples were filtered, enzymatically deconjugated using β-glucuronidase and sulfatase in pH 5.0 acetate buffer, and concentrated using C18 solid-phase extraction with methanol elution.[22] An initial range-finding test found that many samples were cytotoxic at 6.0 mL equivalents per plate; thus, the concentrations used were 0.3, 0.6, 1.2, 2.4, and 4.5 mL equivalents per plate. All concentrations were tested in triplicate with the exception of some samples with limited quantities. A simultaneous positive control (i.e., 0.1 μg/plate 2-aminoanthracene, Molecular Toxicology Inc.), and negative solvent control (i.e., DMSO) were examined to assess assay performance on each test day. Samples were incubated at 37 °C for 72 h before the frequency of revertant (rev) colonies was scored using a ProtoCol automated colony counter (Synbiosis Corporation, Exton, PA). The mean positive control response was 1371.5 ± 65.5 rev/plate, and the mean negative control was 36.7 ± 1.3 rev/plate. Mutagenic potency was calculated as the slope of the initial linear portion of the concentration−response function and expressed as rev/μmol of creatinine.[10,44] Urinary creatinine concentration was used to correct for urinary dilution.

A pair of urinary biomarkers (i.e., CC16 and 8-iso-$PGF_{2\alpha}$) were analyzed using ELISA kits (BioVendor R and D, Asheville, NC and Oxford Biomedical, Abingdon, UK) according to the manufacturer's instructions. Urinary creatinine was measured using a colorimetric assay (Oxford Biomedical, Abingdon, UK).

Air and Dermal-Wipe Samples. This paper focused on comparing urinary metrics with dermal wipe and personal air results. PAH levels on PPE wipes and under-gear clothing will be published separately. Pre- and post-event dermal wipe samples were collected using AlphaWipes prewetted with 70% isopropyl alcohol (Texwipe Inc., Kernersville, NC). Participants received training regarding proper sample collection procedures for all surfaces and areas. Moreover, the importance of sample collection uniformity was explained to each participant. Briefly, participants were instructed to wear clean nitrile gloves and use a 6 cm × 5 cm template to ensure consistent sampling of a 30 cm^2 area. Samples of the right side of the forehead, neck, and wrist were each collected using the 6 cm × 5 cm template. To expedite sample analyses, the wipes from the three locations were pooled. Before- and after-event samples were collected using identical procedures.

Personal air samples were collected using a GilAir Plus pump (Levitt Safety, Ottawa, ON) operating at 2.5 L/min with a polyurethane foam (PUF) cartridge (URG, Chapel Hill, NC) and a QM-A 25 mm quartz filter (Whatman, Maidstone, UK). PUFs were cleaned with a 1:1 v/v mixture of Optima grade acetone and hextane using 2 static cycles of pressurized fluid extraction at 2000 psi for 5 min each at 75 °C. The filters were baked at 400 °C for 5 h. The pumps were calibrated before and after sampling using a Gilian Gilibrator-2 NIOSH primary standard air flow calibrator (Levitt Safety, Ottawa, ON) for quality control. Average pre- and post-calibration pump flow rates were accepted for samples with less than a 5% difference. The sampling pump was placed in the inside pocket of the Bunker gear coat, and polypropylene tubing along the inside of the coat connected the pump and the sample collection

DOI: 10.1021/acs.est.7b02850
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 1. Self-Reported Participant Ages and Health Metrics[a]

			fitness level					weight			overall health				
	N	average age (range)	poor	fair	good	very good	excellent	underweight	just about right	overweight	poor	fair	good	very good	excellent
office workers	17	50 (28–62)	1	3	8	3	2	0	6	11	0	1	10	4	2
firefighters	16	34 (25–50)	0	0	8	6	2	1	11	4	0	1	5	8	2

[a]Values indicate response frequency.

cartridge, which was affixed to the back of the neck with hook-and-loop fastener (Figure S1) at the beginning of each shift.

Air and Skin-Wipe Sample Analysis. Dermal wipe and air samples were analyzed to determine the concentrations of 16 priority PAHs (Table S1).[4] Briefly, samples were spiked with ^{13}C-labeled standards of the U.S. Environmental Protection Agency (U.S. EPA) 16 Priority PAHs (Cambridge Isotope Laboratories Inc., Andover, MA) and extracted using accelerated solvent extraction (ASE-350, Dionex Corporation, Sunnyvale, CA) according to U.S. EPA Method 3640A.[45] Wipe samples required a liquid–liquid extraction step with high-performance liquid chromatography water. PAHs from both wipe and air samples were then isolated on two solid-phase extraction (SPE) cartridges (i.e., 3 mL of alumina (Supelco, Bellefonte, PA) followed by 1 g of silica (Supelco, Bellefonte, PA)). Samples were concentrated to approximately 1 mL before being spiked with an internal standard (i.e., *p*-terphenyl-d14, Cambridge Isotope Laboratories, Tewksbury, MA) and analyzed using an HP 6890 GC coupled with a HP 5973N mass selective detector (Agilent Technologies, Santa Clara, CA). Using a DB5-MS 30 m, 0.25 mm, 250 μm column and single-ion monitoring, the concentrations of PAHs were determined with isotopic dilution calculations via the ^{13}C-labeled PAHs added into the samples at the time of extraction. Table S1 provides a list of the PAHs examined. PAH levels in dermal wipes were expressed as nanograms per square centimeter of the sampled surface. Personal air PAH levels were expressed as micrograms per cubic meter.

Statistical Analyses. Statistical analyses were conducted using SAS v9.2 for Windows (SAS Institute, Cary, NC) and Microsoft Excel. Normality was visually assessed (i.e., box-plot and Q–Q plot). When necessary, values were log-transformed to ensure normality of residuals and variance homogeneity across treatment groups; variance homogeneity was assessed using the Bartlett test. Mutagenic potency values were determined using ordinary least-squares linear regression analysis, and studentized deleted residuals were examined to objectively identify concentration–response outliers.[46] A paired *t*-test was used to compare pre- and post-event levels of creatinine-adjusted urinary PAH metabolites, mutagenicity, CC16, and 8-iso-PGF$_{2\alpha}$.[22,47,48] Single-factor analysis of variance (ANOVA) and Duncan's multiple range tests were employed to compare mean creatinine-adjusted PAH metabolite concentrations between pre-event firefighters, post-event firefighters, OFS office workers who do not participate in fire suppression, and nonsmoking Canadian males aged 25–62 surveyed as part of the Canadian Health Measures Survey (CHMS). Similar analyses were conducted to compare creatinine-adjusted urinary mutagenicity, creatinine-adjusted urinary CC16, and creatinine-adjusted urinary 8-iso-PGF$_{2\alpha}$ levels. Least-squares linear regression was employed to investigate empirical relationships between fold changes in creatinine-adjusted PAH metabolite levels or fold changes in creatinine-adjusted urinary mutagenicity levels and variables that reflect the duration and the magnitude of the exposure (e.g., time at fire, personal air PAH concentrations, and dermal PAH concentrations), as well as demographics (e.g., age) and health status (e.g., weight and fitness level), with levels of urinary biomarkers. Regression diagnostics (i.e., leverage and Cook's distance values and studentized residuals) were calculated according to Neter et al. (1990).[49]

RESULTS AND DISCUSSION

Participants. Samples were successfully collected from 27 firefighters and 17 office worker volunteers between January 2015 and April 2016. Office worker samples (N = 21) were collected from three office locations. In cases in which two samples from the same office worker were collected within 72 h of each other (N = 3 subjects), values were averaged to avoid the possibility of temporally overlapped excretion levels being counted as separate observations. Because all firefighter participants were male, and significant gender-specific differences in urinary creatinine concentrations have been noted in the literature, female office workers were omitted from subsequent analyses (N = 4), leaving a total of 18 office worker samples from 17 individuals.[50]

A total of 31 paired (i.e., pre- and post-fire) samples were collected from 16 firefighters who participated in emergency suppression at 19 fires; there were several instances of multiple participants attending the same fire. Details of variability of exposure between these individuals are detailed at Keir (2017).[51] Most of the paired urine samples (i.e., 29 of 31) had matching wipe (i.e., PPE, clothing, and skin) and personal air samples. All samples were collected during and after structural fires at residential or commercial buildings. There were three instances of multiple fires occurring during one 24 h shift. One incident involved participants attending two fire events before collecting post-fire samples, and another involved participants attending a second fire after starting post-fire sample collection associated with a first fire. A third involved two participants attending a fire, completing the collection of post-fire event samples including the 18 h urine sampling period, and then attending a second fire before their 24 h shift was completed. This latter situation was treated as two separate fire-suppression events.

Details regarding age, weight, and overall health of the subjects are summarized in Table 1. All participants (i.e., office workers and firefighters) indicated that their health and fitness levels were fair to excellent; one office worker indicated a "poor" fitness level. Office workers were significantly older (p < 0.0001), and the majority reported being "overweight". The majority of firefighters reported their body weight as "just about right".

Urinary PAH Metabolites. For most samples, 1-hydroxybenz(*a*)anthracene, 2-hydroxychrysene, 3-hydroxybenz(*a*)anthrancene, 3-hydroxybenzo(*a*)pyrene, 3-hy-

DOI: 10.1021/acs.est.7b02850
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

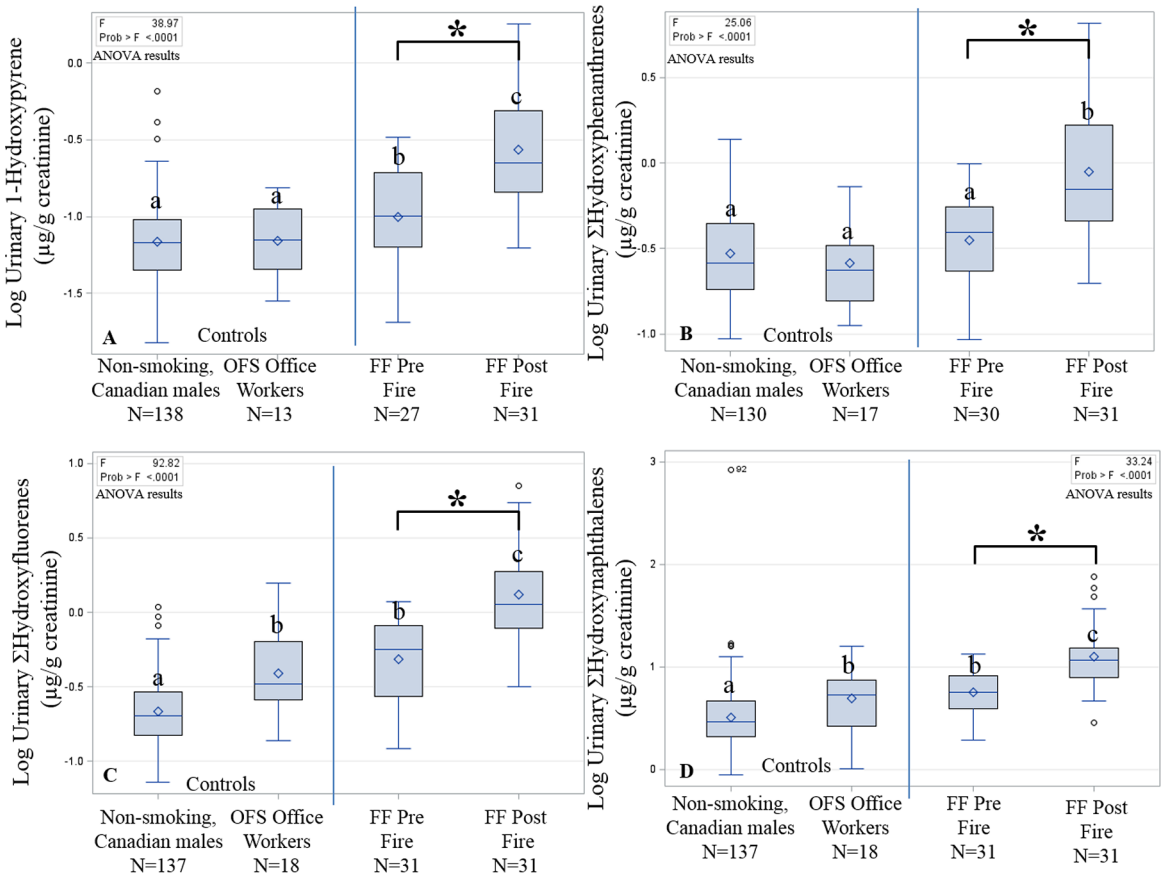


Figure 1. Box-plots summarizing urinary concentrations of (A) 1-hydroxypyrene, (B) Σhydroxyphenanthrenes, (C)Σhydroxyfluorenes, and (D) Σhydroxynaphthalenes in nonsmoking Canadian males aged 25−62, OFS office workers, firefighters before a fire-suppression event (FF Pre-Fire), and firefighters after a fire-suppression event (FF Post-Fire). Boxes with the same letter are not significantly different at $p < 0.05$. The box limits represent the interquartile range (i.e., 25th to 75th percentile), the diamonds represents the mean value, the solid line represents the group median, and the whiskers extend to the 5th and 95th percentiles. Circles represent outliers. The asterisk (*) indicates a significant difference between pre- and post-fire levels at $p < 0.0001$.

droxyfluoranthene, 6-hydroxychrysene, 3-hydroxychrysene, and 4-hydroxychrysene were below the limit of detection. One sample had measurable concentrations of 4-hydroxychrysene. One sample each for 1-hydroxybenz(*a*)anthracene, 2-, 3-, 4-, and 6-hydroxychrysene, and 3-hydroxybenz(*a*)anthrancene could not be measured due to technical difficulties, interference, or poor recovery. In addition, two samples for 3-hydroxybenzo-(*a*)pyrene and three for 3-hydroxyfluoranthene could not be measured due to technical difficulties, interference, or poor recovery. Thus, values for these metabolites were omitted from the analysis. Urinary 1-OHP concentration results for nine samples (i.e., five office workers, four firefighters) as well as two urinary hydroxyphenanthrene results (i.e., one office worker and one firefighter) were unavailable due to analytical difficulties. These samples were omitted from the metabolite-specific analyses.

Urinary PAH metabolite levels (Figure 1) were highest in post-event firefighter samples in comparison with pre-event firefighter samples and two control populations. Urinary PAH metabolite levels in OFS office workers and demographically matched CHMS individuals (i.e., nonsmoking males aged 25−62) were used as study controls. Significant post-fire increases in urinary concentrations of metabolites were observed for all four PAHs examined (i.e., compared to pre-event levels; $p < 0.0001$; Figure 1). Post-event levels of 1-OHP ranged from no increase (NI) to a 38.9-fold increase, with an average post-event increase of 3.7-fold. Similarly, post-event levels of phenanthrene metabolites (ΣOH-Phen) increased by an average of 5.3-fold (NI to 63.4-fold), naphthalene metabolites (ΣOH-Nap) by an average of 2.9-fold (NI to 12.2-fold), and fluorene metabolites (ΣOH-Fluo) by an average of 3.9-fold (NI to 33.2-fold).

Significant post-fire suppression increases in urinary PAH metabolite concentrations have been previously reported. Following participation in training fires, Britz-McKibbin et al. noted significant increases in urinary PAH metabolites, with total PAH metabolite levels increasing by an average of 3.1-fold, a value slightly lower than that observed in the current study. Nevertheless, it is important to note that the comparison with the Britz-McKibbin et al. study is confounded by differences in the measured PAH metabolites. More specifically, Britz-McKibbin et al. reported totals that are sums of eight PAH metabolites; this study included an additional three metabolites and the results are reported as metabolite sums for each parent compound. Interestingly, the totals reported by Britz-McKibbin et al. do not include 1-OHP because levels were below detection in over 50% of the collected samples. In comparison, with the exception of four samples in which technical issues prevented analysis, 1-OHP levels observed in this study exceeded the detection limit in all collected samples.[34] This suggests that, in comparison with training settings, on-shift fire suppression is associated with higher levels of PAH exposures. Such exposure differences may be related to the restricted

DOI: 10.1021/acs.est.7b02850
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nature of the fuels used for training purposes and differences in the firefighting setting and firefighter behavior. Training fires usually do not use flammable or combustible liquids, pressure-treated wood, rubber, plastic, or straw and hay treated with pesticides or harmful chemicals. With respect to the setting and firefighter behavior, training activities require strict adherence to protocols for PPE use, and the suppression activities are scripted, whereas on-shift fires are less predictable, and firefighters may exhibit less-stringent use of PPE.[52]

Differences in the firefighting settings and the recorded levels of urinary PAH metabolites highlights the importance of studies that examine PAH exposures associated with on-shift fire suppression. The only other study that examined urinary PAH metabolites following on-shift fire suppression compared post-event levels with matched reference samples collected several days after the firefighting event.[37] Despite this limitation, significant increases in urinary 1-OHP were still observed. To the best of our knowledge, the current study is the first to compare urinary PAH metabolite levels observed following on-shift, emergency firefighting with pre-event metabolite levels recorded on the same shift and, moreover, a comparison of both pre- and post-event levels with levels in office workers employed by the institution (i.e., the OFS). These comparisons constitute robust documentation of elevated exposures associated with on-shift fire suppression and, moreover, the need for follow-up studies to assess the efficacy of PPE use and post-event decontamination for mitigating PAH exposures.

Interestingly, individuals with some of the highest post-event increases in urinary PAH metabolites (i.e., >10-fold) reported (1) forgetting to wear their flash hood, which protects the head and neck area, or (2) being involved in vertical ventilation, a fire suppression activity that involves the creation of openings in the roof or elsewhere in the lower structure. The latter can contribute to the roof crew becoming engulfed in a column of superheated and pressurized smoke. Britz-McKibbin et al. also noted significantly higher urinary levels of methoxyphenol and PAH metabolites in individuals engaged in different fire suppression activities (i.e., search and rescue compared to fire suppression).[36] Associations between the magnitude of the exposure to toxic combustion by-products and the fire-suppression role should be further scrutinized.

Urinary Mutagenic Potency. Urinary mutagenic potency values reflect overall exposures to organic mutagens.[10,22,53,54] No significant differences were observed between OFS office workers and firefighters before fire suppression (i.e., $p > 0.05$) (Figure 2). Post-fire urinary mutagenic potencies showed a significant average increase of 4.3-fold ($p < 0.001$) relative to pre-event levels, with post-event values ranging from NI to 74.7-fold above pre-event. Long et al. also used *S. typhimurium* strain YG1041 to assess urinary mutagenicity in paired samples collected before and after exposures to combustion emissions.[22] That study examined individuals who use traditional Guatemalan steam baths, known as Temescales, which are heated by wood fires. The Temescales have little ventilation and high levels of wood smoke, and average post-exposure increases in urinary mutagenicity of 1.7-fold were observed.[22] Although the firefighters examined in this study are equipped with PPE, the results revealed average post-exposure increases in urinary mutagenicity that are 2.5-fold greater than that observed in the Long et al. study, in which no PPE was used. Relative differences in the mutagenic potency of the combustion emissions and the intensity and duration of the

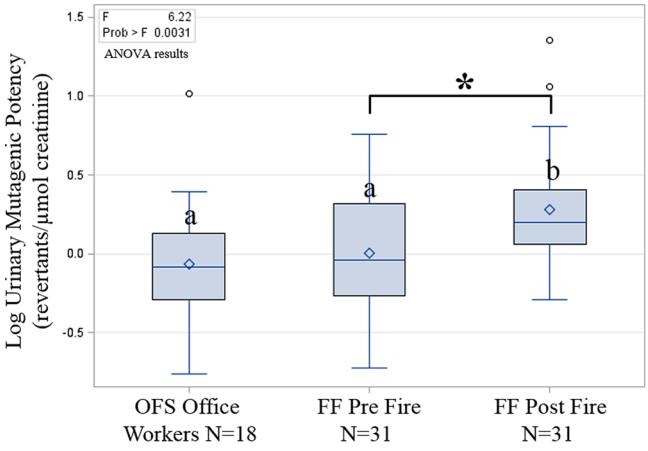

Figure 2. Box-plot showing the differences between the urinary mutagenic potency of OFS office workers, firefighters before a fire-suppression event (FF Pre-Fire), and firefighters after a fire-suppression event (FF Post Fire). The box limits represents the interquartile range (i.e., 25th to 75th percentile), the diamond represents the mean value, the solid line represents the group median, and the whiskers extend to 5th and 95th percentiles. Circles represent outliers. The asterisk (*) indicates a significant difference between pre- and post-fire-suppression urinary mutagenicity levels at $p < 0.0001$.

exposure likely account for the observed differences between firefighters and the subjects examined by Long et al. Although it is not possible to definitively explain the causes of the difference between the firefighters examined in the study and the subjects examined in the Long et al. study, the chemically distinctive nature of firefighters' occupational exposures should be highlighted. For example, Alexander and Baxter (2014 and 2016) noted that firefighters can be exposed to elevated levels of plasticizers and flame retardants.[34,55] However, a paucity of information about the precise identity and physical and chemical properties of putative mutagens in either case prohibit any definitive statements; further investigations are certainly warranted. Similar to the aforementioned need for follow-up examinations of factors that affect PAH exposure levels, there is a need for additional studies that investigate differences between the mutagenic potency of different combustion emissions, as well as the efficacy of PPE and post-event decontamination for the prevention of firefighters' occupation exposures to organic mutagens.

Urinary Biomarkers. Studies of exposure to combustion emissions such as tobacco smoke and wood-smoke particles have documented increases in urinary isoprostanes.[56−58] Firefighters' pre-event urinary 8-iso-$PGF_{2\alpha}$ concentrations were not significantly different from post-event levels, suggesting that fire suppression is not associated with increased oxidative stress. This is surprising because increases in urinary 8-iso-$PGF_{2\alpha}$ have been noted in combustion by-product exposed occupational groups such as welders, women exposed to wood smoke from cooking stoves, and (most importantly for the current study) wildland firefighters in the field or smoke simulators during breathing-apparatus training.[59−62] Interestingly, other studies also failed to detect significant changes in urinary 8-iso-$PGF_{2\alpha}$ following exposure to combustion emissions in wood-smoke chamber studies, and studies of wildland firefighters attending prescribed burns.[63,64] However, in contrast to what was expected, OFS office workers were found to have significantly elevated urinary 8-iso-$PGF_{2\alpha}$ levels compared to firefighters; both overall (i.e., 2.1 versus 1.0 ng/mg

DOI: 10.1021/acs.est.7b02850
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creatinine, $p < 0.007$) as well as firefighters before and after fire suppression (i.e., 1.0 and 1.1, respectively; $p < 0.03$; Table S4).This may be due to office workers being significantly older than the firefighter participants (49.9 ± 2.3 versus 31.8 ± 0.7, $p < 0.0001$). Indeed, after accounting for the effect of age, the difference in urinary 8-iso-PGF2α was no longer statistically significant. Interestingly, rodent studies of urinary 8-iso-$PGF_{2\alpha}$ levels have also shown significant age-related increases. However, this contradicts earlier studies that noted significant age-related decreases in rat and human urinary isoprostane levels.[23,57,65−68] Other factors such as body weight, blood cholesterol, blood glucose, and cardiovascular disease have also been associated with elevated urinary 8-iso-$PGF_{2\alpha}$.[36]

Alternatively, the lack of event-related increases in urinary 8-iso-$PGF_{2\alpha}$ observed herein may be due to hyperoxic conditions resulting from self-contained breathing apparatus (SCBA) use. Oxygen is known to interfere with the formation 8-iso-$PGF_{2\alpha}$, and high oxygen concentrations in inhaled air have been associated with low urinary 8-iso-$PGF_{2\alpha}$.[69] Firefighters routinely use their SCBA, and SCBA use has been shown to create a systemic hyperoxic condition by increasing oxygen consumption by 83%.[70] Moving forward, assessments of oxidative stress in firefighters should be examined using alternative biomarkers. The metabolite of 8-iso-$PGF_{2\alpha}$, 2,3-dinor-5,6-dihydro-15-F2t-isoprostane (15-F2t-IsoP-M), has been shown to be a more reliable and sensitive urinary biomarker of oxidative stress, and it may prove useful for detecting less-pronounced changes.[71] However, if hyperoxic conditions are in fact interfering with 8-iso-$PGF_{2\alpha}$ formation, it may be useful to assess oxidative stress using biomarkers that are not hindered by hyperoxic conditions, such as urinary 8-hydroxy-2′-deoxyguanosine or malondialdehyde.[72,73]

The patterns observed for the urinary lung injury biomarker (i.e., CC16) are similar to what was observed for 8-iso-$PGF_{2\alpha}$. Specifically, OFS office workers had significantly higher urinary concentrations of CC16 compared to firefighters; both overall (i.e., 8.5 versus 3.4 ng/mg creatinine, $p < 0.001$), as well as firefighters before and after fire suppression (i.e., 3.8 and 3.2, respectively; $p < 0.002$; Table S4). Again, after accounting for the effect of age, the difference in urinary CC16 was no longer statistically significant. In comparison with control groups from other studies, the means of both groups examined in this study are well above the control group of 21 athletic males aged 29 ± 6 years (0.65 and 0.68 ng/mg creatinine) examined by Bolger et al. but also well below the control group of another study of 8 males between the ages of 21−37 (27.5 ng/mg creatinine).[31,74] Further work is required to investigate the levels of lung injury associated with fire suppression, if any, and the impacts of age, weight, and fitness on urinary CC16 levels.

Similar to the 8-iso-$PGF_{2\alpha}$ results, paired analyses of the results also failed to show significant event-related changes in firefighters' urinary CC16 concentrations. This may suggest that use of PPE is sufficiently protecting firefighters, eliminating respiratory exposures and minimizing lung injury. However, this contradicts previous firefighter studies that observed significant increases in serum CC16 following exposure to combustion by-products.[32,33] Differences in SCBA use are not thought to be driving this contradiction as Burgess et al. found increases in CC16 in firefighters during overhaul, even with respiratory protection.[32] Analysis of serum CC16 in these studies, rather than urinary CC16, may indicate that the analytical matrix is the source of the discrepancy. Investigations regarding the utility of alternative lung-injury biomarkers, such as those used to monitor firefighters responding to the 9/11 World Trade Center incident (e.g., serum apolipoprotein-AII, C-reactive protein, and macrophage inflammatory protein-4), may be warranted.[33,75]

Relationships between Urinary PAH Metabolite Levels and Variables Reflecting External PAH Contamination Levels. Personal air PAH levels (micrograms per cubic meter), duration of fire suppression, and PAH surface contamination on skin (nanograms per square centimeter) were used as indicators of external exposure to combustion emissions. Personal air samples provide a snapshot of the external environment to which a firefighter is exposed, and the duration of fire suppression provides an indication of fire severity and exposure magnitude. Thus, significant empirical relationships between personal air PAH levels, or the duration of fire suppression, and changes in the urinary concentration of PAH metabolites, would support the supposition that the levels of internal PAH exposures are indeed the result of emergency fire suppression.

Regression analyses investigating the effects of personal air PAH level and fire suppression time on urinary levels of PAH metabolites revealed statistically significant effects on all urinary PAH metabolite levels for personal air PAH concentrations ($p < 0.05$) and on OHP, ΣOH-Phen, and ΣOH-Fluo for fire suppression time ($p < 0.01$). However, close scrutiny of the results revealed that single highly influential observation (i.e., high leverage and Cook's distance), characterized by the highest fire suppression time (i.e., 420 min) and the penultimate air total PAH level (i.e., 21 300 ng/m^3), is driving the relationship. Indeed, after removal of the influential observation, regression results failed to reveal any effect of fire suppression time on urinary levels of PAH metabolites. However, the effect of levels of total PAHs in personal air was statistically significant for total urinary metabolites and ΣOH-Nap (Table S5). The statistically significant effect of personal air total PAHs on the urinary levels of naphthalene metabolites, but not the urinary metabolites of fluorene, pyrene, or phenanthrene, may suggest inhalation exposure is occurring but can only be detected for a compound that exists at high concentrations. Interestingly, a composite exposure variable, calculated as the product of air total PAH level and fire suppression time, explained more of the variation in the aforementioned metabolite metrics than air total PAH level alone (Table S5). Nevertheless, it is clear that personal air total PAHs and fire suppression time have only a modest ability to account for variability of the various urinary metabolite metrics.

Additional regression analyses investigated the effect of post-event increases in dermal PAHs (i.e., total, low molecular weight (LMW) and high molecular weight (HMW)) on urinary levels of PAH metabolites (Table S6). The results revealed statistically significant effects of event-related increases in dermal contamination (i.e., total, LMW, and HMW) on urinary levels of total PAH metabolites and naphthalene metabolites. However, scrutiny of the results revealed a statistically significant outlier (i.e., $p < 0.0005$, determined based on studentized residual) that deviates from the relationships between dermal levels of total, LMW, and HMW PAHs, and urinary levels of pyrene, phenanthrene, and fluorene metabolites. Not surprisingly, the outlier observation is identical to the aforementioned problematic observation (i.e., observation characterized by the highest fire suppression time and the penultimate air total PAH level). In contrast to what was observed for the analyses that examined the effects of personal

DOI: 10.1021/acs.est.7b02850
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air PAHs and fire suppression time, removal of the problematic observation improved several of the statistical relationships with dermal PAH concentrations and, indeed, increased the fraction of explained variation in urinary levels of pyrene, phenanthrene, and fluorene metabolites (Table S6). Additional analyses revealed that a multiple regression model including post-event increases in dermal PAHs and personal air total PAHs showed significant effects of each variable and an r^2 value indicating that the model can account for 54% of the variability in the urinary level of total PAHs (Table S7). These results, along with the lack of post-event increases in the lung injury biomarker, and high post-event increases in PAH metabolites in the individual who attended a fire without a flash hood, suggest that dermal contact is an important route of PAH exposures for firefighters engaged in emergency fire suppression, as previously noted by IARC.[4] Furthermore, naphthalene was the most abundant PAH in personal air samples and is the most volatile, yet had the lowest increase in urinary metabolites, suggesting that inhalation is not the primary route. Fent et al. found similar results, noting matched rankings between PAH concentrations on the neck and urinary PAH metabolite levels.[17] Importantly, elevated environmental temperatures, such as those experienced by firefighters during emergency fire suppression, have been associated with significant increases in dermal permeability and absorption.[76−78] The recognition of dermal contact as a noteworthy route of firefighters' PAH exposures has led to the suggestion of using surface wipes to remove post-fire dermal contamination, and future studies should investigate the efficacy of dermal contact prevention (e.g., improved PPE design and fit and the utilization of gloves for contaminated gear handling), and post-exposure decontamination (e.g., on-scene dermal wiping and timely bathing), on the levels of event-related PAH exposures.[79]

Overall, the results confirm that involvement in on-shift, emergency fire suppression significantly increases urinary concentrations of PAH metabolites and organic mutagens, thus confirming event-related increases in internal exposure. In addition, empirical relationships between urinary levels of PAH metabolites and the concentrations of PAHs in personal air samples collected at the scene supports the assertion that firefighters, as suspected, are significantly exposed to combustion emissions that contaminate the occupational environment at municipal fires. Because firefighters' pre-event levels of PAH metabolites and mutagenicity are not significantly different than those for the office worker and CHMS controls, the post-event increases in these parameters appear to be transient. It is important to note that although urinary levels of PAH metabolites will be transient, and expected to return to within 5% of background within 5 days, the effects manifested by exposures to PAHs and other polycyclic aromatic compounds (PACs) is persistent. For example, Bieler et al. showed that tissue levels of 3-nitrobenzanthrone-induced genetic damage (i.e., stable bulky DNA adducts) can persist for up to several years.[80] Moreover, extensive review by the IARC definitely indicates that PAH- and PAC-induced genetic damage is empirically and mechanistically linked to cancer in humans.[6,81,82] A lack of event-related changes in urinary CC16, a biomarker of pulmonary lung damage, and the observation of significant relationships between urinary PAH metabolite levels and dermal PAH contamination levels suggests that dermal contact is an important route of firefighter exposure to combustion emissions containing PAHs. These results, along with additional scrutiny regarding the influence of fire severity and fire suppression role on urinary levels of PAH metabolites and other organic mutagens (not shown), indicate that an individuals' level of event-related exposures to combustion emissions is determined by the complex, dynamic interplay of variables relating to the duration of fire-suppression activities, the role in fire suppression, and the extent and nature of PPE use. Future research should investigate the efficacy of interventions (e.g., more-effective PPE and decontamination) that may reduce firefighters' exposures to combustion emissions. Given the asserted importance of dermal exposure in determining the internal dose of PAHs, investigations regarding the ability of post-event skin decontamination to reduce exposure should be prioritized. It seems reasonable to hypothesize that adequate post-event skin and PPE decontamination will effectively reduce combustion emission exposures and the associated health risks.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02850.

Figure S1 shows sample collection procedures. Tables showing PAH measurements and concentrations and summaries of potency values and biomarker concentrations. Tables showing the effects of personal air total and dermal PAH levels and general linear models describing event-related increases in total urinary PAH metabolites. (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: Jules.Blais@uottawa.ca.
*E-mail: Paul.White@hc-sc.gc.ca.

ORCID

Jules M. Blais: 0000-0002-7188-3598

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the Ontario Ministry of Labour. We are grateful to Dr. Hugo Lemieux for initiating the project and the Ottawa Fire Services (OFS) for their participation and support. In particular, we are grateful for the support of the OFS senior management and the Ottawa Professional Firefighters' Association. We also thank Dr. Nina Holland (UC Berkeley) for her guidance with the biomarker analyses.

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