The Use of Urinary Biomarkers to Assess Exposures to Polycyclic Aromatic Hydrocarbons (PAHs) and Other Organic Mutagens

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Abstract

Exposure to combustion emissions poses a threat to human health due to the complex mixture of toxic compounds. Polycyclic aromatic hydrocarbons (PAHs) are one group of compounds found within this mixture, and have known carcinogenic and mutagenic properties. Rates of exposure to PAHs depend on a wide range of variables including, but not limited to, demographic, geographical location, dietary habits, smoking habits, and occupation. Understanding magnitude of exposure to these compounds in various groups is imperative to highlight at-risk populations and provide appropriate exposure reduction recommendations. Here, urinary biomarkers are used as a non-invasive, convenient way to assess an individual's exposure to combustion emissions. Urinary measurements of metabolites of individual PAHs as well as compounds indicative of a physiological condition resulting from combustion emission exposure are used to infer exposure. Pairing urinary data with information from questionnaires collecting data on possible sources of combustion by-product exposure was used to determine situations of high exposures.

This thesis investigated the influence of demographic, lifestyle, and occupational factors on urinary levels of PAH metabolites and/or urinary mutagenicity. More specifically, statistical methods were used to analyze population data compiled for the Canadian Health Measures Survey (CHMS). Smoking, age, and sex were identified as the variables most predictive of urinary PAH metabolite concentrations in Canadians. Together with the other demographic and lifestyle variables examined, 24-50% of the variation in the various PAH metabolites was explained. Furthermore, the results obtained illustrated that utilizing PAH metabolites other than the traditionally used 1-

hydroxypyrene may be more suitable for certain exposure scenarios (e.g., fluorene metabolites for tobacco smoke exposure). Occupational exposures to combustion emission were investigated in firefighters as they experience above average risk of cancer, thus paired with their obvious involvement with combustion, are an ideal population to apply the use of urinary biomarkers to assess PAH and combustion exposure. The effect of participating in fire suppression activities (i.e., firefighting) on urinary levels of selected PAH metabolites and organic mutagens was examined. Levels of external PAH exposures were assessed using personal air monitoring and surface wipes of skin. Significant increases in urinary PAH metabolites and mutagenicity were seen after fire suppression events. Empirical relationships between urinary PAH metabolites and duration of fire event and skin concentrations of PAHs suggested that dermal contamination during live fire events is a major route of exposure. Overall, the results from both studies identified factors that may affect an individual's concentrations of urinary biomarkers of combustion emission exposure. This may be used to identify at-risk populations and/or determine effective exposure reduction techniques to these hazardous compounds.

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List of Abbreviations

1-OHP	1-Hydroxypyrene
8-iso-PGF2α	15-Isoprostane F2t
β-HCH	β-Hexachlorocyclohexane
ANOVA	Analysis Of Variance
ASE	Accelerated Solvent Extraction
BaP	Benzo(a)pyrene
CC16	Clara Cell 16
CHMS	Canadian Health Measures Survey
DMSO	Dimethyl Sulfoxide
FF	Firefighter
GM	Geometric Mean
HCB	Hexachlorobenzene
HDPE	High Density Polyethylene
IARC	International Agency for Research on Cancer
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
NFPA	National Fire Protection Association
NHANES	National Health and Nutrition Examination Survey
NI	No Increase
NOCS	National Occupational Classification System
OFS	Ottawa Fire Services
PAH	Polycyclic Aromatic Hydrocarbon
$PM_{2.5}$	Particular Matter ≤ 2.5 μm
p,p′-DDE	p,p′-dichlorodiphenyltrichloroethane
PPE	Personal Protective Equipment
PUF	Polyurethane Foam
SCBA	Self-Contained Breathing Apparatus
SPE	Solid Phase Extraction
STEL	Short Term Exposure Limit
TCE	Trichloroethylene
USEPA	United States Environmental Protection Agency

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Preface

This thesis is written as a compilation of two articles as outlined in the guidelines provided by the Faculty of Graduate and Postdoctoral Studies. Chapter 1 is an introductory chapter which provides an overview of information necessary for understanding the subsequent articles. Chapters 2 and 3 are written in journal article format and contain the primary results of this thesis. Chapter 4 provides final summaries and conclusions, encompassing both research articles.

Chapter 3 study design and methods were approved by the University of Ottawa and Health Canada Research Ethics Boards [certificates H07-14-01B and REB 2014-0035, respectively].

Statement of Contributions

Chapter 2: Relationships between demographic and lifestyle factors and urinary levels of PAH metabolites - empirical analyses of 2009-2011 CHMS data

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Manuscript Preparation…..……...	Jennifer LA Keir
Comments and Feedback on Draft Manuscript …..……	Paul A White Jules M Blais

Chapter 3: Occupational exposures of Ottawa firefighters to PAHs and other organic mutagens

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Urinary Clara Cell 16 Assay…….…….…….	Jennifer LA Keir
Urinary 15-Isoprostane F_{2t} Assay ……..……	Jennifer LA Keir
Analysis of PAHs in Air and Wipe Samples……..…..…..	Umme S Akhtar
Data Analysis and interpretation…….……..…..…….	Jennifer LA Keir Paul A. White Jules M Blais
Manuscript Preparation…..…….	Jennifer LA Keir

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Chapter 1. Introduction

1.1. Combustion Emissions and PAHs

Complex mixtures of toxic substances are formed during anthropogenic and naturally-occurring combustions. These include petrogenic combustion and/or charring of trees (i.e., forests), fossil fuels such as coal and petroleum, tobacco, grilled meats, etc. The mixtures can contain hundreds of compounds such as heavy metals (e.g., antimony, cadmium, lead), inorganic compounds (e.g., SO_2, NO_2, HCl, HF, HCN, dioxins), and organic compounds (e.g., cycloalkanes, cyclopentenes, furans) (Guidotti, 2015). One group of compounds of particular concern are polycyclic aromatic compounds (PACs), defined by their two or more fused aromatic rings. Included in this group are polycyclic aromatic hydrocarbons (PAHs), which contain only carbon and hydrogen, and have known mutagenic, carcinogenic, and teratogenic properties (IARC, 2010b).

In 1976, 16 PAHs were selected by the US Environmental Protection Agency (US EPA) as priorities for concern and control (Figure 1). These compounds, routinely denoted the "priority PAHs", were included in the US EPA's list of 129 pollutants listed in the Clean Water Act (Keith & Telliard, 1979). In addition, the International Agency for Research on Cancer (IARC) has classified certain PAHs as either known, probable, or possible carcinogens. Specifically, benzo[*a*]pyrene (BaP) is currently classified as a known human carcinogen (Group 1), indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and dibenzo[*a,l*]pyrene are classified as probable human carcinogens (Group 2A), and benz[*j*]aceanthrylene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*c*]phenanthrene, chrysene, dibenzo[*ah*]pyrene, dibenzo[*ai*]-pyrene, indeno[1,2,3-*cd*]pyrene and 5-methylchrysene

are classified as possible human carcinogens (Group 2B) (IARC, 2010b). Consequently, contamination of environmental media with PAHs, such as the 16 priority compounds shown in Figure 1, and human exposures to these and other PAHs, is a topic that has attracted considerable attention.

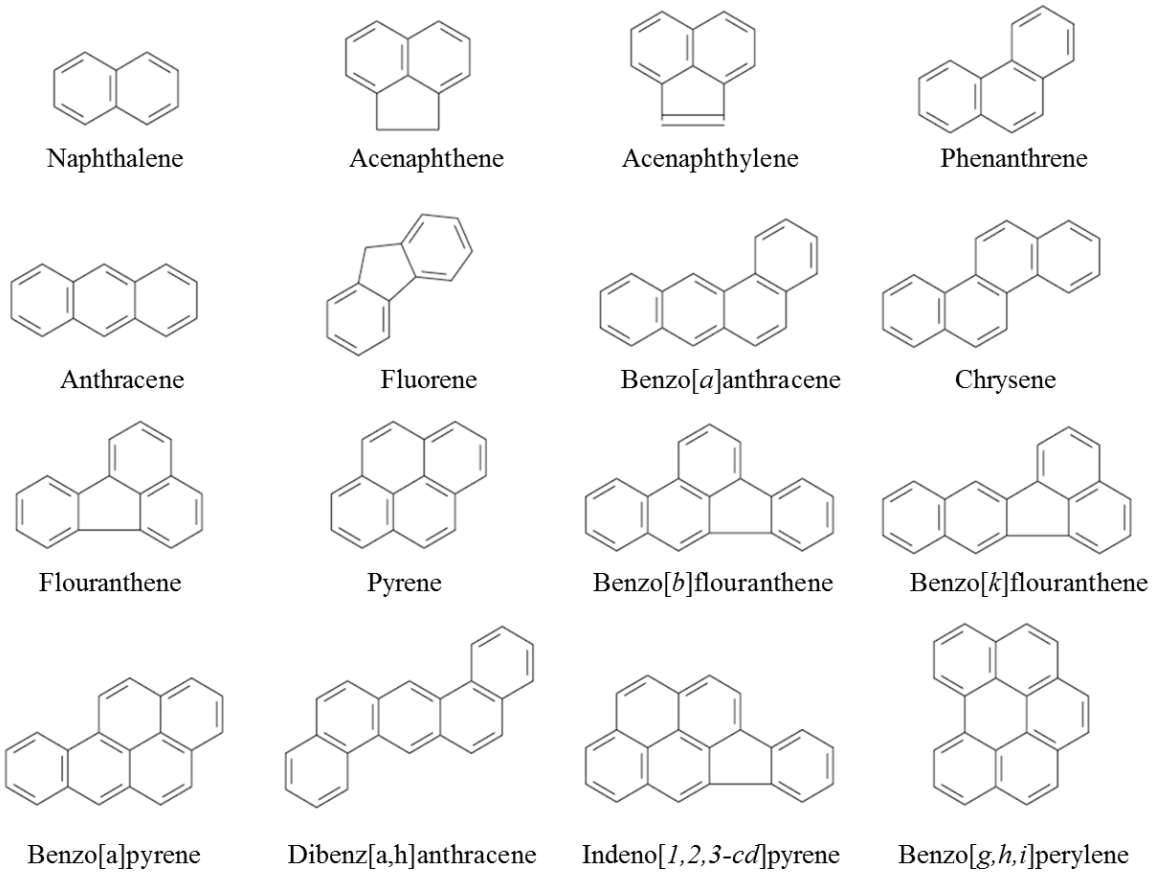

Figure 1 Structures of the US EPA's 16 priority PAHs

1.2. Genetic Toxicity of PAHs

The mechanisms by which PAHs may induce carcinogenicity include metabolism of the parent PAH compound as well as the activation of the receptor responsible for regulating the production of metabolizing enzymes.

1.2.1. Metabolism

PAHs themselves are not carcinogenic or mutagenic; however, metabolism of these compounds converts them into DNA reactive substances that can induce the formation of mutations and initiate cancer (IARC, 2010b). Because of PAHs' non-polar, hydrophobic physical properties, metabolism into more polar, water-soluble metabolites is necessary for removal from the body. PAHs are metabolized via Phase I and II detoxification enzymes, eventually forming conjugates that are excreted through the urine and feces. This can occur through a variety of pathways. Ironically, some of the products of Phase I metabolism are potent electrophiles that can readily react with DNA forming bulky lesions known as adducts, which if unrepaired, can cause permanent sequence changes (i.e., mutations). These intermediates can be seen in the example of BaP in Figure 2 which outlines the various pathways to the formation of epoxides, phenols, quinones, hydroquinones, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, tetrols and other potentially reactive intermediates, on their way to being excreted. Understanding the mutagenic potential of these compounds is important as carcinogenic PAHs generally have a mutagenic mode of action whereas the non-carcinogenic PAHs are generally not mutagenic. Thus, although the pathways ultimately end up in excretion of PAHs, it is in the intermediates of this pathway where genetic damage can occur.

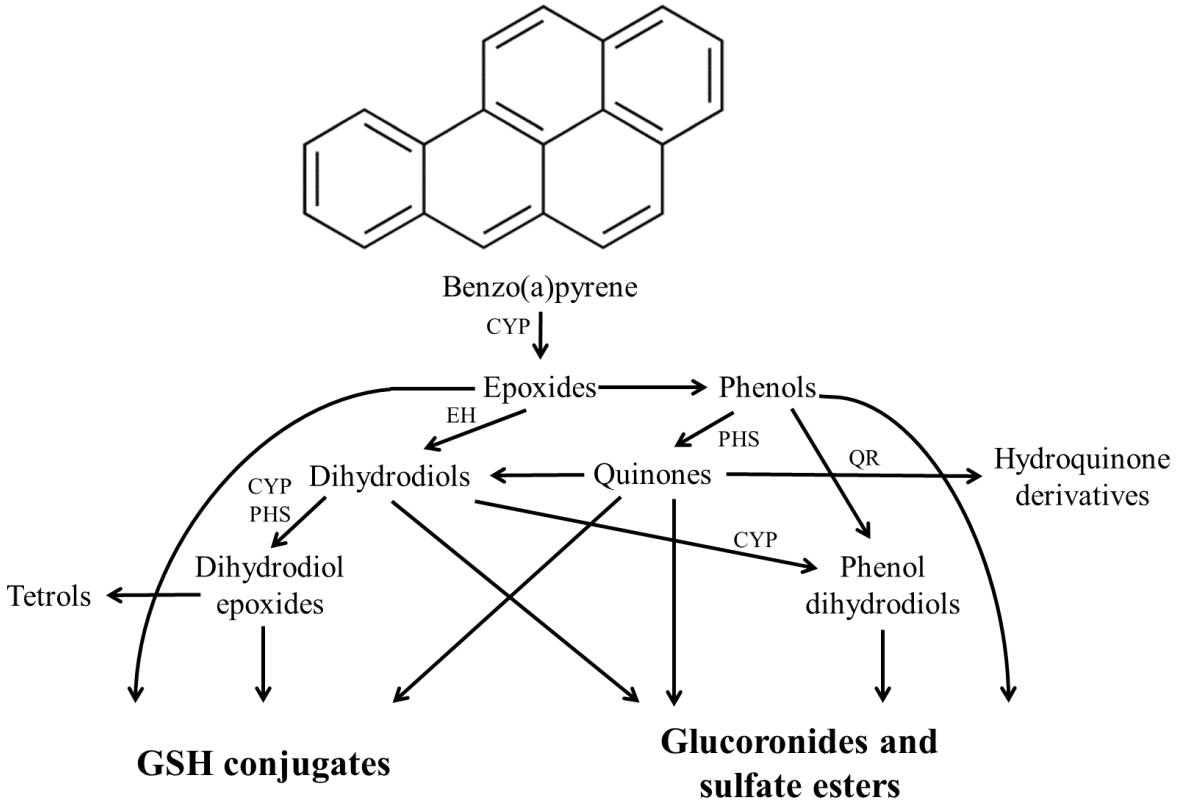

Figure 2 Metabolic scheme for benzo(a)pyrene. CYP, cytochrome P450 enzymes; EH, epoxide hydrolase; PHS, prostaglandin H synthase; QR, quinone reductase; GSH, glutathione. Adapted from IARC monograph, volume 92 (IARC, 2010).

There are three main routes of formation of DNA damaging metabolites: diol-epoxide, radical cation, and quinone-semi-quinones.

Diol-epoxides are thought of as the most prominent pathway of production of DNA damage. In fact, IARC (2010) suggested this pathway as the root cause of the mutagenic and carcinogenic properties of all genotoxic, carcinogenic PAHs (IARC, 2010b). Jerina et al. (1976) first proposed the pathway, in particular to PAHs which contain the bay region. The bay region is an area of a PAH where three benzo rings have fused and created a sterically-hindered region. PAHs may also possess a fjord region (e.g., dibenzo[*a*,l]pyrene), an area even more sterically-hindered as its forms between four angularly fused benzo rings. This formation can sometimes force the compound out of planarity, producing even higher reactive compounds, particularly with adenine nucleotides (Figure 3) (Baird & Ralston, 1997; Muñoz & Albores, 2011). The steric hindrance of these compounds paired with their electrophilic, dihydrodiol epoxides intermediates during metabolism create compounds that are relatively resistant to phase II conjugation and are highly reactive with nucleophilic DNA (Jerina et al., 2012).This results in the formation of bulky, covalent DNA adducts and is the driving cause of genotoxicity in PAHs such as BaP, dibenzo[*a,l*]pyrene, and benz[a]anthracene (IARC, 2010b). Although nucleotide excision repair can often restore DNA, unsuccessful DNA repair or mistakes during DNA synthesis can ultimately lead to mutations in DNA.

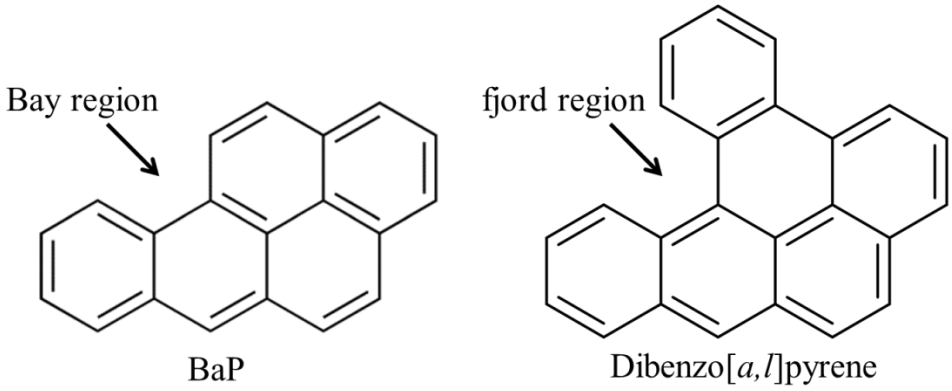

Figure 3 Regions of PAHs that contribute to the ability of metabolites to form DNA adducts that can cause mutations and initiate cancer. BaP's bay region and Dibenzo[*a,l*]pyrene's fjord region are two examples. Adapted from Baird & Ralston (1997) and Baird et al. (2005).

Similarly, recent evidence suggests radical cation formation at the most electrophilic carbon and conversion of dihydrodiols to *o*-quinones also play vital roles in the genotoxicity of PAHs through the formation of bulky adducts (Benigni, 2003). Radical cations are formed when P450 enzymes remove a π electron from a double bond in a PAH whereas PAH-*ortho*-quinones are formed from the dehydrogenation of PAH dihydrodiols by aldo-keto reductases (AKRs). Both mechanisms generate electrophilic compounds with high reactivity with DNA resulting in formation of bulky adducts. Furthermore, PAH-*ortho*-quinones have also been shown to generate reactive oxygen species (ROS) resulting in damage to DNA through the oxidation of purines (Park et al., 2005). These mechanisms are thought to contribute to the carcinogenicity of PAHs such as BaP, dibenzo[a,l]pyrene, 7,12-dimethylbenz[*a*]- anthracene and 3-methylcholanthrene (IARC, 2010b). This demonstrates another way PAHs can induce genetic damage via formation of bulky adducts.

1.2.2. AHR Activation

PAHs are not only metabolized by endogenous enzymes; they also have an influence on the regulation of the expression of these enzymes. The aryl hydrocarbon receptor (AHR) controls the expression of the enzymes required for PAH metabolism. The AHR is activated by planar, non-polar compounds, thus PAHs such as BaP, 7,12-dimethylbenz[a]anthracene and dibenzo[a,l]pyrene, can act as AHR agonists (Hankinson, 1995; Shimada & Fujii-Kuriyama, 2004). For example, mice lacking AHR activation were found to exhibit resistance to ovarian damage caused by PAHs (Matikainen et al., 2001). Because metabolism plays an integral role in the mutagenic and ultimately carcinogenic potential of some PAHs, activation of the enzymes responsible for metabolism also plays an important role.

1.2.3. Mutations arising from DNA adducts

Once DNA adducts are formed, they may induce the formation of mutations, and subsequently initiate cancerous tumors, via a variety of pathways. Bulky stable adducts, as well as oxidative lesions, have been shown to produce deletions, insertions, tandem mutations, and base-pair substitutions (i.e., transitions and transversions) (Baird et al., 2005). Genetic damage arising from mutations, induced by compounds such as PAHs, can contribute to cancer hallmarks such as evasion of apoptosis, replicative immortality, and sustained proliferative signaling, resulting in genetic damage being termed an "enabler of carcinogenesis" (Hanahan & Weinberg, 2011). Non-genotoxic effects related to carcinogenesis have also been documented. These include tumor promotion, DNA methylation, and blocking of DNA repair. More specifically, Bláha et al. (2002) found that environmentally relevant PAHs can obstruct gap-junctional intercellular communication causing cells to exit growth suppression, thus inducing proliferation. Interestingly, some PAHs considered to have no or low mutagenic activity (e.g., pyrene, phenanthrene, fluoranthene) were among the most potent inhibitors of the gap-junction communication (Blaha et al., 2002). In addition, a study looking at promoter methylation of 13 breast cancer-related genes in breast tumor tissue, and global methylation in peripheral blood, suggested DNA methylation may be another mechanism by which PAHs contribute to carcinogenesis (White et al., 2016). Hsu et al., (2005) showed that PAHs can also reduce repair activity through blocking polymerase replication activity. In summary, DNA-reactive PAH metabolites can cause mutations in genes linked to cancer; moreover, they can contribute to carcinogenesis via non-genotoxic mechanisms.

1.3. Human exposure to PAHs

Individuals can be exposed to PAHs by ingestion, inhalation, or dermal contact. The primary route of exposure is often dependent on the number of rings. PAHs with fewer than four aromatic rings are volatile (e.g., high vapour pressure at 25°C) and will be present in air, whereas those with more than four rings are typically bound to particulate material. Pyrene, a four ring PAH, may be present in either phase (F J Jongeneelen, 2001). Environmentally speaking, PAH exposure is assumed to be primarily through inhalation, but dermal absorption is now regarded as an important route of exposure, particularly in occupational settings where high dermal PAH deposition has been observed (Raymond, 1998; VanRooij et al. 1993; VanRooij et al., 1993b). Overall, an individual's exposures to combustion emissions, and thus PAHs, derive mainly from diet (e.g., barbequed or smoked foods), smoking habits, and contaminated air (e.g., smoke from bonfires or fireplaces).

1.3.1. Sources of PAHs

Certain types of cooking can cause the formation of PAHs, and food is the main source of PAH exposure for non-smokers. Fat content and cooking style have been shown to affect PAH concentrations. For example, PAHs were found to be higher in sausages with higher fat content, in meats charcoal barbequed compared to frying, grilling, roasting, and toasting, in meats cooked on coal and flame barbecues compared to electric and gas barbecues, and in breads cooked at higher temperatures (Aaslyng et al., 2013; Ciecierska & Obiedziński, 2013; Gomes et al., 2013; Pöhlmann et al., 2013; Rose et al., 2015). PAH content can also vary with type of food. For example, Aaslyng et al., 2013 found BaP in 90% of barbequed beef samples, 80% of barbequed pork samples, and 70% of barbequed chicken samples. Rye and rye bran have been found to

have significantly higher levels of PAH contamination compared to wheat grain and bran (Ciecierska & Obiedziński, 2013). Where the food item was farmed can also affect PAH content, particularly those in contaminated areas such as near waste burning sites (Wang et al., 2012). These examples illustrate the range of exposures individuals may experience depending on their dietary habits.

Inhalation exposure of PAHs can come from a variety of sources. It is not surprising that smokers are exposed to high levels of combustion emission, including PAHs and other toxic compounds. When PAHs in tobacco smoke were quantified, 14.3 ng of BaP per cigarette was detected in mainstream smoke (i.e., the smoke inhaled) and 91.7 ng per cigarette in side stream smoke (i.e. smoke directly emanating from the end of the burning cigarette). Marijuana smoke has similar amounts, with 8.67 and 101 ng per cigarette found in mainstream and side stream smoke, respectively (Moir et al., 2007). The high levels of smokers' PAH exposures is reflected in the concentrations of PAHs and PAH metabolites detected in various biological samples including serum, lung tissue, follicular fluid and breast milk in women, and urine (Goldman et al., 2001; G. St. Helen et al., 2012; Neal et al., 2008; Zanieri et al., 2007). Non-tobacco related sources can also contribute to airborne PAH exposures. For example, PAH concentrations in traffic polluted air can be 70x higher than indoor air, urban air is 5x higher relative to rural sites, and air near incineration plants is 2.2x higher than air at control sites (Mao et al., 2007; Merlo et al., 1998; Motelay-Massei et al., 2005). Thus, PAH exposures can occur via a variety of pathways, with the magnitude and composition depending on environmental condition, occupation, lifestyle and personal habits.

1.3.2. Factors affecting PAH exposure

The wide range of aforementioned PAH sources indicate that demographic, behavioral, lifestyle, and occupational factors can influence the magnitude and frequency of PAH exposures. For example, traditional gender-specific behaviors such as domestic cooking can contribute to increases in women's exposure to cooking emissions that are known to contain PAHs (IARC, 2010b). Additionally, preschool children who are in intimate contact with settled house dust can be exposed to 2.5-fold more particle bound PAHs in comparison with adults (Maertens et al., 2004). Similarly, certain segments of a population may be more engaged in activities with higher exposures to combustion emissions. For example, smoking status differs significantly depending on socioeconomic status, education, gender, and race (Barbeau et al., 2004). Different lifestyle factors, which are often linked with demographic factors, can have less obvious relationships with combustion emission exposures. For example, factors such as home location (e.g., near an incinerator), use of a domestic fireplace, and frequency of vacuuming, can all influence domestic combustion emission exposures (Alfheim & Ramdahl, 1984; Chuang et al., 1999; Daisey et al., 1989; Maertens et al., 2008). Lastly, since nearly a quarter of most of the population's time is spent at work, occupational setting is also a critically important determinant of PAH exposure that must also be considered.

1.3.3. Occupational exposures to PAHs

Individuals will experience different levels of exposure to the abovementioned PAH sources depending on diet, smoking status, and home location. Occupational PAH exposures are particularly important for those who work in settings that provide opportunities to be exposed to combustion emissions (e.g., welding, firefighting, metal refining and founding, vehicle

maintenance, etc.). Not surprisingly, workplace exposures to combustion sources can dramatically increase an individual's level of PAH exposure.

The first report of an occupational exposure to cancer-causing PAHs was published by Sir Percivall Pott in 1775. Although Dr. Pott, a British surgeon, was not aware that the PAHs in combustion emissions include potent carcinogens, he connected soot exposure in chimney sweeps to their high rates of scrotal cancer (Pott, 1775). Many recent studies have noted significant occupational PAH exposures in coke oven workers, cooks, and road paving workers, relative to unexposed controls; and moreover, that individuals engaged in these occupations have significantly higher rates of several cancers (Britz-McKibbin et al., 2016; Kato et al., 2004; Pan et al., 2008; Serdar et al., 2003; Simioli et al., 2004; J. G. VanRooij et al., 1993). Since most of the population spends nearly a fifth of the week at work, PAH contaminated environments can pose significant exposure-related health concerns.

Firefighting is one occupation of particular interest due to their unavoidable interactions with combustion emissions; moreover, the epidemiological evidence of increased risk of several cancers and diseases. The International Agency for Research on Cancer (IARC) reviewed epidemiological studies of cancer in firefighters, including 19 cohort studies, 11 case–control studies, and 14 studies that used other designs. Higher risks of many types of cancers were identified but few consistent relationships were identified. Nevertheless, meta-analyses found incidence of non-Hodgkin's lymphoma, testicular, and prostate cancer of ~20, 50, and 30%, respectively, as defined by their respective summary risk estimates. Overall, firefighting as an occupation was classified as "possibly carcinogenic" (IARC, 2010a). Since IARC's publication, a survey of the recent literature revealed several additional studies that have noted significantly increased risk of cancer in firefighters. Eight cohort and one case-control study were identified;

these studies are summarized in Table 1 (Bates, 2007; Daniels et al., 2014; Glass, Monaco, et al., 2016; Glass, Pircher, et al., 2016; Ide, 2014; Kang et al., 2008; Paget-Bailly et al., 2013; Pukkala et al., 2014; Tsai et al., 2015). More specifically, nearly all these recent studies noted significant increases in the incidence and mortality from all cancers, as well as cancers of the lung, testicles, brain, esophagus, and kidney. However, other studies describe conflicting results. For example, Bigert et al (2016) examined the results of 14 case-control studies across four countries, and noted no increased in lung cancer risk among firefighters (Bigert et al., 2016). However, this study has been disputed due to its lack of detail on firefighting assignment, firefighting time period, and high reported smoking rates (Guidotti & Goldsmith, 2017). Zeig-Owens et al. (2011) also reported conflicting (i.e., not significant) standardized cancer incidence ratios in firefighters, but this study focused specifically on individuals present at the attacks on the World Trade Center in September 2001 (Zeig-Owens et al., 2011). Thus, both past and current epidemiological evidence suggests a link between firefighting and cancer risk; however more studies are warranted to rigorously address the suspected association. It may be necessary to conduct refined cohort studies that examine statistical association between specific firefighting activities (e.g., attack, vertical ventilation, overhaul, etc.) and cancer risk.

Table 1 Summary of selected post-2010 epidemiological studies of firefighters' cancer risks.

Reference, location	**Cohort Description**	**Cohort Description**	**Exposure Categories**	**Relative risk with 95% confidence interval***
Glass et al., 2016, Australia	17,394 full-time, and 12 663 part-time firefighters	Cohort from Employment records, Australian National Death Index and Australian Cancer Database	Era of first employment, duration of employment, and number and type of incidents attended.	**SIR** - overall risk of all cancers 1.09 (95% CI 1.03 to 1.14), prostate cancer (full-time) 1.23 (95% CI 1.10 to 1.37), prostate cancer (part-time) 1.51 (1.28 to 1.77), melanoma (full-time) 1.45 (95% CI 1.26 to 1.66), melanoma (part-time) 1.43 (95% CI 1.15 to 1.76).
Glass et al., 2016b, Australia	611 male firefighters	Supplied human resources records, supplemented by self-reported information	High (drill operators and instructors)	**SIR** overall cancer risk 1.85 (95% CI 1.20–2.73), testicular cancer SIR = 11.9 (1.44–42.9), melanoma SIR = 4.59 (1.68–9.99)
			Medium (volunteer and paid regional staff instructors)	Brain cancer SIR 5.74 (1.56–14.7).
			Low (paid firefighting trainees)	
Daniels et al 2014, San Francisco, Chicago and Philadelphia, USA	29,993 firefighters	Cohort from National Death Index-Plus (NDI-Plus), the Social Security Administration Death Master File (SSA-DMF), personnel and pension board records, death certificates, and records from the previous studies	Overall	**SMR** overall cancer risk 1.14, (95% CI 1.10 to 1.18), lung cancer 1.10 (95% CI 1.04 to 1.17), esophagus cancer 1.39 (95% CI 1.14 to 1.67), intestine cancer 1.30 (95% CI 1.16 to 1.44), rectum cancer 1.45 (95% CI 1.16 to 1.78), kidney cancer 1.29 (95% CI 1.05 to 1.58), buccal and pharynx cancers 1.40 (95% CI 1.13 to 1.72), malignancies of the liver, gall bladder and biliary tract 1.30 (95% CI 1.06 to 1.57), malignant mesothelioma 2.00, (95% CI 1.03 to 3.49).

				SIR overall cancer risk 1.09 (95% CI 1.06 to 1.12), esophagus cancer 1.62 (95% CI 1.31 to 2.00), large intestine cancer 1.21 (95% CI 1.09 to 1.34) kidney cancer 1.27 (95% CI 1.09 to 1.48), lung cancer 1.12 (95% CI 1.04 to 1.21), buccal and pharynx cancers 1.39 (95% CI 1.19 to 1.62), laryngeal cancer 1.50 (95% CI 1.19 to 1.85), malignant mesothelioma 2.29 (95% CI 1.60 to 3.19).
Tsai et al, California, USA	3,996 male firefighters	Cohort from the California Cancer Registry data (1988–2007)	Overall	**OR** melanoma 1.8 (95% CI 1.4-2.1), multiple myeloma 1.4 (95%CI 1.0-1.8), acute myeloid leukemia 1.4 (95%CI 1.0-2.0), esophagus 1.6 (95%CI 1.2-2.1), prostate 1.5 (95%CI 1.3-1.7), brain 1.5 (95%CI 1.2-2.0), kidney 1.3 (95%CI 1.0-1.6).
Ide 2014, Scotland	2,200 male firefighters	Cohort using service medical records	Overall	**Incidence rate/10^5/year** melanoma and kidney cancer significantly elevated (13.6 and 9.1, respectively, $p< 0.01$) **Mortality rate/10^5/year** kidney cancer significantly elevated (6.5, $p< 0.01$).
Pukkala et al 2014, Noridc countries (Denmark, Finland, Iceland, Norway, and Sweden)	16,422 male firefighters	Cohort from census data from five countries and their respective cancer registries from 1961–2005	Overall	**SIR** All cancers=1.06 (95% CI 1.02 to 1.11)
			30-49 years old	Prostate cancer =2.59, 95% CI 1.34 to 4.52), skin melanoma =1.62 (95% CI 1.14 to 2.23)
			≥70 years old	Non-melanoma skin cancer =1.40 (95% CI 1.10 to 1.76), multiple myeloma =1.69 (95% CI 1.08 to 2.51), adenocarcinoma of the lung =1.90 (95% CI 1.34 to 2.62), and mesothelioma =2.59 (95% CI 1.24 to

				4.77)
Paget-Bailly et al 2013, France	1,833 cases of head and neck cancer, 2,747 controls	French population-based case-control study on Head and Neck cancer (ICARE)	Overall	**OR** head and neck cancer 3.9 (CI, 1.4 to 11.2),
Kang et al, 2008, Massachusetts	258,964 eligible cancer cases , 2,125 firefighters and 2,763 police officers	Cohort from the Massachusetts Cancer Registry from 1986 to 2003	Overall	**SMOR** colon cancer 1.36 (95% CI: 1.04–1.79), brain cancer 1.90 (95% CI: 1.10–3.26)
Bates 2007, California	804,000 eligible records, 3,659 firefighters	Cohort from the California Cancer Registry for 1988–2003	Overall	**OR** testicular 1.54 (95% CI 1.18-2.02), melanoma 1.50 (95% CI 1.33-1.70), brain 1.35 (95% CI 1.06-1.72), esophagus 1.48 (95% CI 1.14-1.91), prostate 1.22 (95% CI 1.12-1.33).

*table only includes studies with recorded risk values, and confidence intervals, which exceed 1.0.
SIR, standard incidence ratio; SMR, standard mortality ratio; OR, odds ratio, SMOR, standard mortality odds ratio

Assessment of occupational PAH exposure levels are particularly important since the magnitude of the exposure will influence worker health, which, in turn, will influence presumptive workplace legislation, workplace policies and regulations, and health insurance coverage. Thus, quantitative examinations of workplace exposures to PAHs are important for identifying at-risk populations and implementing effective exposure reduction procedures.

1.4. Urinary biomonitoring

Biomonitoring is an important aspect of exposure assessment, including exposure to combustion emissions. Current national efforts include the Canadian Health Measures Survey (CHMS), National Health and Nutrition Examination Survey (NHANES), Minnesota Environmental Public Health Tracking, and California Environmental Contaminant Biomonitoring Program (Centers for Disease Control and Prevention, 2014; Minnesota Department of Health, 2016; Office of Environmental Health Hazard Assessment, 2016; Statistics Canada, 2016). All of these involve collection and analysis of specimen samples (e.g., blood, urine) from individuals within the population. These studies provide data on baseline concentrations of various compounds, both endogenous and exogenous, in selected biological matrices (e.g., hair, blood, urine, etc.), and highlight at-risk populations. Biomonitoring can be compound specific, such as analyzing for a certain chemical in blood or chemical metabolite(s) in urine, or it can be nonspecific and examine exposure to the complex mixtures of substances with specific properties.

1.4.1. Compound specific biomonitoring

The presence of PAH metabolites in the urine provides a convenient, non-invasive method to assess PAH exposures, and associatively, combustion emission exposures. 1-

hydroxypyrene (1-OHP) has been widely used as a biomarker of PAH exposure. The US American Conference of Governmental Industrial Hygienists (ACGIH) suggests 1 g 1-OHP/L (0.49 µmol/mol creatinine) for use in biological monitoring as an exposure alert level to indicate need for improvements in industrial hygiene (i.e., not as a health-based biological limit value). This level was determined by examining population levels, and determining the 99th percentile for individuals with no occupational or significant environmental exposure (IARC, 2010b). More recently, Jongeneelen et al. (2014) recommended a benchmark value for occupational PAH exposure of double the ACGIH level. A 1.0 µmol 1-OHP/mol creatinine value is based on previous studies showing a no observable genotoxic effect (Jongeneelen, 2014). An even higher level of 2.3 µmol 1-OHP/mol creatinine was shown to reflect that of individuals exposed to the occupational limit for airborne coal tar pitch volatiles (i.e., the threshold limit value (TLV)) in coke oven workers, equaling a lung cancer risk of approximately 1.3) (Jongeneelen, 1992). Nonetheless, these estimates are important tools for evaluating the significance of an individual's PAH exposure level.

Advances in analytical sensitivities have led to the suggested use of additional urinary PAH metabolites. Moreover, recent works have suggested that alternatives to 1-OHP may be more useful for PAH exposure assessment since pyrene and other higher molecular weight PAHs are primarily excreted through the feces. In contrast, metabolites of lower molecular weight PAHs such as phenanthrene, fluorene, anthracene and naphthalene would be primarily found in urine (Li et al., 2006; Ramesh et al., 2004). For example, Britz-McKibbin et al., 2016 noted that metabolites of naphthalene, fluorene, and phenanthrene (all $\leq$ 3 aromatic rings) are more sensitive biomarkers of combustion emission exposures compared to 1-OHP. Similarly, Polanska et al. (2014) found that metabolites of phenanthrene are most useful for assessing environmental

PAH exposures. It has also been suggested that analysis of multiple PAH metabolites may provide information on route of exposure; urinary 1-OHP is thought to be reflective of respiratory and dermal exposure to particle-bound PAHs whereas urinary metabolites of naphthalene and phenanthrene are reflective of inhalation exposure (Serdar et al., 2003). This multivariate approach adds an additional dimension to the utility of urinary PAH biomonitoring.

1.4.2. Non-specific biomonitoring

Although analytical measurements of specific PAHs, and/or their respective metabolites, can provide information on exposures to selected compounds, nonspecific monitoring can be particularly useful when individuals are exposed to complex mixtures of unknown composition. *A priori* information on the composition of a complex mixture is usually unavailable; moreover, in any case, assessments of exposures hundreds of chemicals in a complex mixture would be excessively labor intensive and costly. However, nonspecific biomonitoring can be used to overcome this issue, and assess total exposure to compounds with specific properties and/or assess total exposure to substances that elicit a specific physiologic response.

Biomonitoring for exposure to overall mutagens in combustion emissions can employ a bacterial mutagen detection system known as the Salmonella reverse mutation assay or Ames test (D M DeMarini et al., 1997; Long et al., 2014; Talaska et al., 1991). The test employs histidine auxotrophs of *Salmonella typhimurium* and assesses a test article's mutagenic activity by virtue of its ability to induce reversion to prototrophic growth. The assay is generally conducted on agar plates and the frequency of revertant colonies at a given test article concentration reflects its mutagenic potency. In the case of urinary biomonitoring, deconjugating enzyme systems are often used to detect compounds conjugated to proteins to aid in excretion. Extracts of the samples are then exposed to the bacteria to assess the mixture's mutagenic

potency (Durston & Ames, 1974). Studies of urinary mutagenicity have shown significantly increased levels in urine of individuals exposed to combustion emissions in cigarette smoke and wood smoke (David M DeMarini, 1981; Kato et al., 2004; Long et al., 2014). This method provides a valuable tool to assess complex mixture of mutagens.

Biomarkers that assess physiologic responses related to exposures to combustion emissions such as cigarette smoke, including urinary or blood levels of Clara Cell 16 (CC16) or 15-Isoprostane F_{2t} (8-iso-$PGF_{2\alpha}$), which have been used to assess epithelial lung injury and overall oxidative stress, respectively (Bernard et al., 1997; G. S. Helen et al., 2013; Montuschi et al., 2000; Morrow et al., 1995). Lung injury can compromise cell integrity and lead to increases in permeability of the epithelial barrier. As a result, above average amounts of CC16 may reach the blood and ultimately the urine after glomerular filtration. Measurement of this compound in serum has been used to assess lung epithelium injury related to ozone (O_3) in cyclists and smoke inhalation in firefighters (Bernard et al., 1997; Broeckaert et al., 2000). However, measurement in urine provides a less invasive, more convenient method of sample collection. Studies have shown urinary CC16 to also be effective as a biomarker of lung injury from sources such as secondhand smoke, air pollution (as seen in a 20% increase in urinary CC16 per 10 $\mu g/m^3$ increase in particulate matter ($PM_{2.5}$) concentration), and exercise-induced bronchoconstriction in athletes (Bolger et al., 2011; G. S. Helen et al., 2013; Timonen et al., 2004). 8-iso-$PGF_{2\alpha}$ is another urinary biomarker of physiological changes but for oxidative stress. It is formed by the oxidative metabolism of arachidonic acid and analysis of urinary 8-iso-$PGF_{2\alpha}$ concentrations has been shown to be a useful indicator of cellular injury by substances that elicit oxidative stress (e.g., production of reactive oxygen species). For example, previous studies have used this urinary biomarker to demonstrate *in vivo* oxidative stress caused by smoking and $PM_{2.5}$ exposure from

welding (Morrow et al., 1995; Nuernberg et al., 2008; Pilz et al., 2000; Roberts & Morrow, 2000). It is important to note that non-specific biomarkers have noteworthy drawbacks. For example, for the biomarkers noted above, there is no consensus regarding levels that indicate a significantly-elevated risk of adverse health effects. Nevertheless, use of these biomarkers avoids the need for *a priori* information regarding the composition of the mixture, thereby providing a means to more expeditiously examine exposures and/or effects of combustions emissions

1.5. Hypotheses and Objectives

Examinations of empirical relationships between biomarker measurements that reflect exposure and/or effect and variables that relate to demography, lifestyle, and occupation can improve understanding on the route(s) of exposure to PAHs and other organic mutagens; and moreover, factors that augment or minimize exposure magnitude. Increased knowledge regarding factors that augment exposure magnitude can be used to develop and implement intervention strategies that minimize exposure and risk.

The main objective of this thesis is to investigate the influence of occupational, demographic and lifestyles factors on urinary levels of PAH metabolites and/or urinary mutagenicity and/or urinary levels of selected physiologic biomarkers. More specifically, the second chapter uses statistical methods to analyze population data compiled for the Canadian Health Measures Survey (CHMS). The third chapter investigates the effect of participating in fire suppression activities (i.e., firefighting) on urinary levels of selected PAH metabolites and organic mutagens. Levels of external PAH exposures were assed using personal air monitoring and surface wipes of skin.

More specifically, it is hypothesized that (i) in regards to the typical Canadian, exposure to tobacco smoke, age, occupation, and home characteristics significantly influence urinary

concentrations of PAH metabolites; (ii) fire suppression is associated with significant increases in urinary biomarkers of combustion emission exposure; and (iii) post-suppression levels of urinary biomarkers are determined by a firefighter's role in fire suppression, his/her duration at the scene, and the nature of the fire itself.

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Chapter 2. The influence of demographic and lifestyle factors on urinary levels of PAH metabolites - empirical analyses of 2009-2011 Cycle 2 CHMS data

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2.1. Abstract

Biomonitoring programs, such as the Canadian Health Measures Survey (CHMS), are used to establish baseline levels of chemical exposures within a population, flagging problematic levels for certain chemicals and assessing the effectiveness of risk management programs. Urinary metabolites of polycyclic aromatic hydrocarbons (PAHs) are among the often monitored compounds, and levels can be used to assess exposures to this group of combustion by-products that includes several potent carcinogens. Pairing this with questionnaire demographic and lifestyle information, it is therefore possible to investigate empirical relationships between urinary levels of PAH metabolites and variables such as smoking status, workplace smoking restrictions, age, sex, household income, home age, and occupation. We obtained urinary PAH metabolite levels and questionnaire data from CHMS Cycle 2 and used a host of statistical methods to investigate empirical relationships between biomarkers of PAH exposure and a series of demographic and lifestyle factors. The results show that smoking status, age, and sex have the strongest influence on urinary concentrations of metabolites of pyrene (1-OHP), phenanthrene

(ΣOH-Phen), fluorene (ΣOH-Flu), and naphthalene (ΣOH-Nap) ($p<0.0001$). In addition, in some cases the results revealed significant effects of home age, occupation, and workplace smoking restrictions ($p<0.05$). Despite data analysis restrictions related to the unbalanced nature of the dataset and the intercorrelation of independent variables, the analyses identified the predominant factors that determine urinary PAH metabolites levels in Canadians. The results can be used to identify remedial measures to reduce exposure and concomitant risk, and/or design follow-up cohort studies to test hypotheses regarding the causes of exposure differences related to sex, age, home age and occupation.

2.2. Introduction

The Canadian Health Measures Survey (CHMS) is a Canadian national biomonitoring program that uses interviews and physical examinations to monitor population health and flag emerging concerns. It provides baseline levels of chemical exposures within the population, and pinpoints problematic levels of certain chemicals. Included in the list of chemicals that were monitored for 2009-2011 Cycle 2 of CHMS are the metabolites of polycyclic aromatic hydrocarbons (PAHs). These compounds are ubiquitous in the environment, being formed during the incomplete combustion of organic matter (e.g., fossil fuels, biomass, etc.). Many PAHs have known mutagenic, carcinogenic, and teratogenic properties (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010). Humans can be exposed to these compounds from a variety of sources (e.g., via exposure to virtually any combustion source); the bulk of the exposure occurs via dietary intake and contact with tobacco smoke.

The large CHMS datasets provide an opportunity to explore empirical relationships between selected biological indicators of contaminant exposure (i.e., concentrations of chemicals in biological fluids) and demographic, geographic, and/or lifestyle factors. For example, a recent study that examined Cycle 1 CHMS data noted a significant sex-related difference in urinary concentrations of Bisphenol A (BPA) (Haines & Murray, 2012). Although population biomonitoring data from the American National Health and Nutrition Examination Survey (NHANES) program have been used to examine the impact of age, sex and smoking status on urinary levels of PAH metabolites, (Li et al., 2008), to the best of our knowledge, no study has analyzed CHMS data to investigate empirical relationships between urinary PAH metabolite levels and Canadian demographic and/or lifestyle factors.

Quantification of urinary PAH metabolites permits convenient, non-invasive insight into an individuals' PAH exposures, and relatedly, combustion emission exposures. 1-hydroxypyene (1-OHP) has been widely used as a biomarker of PAH exposure, yet there is disagreement regarding a recommended benchmark value, particularly for occupation exposures, with values ranging from 1.0-2.3 µmol 1-OHP/mol creatinine (Jongeneelen, 1992; Jongeneelen, 2014; Jongeneelen, 2001). More recently, other urinary PAH metabolites have also been used for exposure monitoring. Quantification of metabolites other than 1-OHP commonly examine the metabolites of lower molecular weight (MW) PAHs such as phenanthrene, fluorene, anthracene, and naphthalene. Metabolites of these compounds are commonly found in the urine, whereas metabolites of the higher MW PAHs are more often found in the feces (Guo et al., 2013; Ruby et al., 2016). Recent studies have found that metabolites of these smaller MW PAHs are more sensitive biomarkers for assessments of combustion emission exposure, more specifically, exposures related to fire suppression and environmental PAHs (Britz-McKibbin et al., 2016; Polanska et al., 2014). Use of different PAH metabolites to discriminate between routes of exposure has also been investigated; some studies have shown urinary 1-OHP to be reflective of respiratory and dermal exposure to particle-bound PAHs whereas urinary metabolites of naphthalene and phenanthrene reflect inhalation exposures (Serdar et al., 2003). Thus, assessing multiple PAH metabolites, rather than only the more commonly examined 1-OHP, may be more useful; providing more insight into the exact route of PAH exposure.

This study used statistical methods to examine empirical relationships between urinary levels of PAH metabolites, and variables related to tobacco smoke exposure, population demographics, residence age, income, and occupation. In addition, comparison of results across

numerous metabolites is used to objectively evaluate the utility of urinary 1-OHP for human biomonitoring.

2.3. Methods

Data from Cycle 2 (of 4) of CHMS was obtained from Statistics Canada Health Statistics Division's Data Access and Information Services (DAIS). Detailed survey methodology is available at the CHMS website (Statistics Canada, 2016a). Briefly, personal interview, physical measurements, and blood and urine samples were collected between August 2009 and November 2011 from participants at 18 sites across seven provinces (i.e., Newfoundland and Labrador, Nova Scotia, Quebec, Ontario, Manitoba, Alberta, and British Columbia) using mobile examination centers. The dataset contains approximately 6395 observations representing Canadians aged 3 to 79. A subset of individuals was selected to provide urine samples (N~2500). Spot urine samples were collected and stored at -20°C before being shipped on dry ice. Analyses for the various PAH metabolites were conducted using enzymatic deconjugation followed by GC-MS/MS (Gaudreau, Bérubé, Bienvenu, & Fleury, 2016).

2.3.1. Data Analysis

Metabolite concentrations were combined by parent molecule and analyzed as total concentration of metabolites for each parent compound (i.e. pyrene is metabolized to 1-hydroxypyrene (1-OHP), 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene are summed and reported as Σhydroxyphenanthrenes (ΣOH-Phen), 2-, 3-, and 9-hydroxyfluorene are summed as reported as Σhydroxyfluorenes (ΣOH-Fluo), and 1- and 2-hydroxynaphthalene are summed and reported as Σhydroxynaphthalenes (ΣOH-Nap)). Observations were omitted if one or more of the metabolites in a group were not available. Metabolites of chrysene, fluoranthene, and

benzo[*a*]pyrene were not analyzed since preliminary analysis revealed >85% of the samples were below the limit of detection (LOD).

Multiple variables were initially investigated and variables with the most significant relationships and/or relevance were selected from questionnaire data. Demographic variables such as age, sex, occupation, income, and residence age, as well as lifestyle factors related to tobacco smoke exposures (i.e., smoking status, workplace restrictions) were selected. The 2001 National Occupational Classification System was used to categorize occupation. More information about the NOCS systems can be found on the Statistics Canada website (Statistics Canada, 2016b). A summary of the variables examined and the levels for each are provided in Table 1. In some cases, variable levels were adjusted to improve the number of observations in each level and effectively reduce the complexity of the analyses. Data points where the independent variable was “N/A” (i.e., not available) were omitted unless they represented >%5 of the whole dataset at which point an “N/A” variable level was included in the analysis. For example, the Smoking Status variable has three levels, “Current Smoker”, “Former or Never Smoker”, and “Other”. The first level includes "Daily", "Occasional", "Always occasional" smokers, the second includes "Former daily", "Former occasional", "Never smoked", and the “N/A” category is defined as those answering "Not applicable", Do not know", "Refusal", and "Not stated".

Table 1 Categorization of independent variables used in analysis.

Variable	Variable Levels
Smoking status (3 levels)	Current Smoker, including daily, occasional, or always occasional smokers.
	Former or Never smoker, including former daily, former occasional, never smokers.
	N/A, including not applicable, do not know, refusal to state, and not stated.
Work Smoking Restrictions (3 levels)	Limited Restrictions, including some restrictions and no restrictions.
	Restricted, including restricted, and designated areas only.
	N/A, including not applicable, do not know, refusal to state, and not stated.
Sex	Male
	Female
Age (years)	0-18
	19-49
	≥50
Household Income	<$35,000
	≥$35,000 to <$80,000
	≥$80,000
Age of home (years)	<20
	20 to <40
	≥40
	NA, including not applicable, do not know, refusal to state, and not stated.
Occupation	Blue Collar, including occupations in art, culture, recreation and sport, sales and service occupations, trades, transport and equipment operators and related occupations, occupations unique to primary industry, and occupations unique to processing, manufacturing and utilities.
	White Collar, including management occupations, business, finance and administrative occupations, natural and applied sciences and related occupations, health occupations, and occupations in social science, education, government service and religion.
	N/A, including not applicable, do not know, refusal to state, and not stated.

Urinary levels of each PAH metabolite, in µg per g creatinine, were defined as dependent variables, and the effects of the variables listed in Table 1 were examined using SAS 9.4 for Windows 7 (SAS Institute, Cary, NC, USA). Creatinine corrected values were used to account for interindividual variability in glomerular filtration (i.e., dilution of urine). Urinary metabolite levels were log transformed to equalize the variance across the range of observations and meet the assumption of the statistical methods employed. Empirical relationships were calculated using the SAS Proc Surveyreg procedure, specifically designed for regression analysis of survey data. The SAS Proc Surveyfreq procedure was used to examine correlations using Pearson Chi-Square test and SAS Proc Surveymeans was used to calculate descriptive statistics. These procedures were designed specifically for use with survey data to generate frequency and cross tabulation tables and to provide estimates of population means, respectively. Standard errors of geometric means were calculated as defined by (Wolter, 1985)). WEIGHT and REPWEIGHTS statements were used in all three procedures to incorporate weighting factors adjusting for subject selection as recommended by (Statistics Canada, 2011). A VARMETHOD statement was also used to specify the balanced repeated replication (BRR) method as the variance estimation model. Forward, backward, and stepwise variable selection was used to determine the most parsimonious model relating selected variables to urinary PAH metabolite concentrations. Variable selection employed a Proc Surveyreg macro developed by (Wang, F. & Shin, H.C., 2011). Briefly, variables were iteratively added or removed, and models selected based on effect significance and iterative model improvement. Figures were generated by Microsoft Excel.

2.4. Results & Discussion

Table 2 contains descriptive statistics for each urinary metabolite, across the various levels of the independent variables examined in this study. Differences in the unweighted sample

sizes arise from certain metabolite sums being incomplete (i.e., one or more of the metabolites was not stated), and thus omitted. Differences in the mean and median for all metabolites reflect the skewed nature of the data, thereby justifying log transformation for analysis of urinary PAH metabolite data. Consequently, the geometric mean provided in Table 2 is a superior measure of central tendency for each level of each independent variable. For example, it is readily apparent that increased tobacco smoke exposure (i.e., current smoke and less restrictive workplace) increases PAH metabolite levels; conversely, increased age and household income appear to be negatively correlated with PAH metabolites levels (Table 2). Occupation also appears to be related to urinary PAHs, with levels in Blue Collar workers being elevated compared with White Collar (Table 2). Sex and home age appear to have minimal influence on PAH metabolite levels. Thus, it is evident from the descriptive statistics alone that several factors are empirically related to urinary PAH metabolite concentrations.

Collinearity amongst both the independent (i.e., smoking status, occupation, age, etc.) and the dependent variables (e.g., 1-OHP, ∑OH-Phen, etc.) indicates that the results obtained for models with more than one independent variable will depend on the order in which the variables are entered into the model. Moreover, with respect to the dependent variables, statements regarding the utility of any particular urinary metabolite to assess PAH exposures from particular sources must be interpreted with caution. Indeed, the correlation matrix for the independent variables, which is presented in Table 4, revealed multicollinearity for most of the variables examined. Consequently, a variable selection process was required to identify the most parsimonious model. The procedure involves iterative inclusion or exclusion of variables, and the use of F statistics to evaluate sequential model improvement. The parsimonious model is that which contains the most explanatory power with the minimum number of variables; Table 6

summarizes the results of the model selection analyses. The results obtained revealed that the variables investigated can explain between 24 – 50% of the variation in the PAH metabolites investigated. More rigorous handling of the multicollinearity amongst the independent variables could involve partitioning of the dataset into more balanced subsets of the available data (e.g., smoking effect for males only, occupational effect for smokers only, etc.). The downside to this type of approach is over-stratification of the dataset will lead to a decline numbers of observations and statistical power.

Multicollinearity amongst the dependent variables was also investigated. Collinearity amongst the metabolites was assessed using Pearson correlation coefficients; the results revealed low to moderate correlation (i.e., Pearson r of 0.02-0.56, Table 4). Furthermore, correlation analysis of the residuals associated with the models described in Table 6 also showed low to moderate correlation (i.e., Pearson r of 0.28-0.67, Table 5). Residuals associated with of the phenanthrene and fluorene models were noticeably more correlated than the others, thus making it difficult to confidently that the described empirical relationship with each metabolite are unique. However, the magnitude of the Pearson coefficients for nearly all the correlations between model residuals would not considered severe (i.e., are below 0.6) (Grewal et al., 2004). Nevertheless, future work should investigate the use of other alternative methods to handle dependent variable collinearity. For example, principal component analysis (PCA) could be used to derive a metabolite score for each individuals, with subsequent analyses examining empirical relationships between the independent variables and the unique axis scores.

Overall, the results clearly indicate that smoking status has the most consistent and greatest influence on PAH metabolites levels. This is followed by the effect of age for all metabolites except the sum of naphthalene metabolites. Those less influential, sex and home age,

also significantly related to PAH metabolite levels. The sections following Table 6 present a more detailed interpretation of the results; and moreover, discussions regarding the influence of the various variables examined on urinary PAH metabolite levels.

Table 2 Descriptive statistics for urinary PAH metabolite levels in Canadians. Summary is based on the CHMS 2009-2011 data, and values are presented for the various levels of the independent variable examined.

		1-Hydroxypyrene (1-OHP) (µg/g creatinine)								∑Hydroxyphenenathrenes (∑OH-Phen) (µg/g creatinine)							
Variable	Categories	Unweighted sample size	Min.	Median	Median SE	Max	Mean	Mean SE	GM	Unweighted sample size	Min.	Median	Median SE	Max	Mean	Mean SE	GM
	Total	2415	0.010	0.10	0.0016	5.3	0.15	0.0067	0.11	2319	0.090	0.37	0.017	6.4	0.53	0.021	0.41
Smoking status	Smoker	237	0.018	0.19	0.020	2.0	0.28	0.030	0.21	247	0.12	0.69	0.080	5.1	0.95	0.080	0.73
	Former or never smoker	1157	0.011	0.087	0.0017	5.3	0.11	0.0044	0.090	1154	0.090	0.32	0.020	5.6	0.43	0.020	0.34
	N/A	1021	0.024	0.15	0.0072	2.4	0.20	0.0078	0.16	918	0.11	0.37	0.020	6.4	0.49	0.030	0.41
Work Smoking Restrictions	Limited Restrictions	63	0.034	0.12	0.035	1.9	0.22	0.044	0.15	66	0.090	0.32	0.13	2.2	0.58	0.15	0.39
	Restricted	785	0.019	0.10	0.0023	2.0	0.14	0.0081	0.10	803	0.090	0.37	0.022	5.1	0.53	0.024	0.40
	N/A	1567	0.011	0.11	0.0057	5.3	0.16	0.0067	0.12	1450	0.090	0.37	0.020	6.4	0.54	0.030	0.31
Sex	Male	1203	0.011	0.098	0.0031	2.4	0.15	0.013	0.11	1174	0.090	0.33	0.023	5.6	0.52	0.030	0.38
	Female	1212	0.019	0.099	0.0026	5.3	0.15	0.0061	0.11	1145	0.090	0.39	0.022	6.4	0.55	0.030	0.43
Age (years)	0-18	1453	0.018	0.13	0.0056	2.4	0.18	0.008	0.14	1334	0.090	0.31	0.014	6.4	0.43	0.026	0.35
	19-49	563	0.023	0.099	0.0029	2.0	0.15	0.010	0.11	582	0.090	0.34	0.030	3.0	0.51	0.030	0.39
	≥50	399	0.011	0.084	0.0038	5.3	0.13	0.0098	0.089	403	0.10	0.42	0.016	5.6	0.63	0.043	0.47
Household Income	<$35,000	364	0.018	0.12	0.012	2.0	0.20	0.021	0.13	344	0.10	0.47	0.050	4.2	0.66	0.050	0.49
	≥$35,000 to <$80,000	919	0.011	0.097	0.0020	5.3	0.14	0.0074	0.11	890	0.090	0.36	0.020	6.4	0.55	0.044	0.41
	≥$80,000	1132	0.022	0.10	0.0030	1.8	0.13	0.007	0.10	1085	0.090	0.35	0.020	5.2	0.46	0.022	0.37
Age of home (years)	<20	752	0.011	0.096	0.0041	1.8	0.13	0.0091	0.10	716	0.090	0.33	0.030	6.4	0.45	0.028	0.36
	20 to <40	817	0.018	0.097	0.0051	5.3	0.15	0.014	0.11	796	0.10	0.35	0.027	5.2	0.50	0.050	0.38
	≥40	740	0.019	0.099	0.0044	2.4	0.17	0.012	0.12	707	0.10	0.40	0.039	5.6	0.65	0.053	0.47
	NA	106	0.024	0.13	0.029	0.67	0.17	0.014	0.14	100	0.16	0.47	0.056	1.9	0.54	0.040	0.47
Occupation	Blue Collar	459	0.020	0.099	0.0072	2.0	0.18	0.015	0.12	468	0.090	0.35	0.043	5.1	0.60	0.051	0.42
	White Collar	390	0.019	0.091	0.0033	0.98	0.11	0.0074	0.090	402	0.10	0.37	0.028	4.2	0.46	0.020	0.38
	N/A	1566	0.011	0.11	0.0057	5.3	0.16	0.0067	0.12	1449	0.090	0.37	0.018	6.4	0.54	0.031	0.41

N/A, not applicable or not stated; SE, standard error; GM, geometric mean.

		∑Hydroxyfluorenes (∑OH-Fluo) (µg/g creatinine)								∑Hydroxynaphthalenes (∑OH-Nap) (µg/g creatinine)							
Variable	Categories	Unweighted sample size	Min.	Median	Median SE	Max	Mean	Mean SE	GM	Unweighted sample size	Min.	Median	Median SE	Max	Mean	Mean SE	GM
	Total	2505	0.11	0.46	0.022	16	0.92	0.053	0.56	2475	0.66	5.2	0.33	7520	19	5.9	6.1
Smoking status	Smoker	254	0.17	2.33	0.28	16	2.8	0.20	1.94	252	1.2	19	2.9	7520	53	29	17.3
	Former or never smoker	1226	0.11	0.40	0.010	5.6	0.49	0.02	0.41	1209	0.66	4.1	0.23	4105	12	0.23	4.6
	N/A	1025	0.12	0.50	0.030	7.0	0.63	0.04	0.54	1014	0.96	6.1	0.32	400	9.5	0.82	6.4
Work Smoking Restrictions	Limited Restrictions	68	0.15	0.43	0.56	16	1.7	0.64	0.75	67	1.2	4.6	6.7	78	14	4.6	7.3
	Restricted	852	0.11	0.44	0.030	9.5	0.92	0.07	0.55	842	0.66	5.1	0.45	2403	13	3.9	6.0
	N/A	1585	0.11	0.48	0.031	9.0	0.83	0.053	0.57	1566	0.70	5.3	0.3	7520	30	15	6.1
Sex	Male	1255	0.11	0.44	0.030	9.4	1	0.10	0.57	1242	0.70	4.4	0.38	843	9.5	0.90	5.4
	Female	1250	0.11	0.49	0.020	16	0.84	0.056	0.55	1233	0.66	5.9	0.29	7520	29	12	6.8
Age (years)	0-18	1470	0.12	0.43	0.024	8.1	0.58	0.041	0.47	1454	0.70	5.2	0.3	400	8.1	0.54	5.5
	19-49	613	0.11	0.44	0.030	16	0.96	0.10	0.57	601	0.66	4.7	0.62	2403	14	5.3	5.9
	≥50	422	0.11	0.50	0.036	9.5	1.1	0.068	0.61	420	0.71	5.3	0.33	7520	33	16	6.6
Household Income	<$35,000	374	0.15	0.58	0.063	9.4	1.5	0.17	0.81	373	0.79	6.9	1.1	7520	49	28	7.9
	≥$35,000 to <$80,000	952	0.11	0.48	0.023	16	0.96	0.06	0.58	943	0.70	5.2	0.38	4105	12	3.7	6.1
	≥$80,000	1179	0.11	0.41	0.018	6.8	0.66	0.06	0.47	1159	0.66	4.8	0.33	2403	12	5.1	5.4
Age of home (years)	<20	778	0.11	0.40	0.010	6.3	0.65	0.073	0.46	770	0.66	4.6	0.35	2403	16	8.8	5.4
	20 to <40	851	0.12	0.43	0.050	8.1	0.87	0.14	0.53	840	0.71	5.1	0.48	4105	15	4.8	6.0
	≥40	767	0.11	0.54	0.027	16	1.2	0.11	0.67	756	0.79	5.3	0.41	400	12	1.6	6.4
	NA	109	0.15	0.54	0.13	5.4	1.0	0.16	0.74	109	0.98	7.7	2.6	7520	115	109	9.0
Occupation	Blue Collar	499	0.11	0.45	0.060	16	1.3	0.16	0.65	494	0.88	5.6	0.72	2102	14	2.2	6.7
	White Collar	422	0.11	0.44	0.030	8.1	0.64	0.039	0.48	416	0.66	4.9	0.31	2403	12	7.3	5.4
	N/A	1584	0.11	0.48	0.031	9.0	0.83	0.053	0.57	1565	0.70	5.3	0.3	7520	30	15	6.1

N/A, not applicable or not stated; SE, standard error; GM, geometric mean.

Table 3 Correlations between independent variable investigated in this study. Correlations were examined using the Pearson Chi-square method, and values shown are Chi-squared values.

	Sex	Age	Smoking Status	Work Smoking Restrictions	Household Income	Age of home	Occupation
Sex	1	1.5	22.8**	58.8***	12.4	12.2	74.8***
Age		1	1173.2***	809.0***	70.1***	44.9	831.9***
Smoking Status			1	18.5*[a]	104.8***	52.0**	52.0***[a]
Work Smoking Restrictions				1	188.6***	8.5	15.4**[b]
Household Income					1	240.9***	250.5***
Age of home						1	37.1
Occupation							1

***p<0.001, **p<0.01, *p<0.05

[a] "N/A" answers for Smoking Status had to be omitted to avoid cell frequencies of zero, and allow computation.

[b] "N/A" answers for Work Smoking Restrictions had to be omitted to avoid cell frequencies of zero, and allow computation.

Table 4 Correlations between dependent variable investigated in this study. Values shown are Pearson correlation coefficients.

	1-OHP	ΣOHPhen	ΣOHFluo	ΣOHNap
1-OHP	1	0.44***	0.39***	0.02
ΣOHPhen		1	0.56***	0.06**
ΣOHFluo			1	0.11***
ΣOHNap				1

*p <0.05,**p<0.01,***p<0.001

Table 5 Correlations between the residuals of dependent variables investigated in this study. Values shown are Pearson correlation coefficients.

	1-OHP Residuals	ΣOHPhen Residuals	ΣOHFluo Residuals	ΣOHNap Residuals
1-OHP Residuals	1	0.59***	0.55***	0.28***
ΣOHPhen Residuals		1	0.67***	0.32***
ΣOHFluo Residuals			1	0.40***
ΣOHNap Residuals				1

*p<0.05, **p<0.01, ***p<0.001

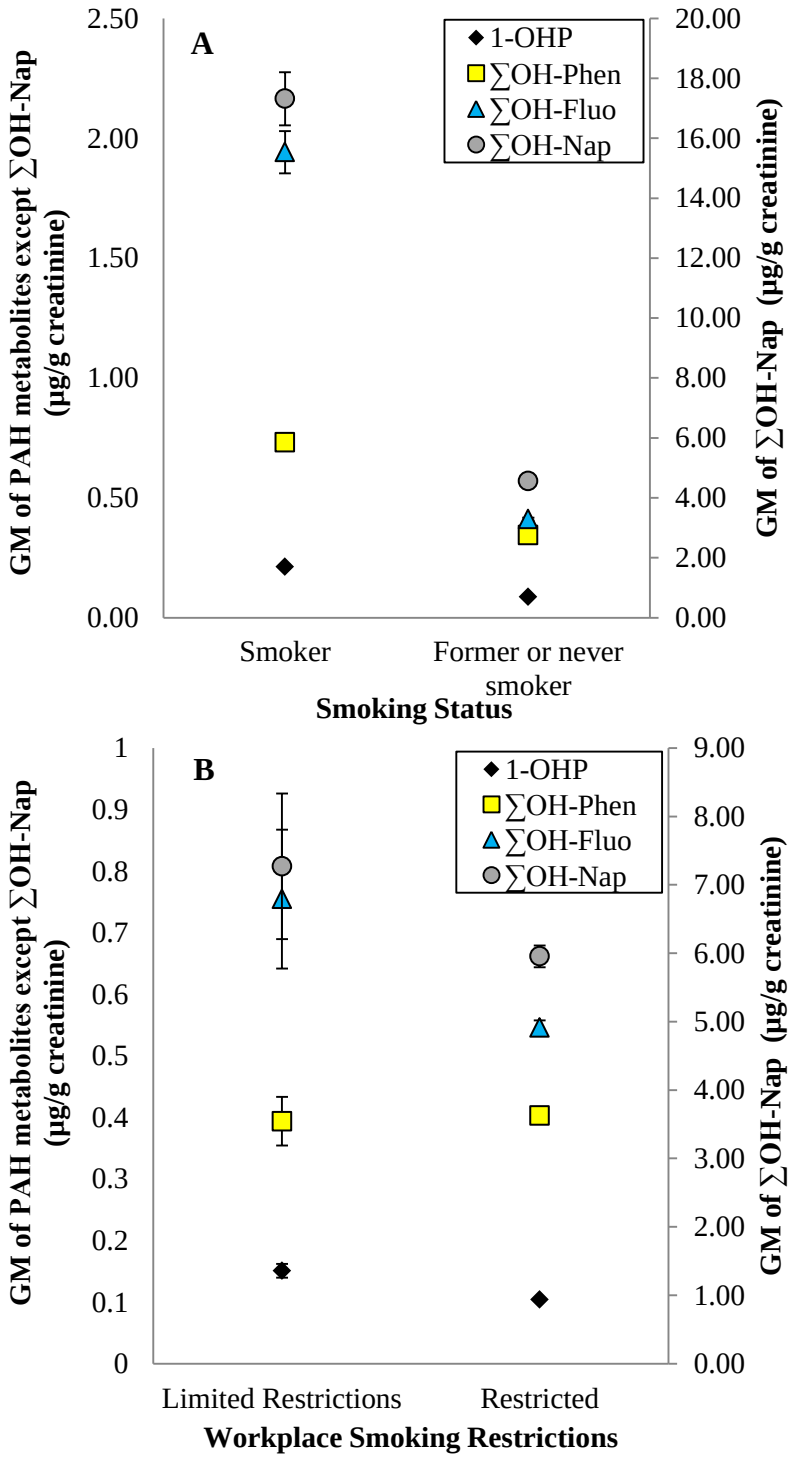

Figure 1 Relationship between urinary PAH metabolites and (A) smoking status and (B) workplace smoking restrictions. Error bars show standard error of the geometric mean (GM). Where error bars cannot be seen, they were smaller than the plotting symbol.

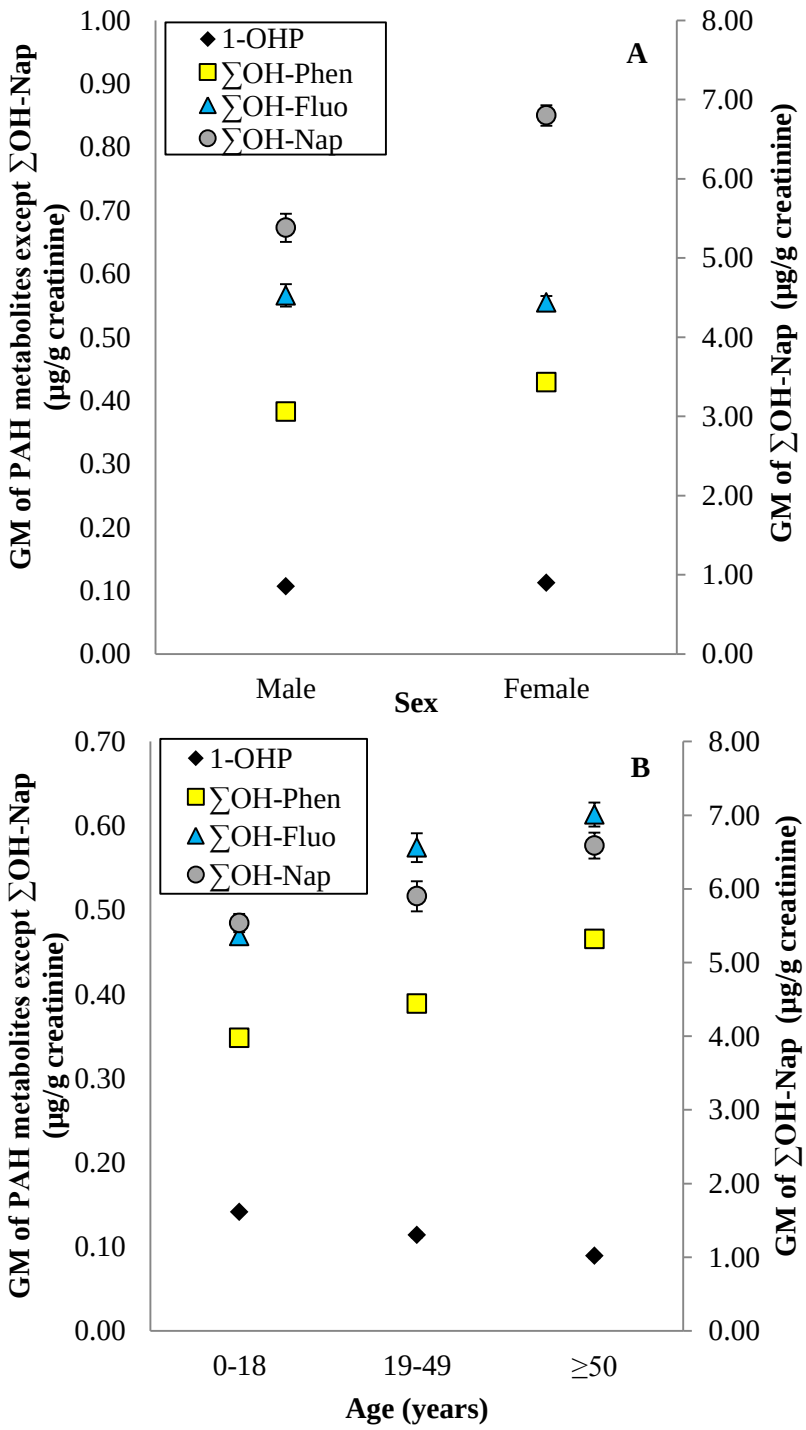

Figure 2 Relationship between urinary PAH metabolites and (A) sex and (B) age. Error bars show standard error of the geometric mean (GM). Where error bars cannot be seen, they were smaller than the plotting symbol.

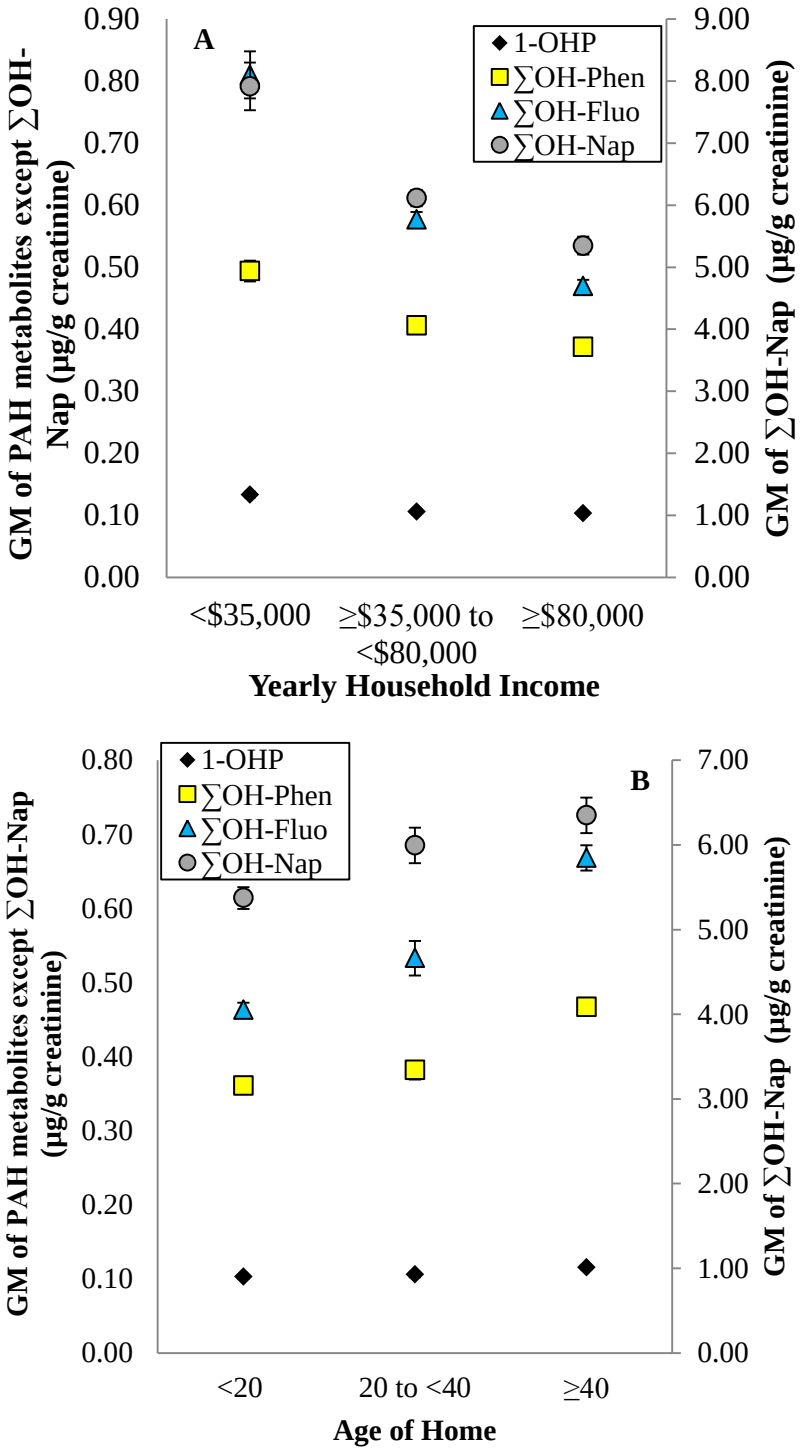

Figure 3 Relationship between urinary PAH metabolites and (A) yearly household income and (B) age of home. Error bars show standard error of the geometric mean (GM). Where error bars cannot be seen, they were smaller than the plotting symbol.

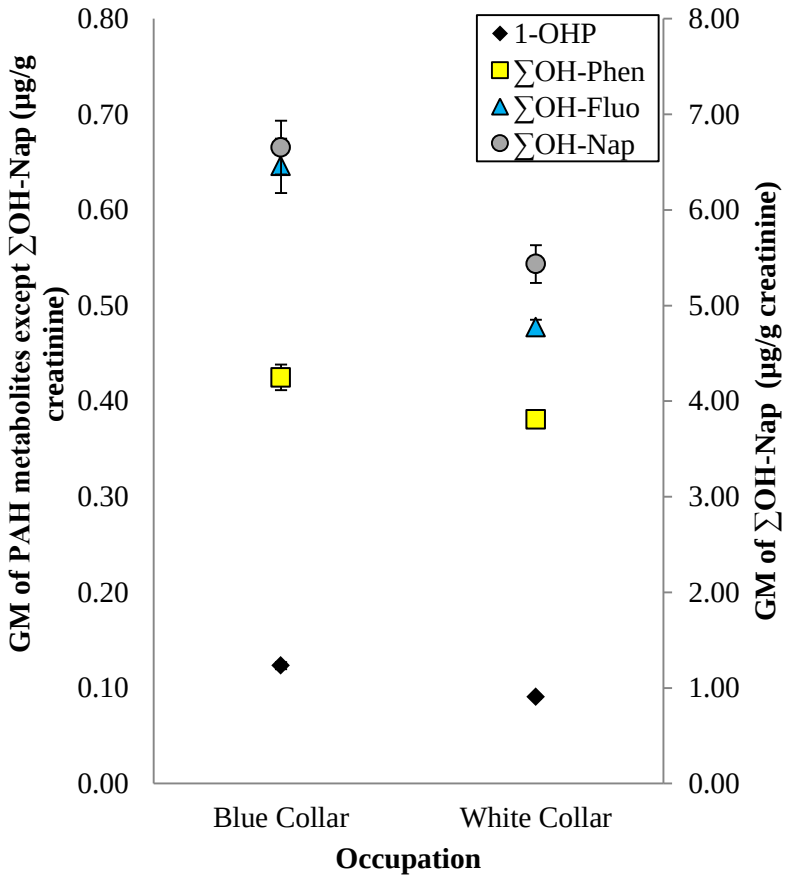

Figure 4 Relationship between urinary PAH metabolites and occupation. Error bars show standard error of the geometric mean (GM). Where error bars cannot be seen, they were smaller than the plotting symbol.

Table 6 Forward, backward, and stepwise weighted model selection for OHP, ∑OH-Phen, ∑OH-Fluo, and ∑OH-Nap using a $p<0.05$ criterion for variables to enter or depart a model. In all cases urinary metabolites levels were log transformed and N/A groups were included (i.e., for Smoking Status, Work Smoking Restrictions, Age of Home, and Occupation.

	Forward Model Selection				Backward Model Selection				Stepwise Model Selection			
PAH Meta-bolite	Variable	r^2	F Value	p Value	Variable	r^2	F Value	p Value	Variable	r^2	F Value	p Value
1-OHP	Smoking Status	0.25	59.66	<0.0001	Smoking Status	0.25	59.66	<0.0001	Smoking Status	0.25	59.66	<0.0001
	Age	0.03	19.39	<0.0001	Age	0.03	19.39	<0.0001	Age	0.03	19.39	<0.0001
	Sex	0.01	6.83	0.009	Sex	0.01	6.83	0.009	Sex	0.01	6.83	0.009
	r^2=0.29				r^2=0.29				r^2=0.29			
∑OH-Phen	Smoking Status	0.18	53.43	<0.0001	Smoking Status	0.18	53.43	<0.0001	Smoking Status	0.18	53.43	<0.0001
	Age	0.03	17.91	<0.0001	Age	0.03	17.91	<0.0001	Age	0.03	17.91	<0.0001
	Sex	0.01	11.89	0.0006	Sex	0.01	11.89	0.0006	Sex	0.01	11.89	0.0006
	Age of Home	0.02	2.9	0.0339	Age of Home	0.02	2.9	0.0339	Age of Home	0.02	2.9	0.0339
	r^2=0.24				r^2=0.24				r^2=0.24			
∑OH-Fluo	Smoking Status	0.48	141.9	<0.0001	Smoking Status	0.48	141.9	<0.0001	Smoking Status	0.48	141.9	<0.0001
	Age	0.01	8.58	0.0002	Age	0.01	8.58	0.0002	Age	0.01	8.58	0.0002
	Age of Home	0.01	5.73	0.0007	Age of Home	0.01	5.73	0.0007	<0.0001	0.01	5.73	0.0007
	Sex	0.00	4.82	0.0282	Sex	0.00	4.82	0.0282	<0.0001	0.00	4.82	0.0282
	r^2=0.50				r^2=0.50				r^2=0.50			
∑OH-Nap	Smoking Status	0.27	104.7	<0.0001	Smoking Status	0.30	99.82	<0.0001	Smoking Status	0.27	104.7	<0.0001
	Sex	0.03	30.32	<0.0001	Sex	0.00	32.24	<0.0001	Sex	0.03	30.32	<0.0001
	Age	0.01	3.29	0.0375	Occup-ation	0.00	12.69	<0.0001	Age	0.01	3.29	
	r^2=0.31				Work Smoking Restr-ictions	0.00	6.27	0.0019	r^2=0.31			
					Age	0.01	3.5	0.0303				
					r^2=0.31							

2.4.1. Tobacco smoke exposure

Tobacco smoke can be a major source of PAH exposure with up to thousands of Enanograms of PAHs being produced during the combustion of a single cigarette (Moir et al., 2007). With cigarette smoking recognised as the leading cause of preventable disease, and PAHs recognized as possible and probable known human carcinogens, assessment of tobacco smoke-derived PAH exposures is a laudable objective (US Department of Health and Human Services, 2014). Indeed, urinary PAH metabolites have been used extensively to investigate individuals' tobacco smoke exposure (Benowitz et al., 2012; Hecht, 2002; Levine et al., 2015; Strickland & Kang, 1999; van Schooten et al., 1995). Here, not surprisingly, the levels of all four PAH metabolite groups were elevated (i.e., higher medians, means, and geometric means) compared to non-smokers (Table 2). Moreover, in comparison with the other variables examined, smoking status was selected as the best explanatory variable across all selection models presented inTable 6, explaining 18-30% of the variability. Since tobacco smoke is known to have differing levels of the PAHs examined, the effect of smoking status on the levels of each metabolite or metabolite group were investigated to determine if specific metabolites may be superior biomarkers of tobacco smoke exposure, and thus better indicators of PAH source (Menzie, Potocki, & Santodonato, 1992; Moir et al., 2007). Personal smoking habits accounted for more of the variance in ∑OH-Fluo levels (i.e., 48%) in comparison to ∑OH-Nap, 1-OHP, and ∑OH-Phen (i.e., 27-30%, 25%, 18%, respectively), possibly due to the relative amounts of the parent PAHs found in tobacco smoke. Compared to phenanthrene and pyrene, fluorene is found in higher amounts in mainstream cigarette smoke (Moir et al., 2007). This agrees with St. Helen et al. (2012) who noted an enhanced effect of smoking status on urinary 1-hydroxyfluorene, compared to other PAH metabolites; and moreover, a strong correlation between urinary 1-hydroxyfluorene

and urinary nicotine metabolites (St. Helen et al., 2012). Although the concentration of naphthalene in tobacco smoke is higher than fluorene, its high volatility likely renders its metabolites unreliable biomarkers of cigarette smoke exposure. Thus, the results obtained here and elsewhere suggest that metabolites of fluorene are superior biomarkers of tobacco smoke-derived PAH exposures compared to other urinary PAH metabolites.

Because individuals spend a considerable amount of their time in the workplace, the relationships between smoking restrictions at the workplace and urinary PAH metabolites were also investigated. To the best of our knowledge, this is the first study to examine relationships between urinary PAH metabolite levels and reported smoking restrictions in the workplace. Individuals with Limited Restrictions (i.e., no or some smoking restrictions) had the highest means for 1-OHP, ∑OH-Phen, and ∑OH-Fluo, the highest GM for 1-OHP, ∑OH-Fluo, and ∑OH-Nap (Table 2).Interestingly, significant relationships between workplace smoking restrictions and urinary metabolites levels were only observed for ∑OH-Nap in the Backwards Selection Model (Table 6). This suggests that although metabolites of fluorene appear to be a superior biomarker of cigarette smoking (i.e., personal use), it does not appear to be an effective biomarker of environmental tobacco smoke exposure. Correlations between Workplace Smoking Restrictions and all six other variables investigated (Table 3) complicates robust examination of a workplace smoking restriction effect on urinary PAH metabolite levels. Indeed, model selection failed to indicate a significant effect of workplace smoking restrictions for nearly any of the PAH metabolites examined. More specifically, after accounting for the effects of other variables such as smoking status, age, sex, age of home, and occupation, the effect of workplace smoking restrictions was not significant except for the Backward Model Selection for ∑OH-Nap levels. Thus, although the analyses of each variable individually do seem to suggest that

workplace smoking restrictions does have a significant impact of urinary PAH levels, the variable is not sufficiently orthogonal to the other variables to be detected when multiple variables are examined simultaneously. The results presented in Table 3 suggest that the strong correlations between workplace restrictions and both age and household income interfere with robust, meaningful detection of a workplace smoking restriction effect. Robust examination of a workplace smoking restriction effect would require examination of populations that are matched for age and household income (i.e., a dataset that is effectively balanced for age and household income).

2.4.2. Demographic and lifestyle factors

The influence of demographic and lifestyle factors, such as sex, age, income, and age of home, on PAH exposure and urinary metabolites are less intuitively obvious. With respect to sex, a lack of association between sex and metabolites of pyrene, phenanthrene, and fluorene has been previously noted in study results from Australia, Israel, Spain, and the United States (Bartolomé et al., 2015; CDC, 2015; Levine et al., 2015; Thai et al., 2016). However, the results of the model selection (Table 6) indicate that sex has a significant effect after accounting for the effects of smoking status and age. This apparent contradiction is likely caused by the unbalanced nature of the dataset, which is confirmed by the strong correlation between sex and variables such as smoking status and occupation (Table 3). More specifically, smoking status, work smoking restrictions, and occupation were significantly correlated with sex, with women more frequently falling into the categories associated with lower levels of combustion exposure (i.e., fewer women smokers, fewer women working in workplaces with limited smoking restrictions, fewer women in blue collar occupations). Thus, sex does appear to be significantly related to urinary levels of PAH metabolites; however, only after accounting for the confounding and more

predominant effects of smoking status and/or age and/or occupation (see Table 6). This result is consistent with biomonitoring results for several other substances. For example, analysis of CHMS Cycle 1 data by (Haines & Murray, 2012) revealed significantly higher concentrations of several compounds in the biological fluids of Canadian women compared to Canadian men from (i.e., blood cadmium, manganese, p,p′-dichlorodiphenyltrichloroethane (p,p′-DDE), hexachlorobenzene (HCB), and β-Hexachlorocyclohexane (β-HCH)). The aforementioned results also indicate that tobacco smoke exposure and occupation are contributing to sex-specific differences in combustion emission exposures and urinary PAH metabolite levels. However, it is not clear what is driving the sex effect that was detected after accounting for the effects of smoking status and age (Table 6), in particular, the strong effect on urinary levels of ∑OH-Nap. One hypothesis is that traditional gender roles alter exposures to combustions emissions that are not related to cigarette smoking such as diet, use of personal care and cosmetic products, and use of home cleaning products. However this hypothesis does not explain why naphthalene specific metabolites are elevated. Alternatively, differences in absorption, metabolism, storage, and excretion may be influenced by sex-related differences. Indeed, this has been observed for other compounds such as cadmium (i.e., depleted iron stores in menstruating women lead to increased uptake and accumulation of cadmium), trichloroethylene (TCE) (i.e., increased testosterone levels lead to decreased dermal absorption of TCE), and arsenic (i.e., hormonal influences, drinking water consumption, and nutrition have been suggested as possible reasons for differences in toxicokinetics between sexes) (Loffredo, Aposhian, Cebrian, Yamauchi, & Silbergeld, 2003; McCormick & Abdel-Rahman, 1991; Vahter, Berglund, Åkesson, & Liden, 2002). More specific to PAHs, females have been reported to exhibit higher expression of genes for PAH metabolizing enzymes (i.e., CYP1A1). It has even been suggested that women's

elevated enzymatic capacity increases their susceptibility to DNA adduct formation and lung cancer (Mollerup et al., 2006; Mollerup, Ryberg, Hewer, Phillips, & Haugen, 1999). Thus, if the sex-specific effects are robust, and females are indeed experiencing higher exposures to an individual PAH or multiple PAHs, identification of the putative sources is of utmost importance. Similar to the earlier suggestion regarding investigations of workplace smoking restriction effects, future work to robustly investigate the effect of sex on urinary levels of PAH metabolites should examine a dataset that is balanced with respect to age, smoking status and occupation. This is particularly pertinent for metabolites of naphthalene.

The results obtained revealed significant relationships between age and urinary concentrations in the iterative selection models summarised in Table 6 for all metabolites and all selection strategies. For 1-OHP, the highest median, mean, GM (Table 2), were observed for individuals aged 0-18 years. Upon further investigation, individuals under 6 years old (i.e., children) seem to be driving this trend. This result agrees with the analyses of American biomonitoring data that also showed higher levels of 1-OHP in children (Li et al., 2008). This elevation may reflect increased toddler exposures to PAHs adsorbed to settled house dust. Indeed, (J. W. Roberts et al., 1991) have shown that hand-to-mouth behaviour is associated with higher children's PAH ingestion rates, and concomitant urinary 1-OHP concentrations that are 2.5-fold greater than adults. For the remaining metabolites (i.e., ∑OH-Phen, ∑OH-Fluo, and ∑OH-Nap), adults ≥50 years old had the highest median, mean, and GM (Table 2). This could be related to differences in daily activities that lead to increased exposures related to occupation, recreation, and/or transportation. Interestingly, the results obtained here are not consistent with a large scale Australian study that noted lower concentrations of all measured PAH metabolites in children under 15 years old and adults over 60 (Thai et al., 2016). However, the urinary

metabolite values analysed in that study were not creatinine corrected. Thus, this discrepancy may be a reflection of lower urinary creatinine excretion rates in children and elderly individuals related to their relatively smaller muscle mass (Barr et al., 2005). Due to the lower creatinine excretion rates, creatinine correction would lead to higher creatinine-corrected urinary PAH concentrations and the opposite conclusion (i.e., higher values in children and elderly). The inconsistency between the results obtained herein and previously published results underscores the importance of creatinine correction (i.e., correction for urinary dilution) when assessing age related differences in urinary concentrations of contaminant metabolites. Creatinine correction accounts for differences in urinary output; however, differences in urinary creatinine related to variations in muscle mass across different demographic groups can skew the results and should be kept in mind. Nonetheless, the influence of age-related activities on urinary PAH metabolites warrants further investigation.

The results obtained also revealed a significant effect of home age on urinary PAH metabolite concentrations as well as for household income but not significantly. Table 2 and Figure 3show the differences in the GMs of the various urinary PAH metabolites for different categories of household income and age of home. With respect to household income, the highest median and GM across all metabolites are associated with individuals with a yearly household income of less than $35,000, and as income increases there is a decrease in urinary PAH metabolite concentrations (Table 2, Figure 3a). This is consistent with previous findings indicating that low-income individuals experience increased PAH exposures from poor air quality, which in turn are likely influenced by home location (i.e., proximity to industry or heavy traffic) and environmental tobacco smoke (Chuang et al., 1999). Smoking status may also be driving this relationship; higher smoking rates having been previously noted in low-income

individuals relative to individuals with higher socioeconomic status (Adler et al., 1994; Barbeau et al., 2004; Emmons et al., 2001; Sreeramareddy, Harper, & Ernstsen, 2016). Furthermore, on a more global scale, although tobacco-attributable deaths in countries with higher per capita income are expected to decrease by 9% between 2002 and 2030, they are predicted to double in countries with lower per capita income (Mathers & Loncar, 2006). Although the iterative model selection procedure failed to include household income (Table 6), this is not surprising given the strong correlations between household income and each of the other variables except sex (Table 3). Thus, the unbalanced nature of the data, and the degree of correlation between income and other variables, prohibits robust assessment of the influence of household income on urinary PAH metabolites. Given the nature of the relationships between household income and smoking, occupation, age of home and age, robust assessment of an income effect would require a large dataset that is appropriately balanced.

A significant effect of home age was seen, a variable that is inversely correlated with household income. The data summarised in Table 2 and Figure 3indicate that those who live in the oldest homes (i.e., 50 years old or older) have the highest median and GM levels of metabolites. The results summarised in Table 6 indicate a significant effect of home age on urinary concentrations of ∑OH-Phen and ∑OH-Fluo, explaining 2% and 1% of the variation in urinary metabolite concentrations, respectively. Nevertheless, it should be noted that correlations between home age and both household income and smoking status (Table 4), and the unbalanced nature of the dataset, complicates robust detection of a home age effect. However, iterative model selection did reveal significant, robust effects of home age on ∑OH-Phen and ∑OH-Fluo, after accounting for the effects of smoking status and age (i.e., ∑OH-Fluo) or smoking status, age and sex (∑OH-Phen) (Table 6). Household dust samples from older homes have been

previously shown to have significantly higher PAH concentrations, and it has been suggested that PAHs can accumulate in household carpets, possibly contributing to higher exposures (J. W. Roberts et al., 2009; Whitehead et al., 2011). It is also possible that individuals in older homes are more exposed to PAHs due in part to older styles of residential heating (e.g., wood and wood pellets). Indeed, several studies have shown decreases in household PAHs after transitioning to cleaner heating fuels (Gustafson, Östman, & Sällsten, 2008; Van Metre, Mahler, & Furlong, 2000). Alternatively, income may be the driving factor as income and age of home are inversely related (Figure 3). Thus, it seems clear that home age has a significant, albeit relatively weak, effect on urinary concentrations of ∑OH-Fluo and ∑OH-Phen, and although the cause is not immediately obvious, previous studies provide support for the influence of residential heating fuel. Interestingly, the lack of relationship between home age and urinary 1-OHP suggests that 1-OHP is not a suitable biomarker of domestic PAH exposures. In contrast, fluorene or phenanthrene may be more appropriate.

2.4.3. Occupation

PAHs are formed during incomplete combustion or pyrolysis of organic matter and several occupational setting are known to be contaminated with PAHs. Concomitantly, certain occupations have been associated with an increased likelihood of PAH exposures. These include road paving, roofing, and firefighting, as well as work associated with production of manufactured gas, coke, refined metals, and rubber and other chemicals (Britz-McKibbin et al., 2016; Fent et al., 2014; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans & International Agency for Research on Cancer, 2010; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010; Lee, Lim, & Kim, 2014; Peters, Talaska, Jonsson, Kromhout, & Vermeulen, 2008; Robinson et al., 2008; Sellappa, Mani, & Keyan, 2011;

Simioli et al., 2004; Siwinska, Mielzynska, & Kapka, 2004; Tsai, Shieh, Lee, & Lai, 2001). The results obtained here indicate that individuals with Blue Collar occupations (defined in Table) have markedly higher levels of urinary PAH metabolites (Table 2, Figure 4). In fact, the Blue Collar occupational category is associated with some of the highest median and mean values for many of the metabolites, including the highest mean and GM for 1-OHP, ∑OH-Phen and ∑OH-Fluo, and highest median and GM for ∑OH-Nap. Interestingly, one of the iterative model selection methods revealed a significant effect of occupation on urinary concentration of naphthalene metabolites (Table 6). This result likely reflects differences in occupational exposure; indeed, as noted, individuals with Blue Collar occupations have been shown to experience higher levels of PAH exposures. For example, individuals working in the aluminum industry and anode-manufacturing, compared to controls, have been found to have significantly elevated urinary PAH metabolites (Alexandrie et al., 2000; Bentsen-Farmen, Botnen, Notø, Jacob, & Øvrebø, 1999; Göen, Gündel, Schaller, & Angerer, 1995; Schoket et al., 1999). Here, however, the effect of occupational classification may be confounded with effects related to tobacco smoke, diet, sex, age, and household income. The significant correlations between occupation and all other variables except home age (Table 3), and the inability to detect an occupation effect for two of the metabolites when each variable was tested individually (Table 6), strongly suggests that it was not possible to detect a robust effect of occupational classification. Indeed, Canadian and American studies have noted relationships between occupation and smoking status (Bang & Kim, 2001; Siemiatycki et al., 1988), thus increasing the difficulty of investigating occupational effects that are not confounded with the increased smoking rates that have been observed in Blue Collar cohorts. Investigations regarding the influence of occupation on urinary levels of PAH metabolites may permit the identification of

problematic occupational settings that require intervention to improve worker health and safety. However, it is clear that detection of occupational effects in population-scale biomonitoring data will require careful control of confounding variables. Detection of an occupation effect likely requires examination of specific cohorts of individuals and careful matching of the individuals with appropriate controls.

2.4.4. Influence of Diet

Although this study did not investigate diet, it is a noteworthy factor that can contribute considerably to an individual's urinary PAH metabolite concentrations. For nonsmoking, non-occupationally exposed individuals, dietary intake is considered a major route of exposure (Ramesh et al., 2004). The variables examined here explained 24-50% of the variation for the various PAH metabolites (Table 6), leaving 50-76% unexplained and likely a majority diet related. PAHs can be found in food both endogenously and from introduction during cooking (e.g., pyrolysis during the grilling or barbecuing, smoking). For example, green leafy vegetables such as kale and collards tend to have greater PAH content compared to other vegetables, thought to be due to greater surface area thus greater deposition of airborne contaminants (Kazerouni et al., 2001; Ramesh et al., 2004). Cooking and food preparation can introduce PAHs depending on a variety of factors such as food type (e.g., smoked salmon versus moose), content (e.g., fat content and casing type for sausages), type of heat source (e.g., gas versus charcoal barbeque), and temperature (e.g., different baking temperatures for bread) (Ciecierska & Obiedziński, 2013; Gomes et al., 2013; Kitts, Chen, & Broda, 2012; Rose et al., 2015). Increases in urinary PAH metabolites have been noted after ingestion of PAH containing foods such as smoked salmon , barbequed chicken, and charbroiled beef (Buckley & Lioy, 1992; Li et al., 2012; Motorykin et al., 2015). However, variation of dietary habits and estimation of content of

PAHs in food can make estimation of dietary PAH exposure difficult. Furthermore, diet estimates from food questionnaires can contain significant reporting biases and error, with individuals often underreporting consumption sometimes at implausibly low intake levels when compared with estimated energy needs (Bedard, Shatenstein, & Nadon, 2004; Ferrari et al., 2002; Livingstone et al., 1990; Schaefer et al., 2000). In fact, one study found that 70% of individuals' reported dietary habits disagreed with results from stool analysis (Comino et al., 2016). Because of the unbalanced nature of the dataset and the type of data required to effectively examine the effects of diet, diet was not examined at this time. However, follow-up analyses could investigate the influence of diet that likely has an effect on urinary levels of PAH metabolites apart from the variables examined here in both population data and in more controlled studies.

2.5. Conclusions

Levels of urinary PAH metabolites are useful biomarkers of PAH exposure, as such they permit identification of groups within a population that may have an enhanced risk of PAH-related effects (e.g., cancer). This study, which examined CHMS Cycle 2 data, investigated empirical relationships between urinary levels of various PAH metabolites and several demographic and lifestyle variables. Using a variety of statistical methods, the results were scrutinised to identify the variables that are important determinants of PAH exposures. Moreover, where possible, the results were used to evaluate the utility of urinary 1-OHP, the metabolite most commonly used for PAH biomonitoring, relative to urinary levels of other PAHs. Although the dataset is unbalanced and intercorrelation of independent variables complicates investigations of variable effects, iterative model selection procedures did permit identification of important determinants for PAH exposures. More specifically, the results

revealed that individuals who are current smokers, work in places with limited workplace smoking restrictions, live in households making less than $35,000 a year, live in older homes, and work in Blue Collar occupations are likely to experience the highest levels of PAH exposures. In addition, exposures are generally highest for the youngest and oldest age classes, and generally higher for women compared to men. Together, the variables examined were able to explain 24-50% of the variation on the various PAH metabolites.With respect to the utility of the various PAH metabolites as biomarkers of PAH exposure, the results suggest that some of the PAH metabolites are particularly useful for examination of particular exposure scenarios. For example, the results revealed that smoking status, income, and age of home have a stronger influence on ∑OH-Fluo (i.e., higher r^2) as compared with 1-OHP. Thus, it is reasonable to assert that 1-OHP, the traditional PAH biomonitoring tool, may not be effective for all exposure scenarios; and moreover, that other metabolites may be more suitable (e.g., ∑OH-Fluo for tobacco smoke exposure). Overall, the analyses presented herein exemplify the use of statistical methods to identify demographic and lifestyle variables that influence PAH metabolite levels in the urine of a typical Canadian.

Although the study could not specifically investigate exposures to carcinogenic PAHs, which are excreted in the feces, the results obtained can presumably be used to design and implement remedial measures that reduce exposures to all PAHs. An obvious example would be implementation of smoking cessation programs and increased workplace smoking restrictions. Although it is difficult to identify remedial measures to reduce sex-specific exposures, or exposures related to home age or income, follow-up studies could be designed to determine the causes underlying the empirical relationships identified herein. With respect to the effect of occupational classification, it may be possible to further scrutinise the CHMS data in order to

identify specific occupations that are worthy of further investigation. Follow-up analyses of urinary metabolite levels in specific occupational cohorts can be used to address hypotheses about exposure sources and levels in selected occupational settings. As noted earlier, numerous studies have investigated occupational settings associated with extensive use of high-temperature combustion processes (e.g., metal founding, coke production, etc.). However, few studies have investigated exposures in other Blue Collar occupational settings (e.g. trades, transport and equipment operators). Moving forward, future work should analyse data from other CHMS cycles (where urinary PAH metabolites were measured). As well, analyses should be conducted from other population survey datasets to investigate relationships between PAH metabolite levels and demographic and lifestyle variables, and compare the results with those obtained here. This may include the NHANES from the United States or the BIOAMBIENT.ES project in Spain. Lastly, because empirical analyses do not tell of exact cause, hypotheses generated should be investigated regarding the effect of specific variables on levels of PAH metabolites (e.g., follow-up cohort studies to specifically address hypotheses generated here). For example, if there is in fact a sex effect, gender roles or physiologic differences between males and females could be investigated. Ultimately, this will generate greater understanding of a population's PAH exposure on both a broad and grouped scale, thus allowing for improved exposure reduction programs.

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Chapter 3. Occupational exposures of Ottawa firefighters to polycyclic aromatic hydrocarbons (PAHs) and other organic mutagens

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3.1. Abstract

Firefighters experience above average risks of injury and chronic disease. Few studies have examined firefighters' exposures to combustion emissions, and those that have examined exposures during training exercises. This study examined firefighters' occupational exposures to combustion emissions during "on-shift" fire suppression. Paired urine samples (i.e., pre- and post-event) and fire event questionnaires were collected from 27 male Ottawa Fire Service (OFS) volunteers and 20 office worker controls. PAH exposures were quantified by analysis of urinary PAH metabolites, and urinary mutagenicity was measured using the Salmonella mutagenicity assay (Ames Test). Urinary Clara Cell 16 (CC16) and 15-Isoprostane F_{2t} (8-iso-$PGF_{2\alpha}$) were measured to assess lung injury and overall oxidative stress, respectively. The results show that post-event urinary levels of 1-hydroxypyrene (1-OHP), a metabolite of pyrene, significantly increased by an average of 3.7-fold. Similarly, post-event levels of phenanthrene, fluorene, and naphthalene metabolites showed significant average increases of 5.3-, 3.9- and 2.9-fold, respectively. Post-event levels of urinary mutagenicity showed a significant average increase of

4.3-fold, whereas urinary CC16 and 8-iso-$PGF_{2\alpha}$ did not. Duration of fire suppression was found to be empirically related to post-event concentrations of urinary PAH metabolites. The results indicate that on-shift firefighting is associated with significant exposures to combustion emissions.

3.2. Introduction

Compared to the general population, firefighters experience above average risk of injuries and chronic disease including kidney, ureter, and pancreatic cancers, respiratory diseases, and heritable genetic effects (Guidotti, 1993; LeMasters et al., 2006; Olshan, Teschke, & Baird, 1990). In fact, the International Agency for Research on Cancer (IARC) has declared occupational exposures from firefighting as possibly carcinogenic to humans (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans & International Agency for Research on Cancer, 2010). Exposures to carcinogens during firefighting can occur via contact with combustion by-products, with the magnitude of the exposure varying with the nature of the fire and the individual's role in fire suppression. Many hazardous compounds have been detected at municipal structural fires including formaldehyde, benzene, benzyl chloride, freon, acetic acid, and polycyclic aromatic hydrocarbons (PAHs) (Golka & Weistenhöfer, 2008). PAHs are of concern due to their ubiquitous formation during incomplete combustion of organic matter, and their mutagenic, carcinogenic, and teratogenic properties (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010).

Assessing PAH exposure is imperative to the determination of magnitude and route of exposure. Exposure can be assessed in a number of different ways including urine samples, personal air monitoring, and dermal wipe sampling. Urine can be used to analyze for specific metabolites of combustion emissions or for outcomes associated with combustion emission exposure. Metabolites of PAHs in urine can be measured to provide a non-invasive way to assess whole body exposure. Numerous studies have employed urinary concentrations of PAH metabolites to assess occupational exposures to combustion emissions and PAHs in charcoal, road paving, rubber manufacturing and coke oven workers, cooks, and wildland firefighters

(Kato et al., 2004; Pan, Chan, Huang, & Wu, 2008; Peters et al., 2008; Robinson et al., 2008; Sellappa et al., 2011; Siwinska et al., 2004). Assessment of exposures in individual subjects can be especially important since individuals in an occupation such as firefighting can be involved in a variety of activities, with each activity providing vastly different opportunities for exposure. Given that combustion emissions are complex mixtures, biomarkers that do not require *a priori* information regarding the composition of the mixture and/or the identity of the putative mutagens can be very useful. The Ames/Salmonella reverse mutation assay (Ames Test) can readily assess the mutagenicity of complex mixtures including organic extracts of human urine. Although chemical-specific biomonitoring for PAH metabolites can be useful, they cannot assess the total exposure to a complex mixture of organic mutagens. In contrast, a bioassay such as the Ames test can be used to provide a comprehensive assessment of overall mutagen exposure. This assay has been previously employed to assess the mutagenic activity of complex mixtures such as combustion emissions from coal and biomass fuels, and airborne particulate matter collected from urban and nonurban locations (Bell & Kamens, 1990; Pitts Jr., Grosjean, Mischke, Simmon, & Poole, 1977; Vu et al., 2012). Moreover, several studies have used the Ames test to assess urinary mutagenicity of benzidine-exposed workers in India, wood smoke-exposed charcoal workers in Brazil, and wood smoke-exposed individuals using traditional Native American saunas. All of these studies showed that exposure (i.e., pre- versus post or exposure duration) is positively correlated with urinary mutagenicity (DeMarini et al., 1997; Kato et al., 2004; Long et al., 2014). In addition to chemical metabolites, biomarkers of physiological condition can also be used for occupational monitoring. More specifically, 15-Isoprostane F_{2t} (i.e., 8-iso-$PGF_{2\alpha}$) and Clara Cell 16 (i.e., CC16) can be used to indicate oxidative stress and pulmonary injury, respectively. Isoprostanes are products of free-radical fatty acid peroxidation

of which F_2-isoprostanes can be generated and excreted in the urine. 8-iso-$PGF_{2\alpha}$ has been shown to be a reliable biomarker of lipid peroxidation and oxidative stress *in vivo* in smokers, individuals with systemic sclerosis disorder, and Olympians exposed to air pollution during the Beijing Olympics (Cracowski et al., 2002; Huang et al., 2012; Morrow et al., 1995; L. J. Roberts & Morrow, 2000). Moreover, Wang et al. (2015) showed that co-exposure to PAHs and toxic metals contributed to dramatic increases in oxidative stress, demonstrated by increases in urinary 8-iso-$PGF_{2\alpha}$ (T. Wang et al., 2015). Previous studies of CC16 have shown increases in urinary levels in rats exposed to O_3 and elite swimmers exposed to chloramine,; however, no significant changes in individuals exposed to secondhand tobacco smoke or wood smoke (Arsalane et al., 1999; Barregard et al., 2008; Romberg, Bjermer, & Tufvesson, 2011; St Helen et al., 2013). To the best of our knowledge neither biomarker in urine has been used to examine firefighters before and after fire suppression.

Several studies have looked at firefighters' exposures to toxicants via analyses of wipes (e.g., skin or gear), air, or urine. (Alexander & Baxter, 2014) analysed wipes of personal protective equipment worn during live fires and found 17 PAHs, including the known carcinogen benzo(*a*)pyrene, on at least one piece of firefighters' clothing. Analyses of face and neck wipes from 10 firefighters after 5 fire events by (Baxter, Hoffman, Knipp, Reponen, & Haynes, 2014) found benzo[bjk]fluroanthene (i.e., three benzofluoranthene isomers) in 65% of samples, pyrene in 30% of the samples, and various other PAHs in other samples. Only three PAHs (acenaphthylene, naphthalene, and benzofluoranthene) were found in air samples from the fire events, and were at concentrations well below the recommended short-term exposure limit (STEL). (Fent et al., 2014) conducted personal air, dermal wipe, and urine sampling at training fires. They noted that carcinogenic and probably carcinogenic PAHs represented 0.8-5.7% of

PAHs found in personal air samples, and post-fire neck wipes showed significant increases in total PAH concentrations. Although increase in urinary PAH metabolites were not statistically significant, when changes of PAH levels on the neck and urinary PAH metabolites were ranked from highest to lowest, the two matched. Similarly, (Britz-McKibbin et al., 2016) looked at the same types of firefighter samples collected at Ontario training fires, and also found significant increases in urinary PAH metabolites and dermal PAH concentrations ($p<0.05$). Few studies have examined exposures at on-shift, live fire events. One study that examined urinary PAH metabolite changes after live fire events, found significant increases in urinary 1-hydroxpyrene. However, *unexposed* reference samples from the individuals were collected several days after the actual fire event. This is an unfavourable practice since it may lead to inaccurate baseline for comparison with post-fire values (Caux, O'Brien, & Viau, 2002). It can be argued that the true nature of a firefighter's exposures experienced over a career would be best understood using on-shift, live fire events

To the best of our knowledge, no published study has examined occupational on-shift exposures of municipal firefighters' to PAHs and other organic mutagens. With Ottawa firefighters attending 750 fires in 2015 alone, the opportunity for exposures to PAH-contaminated combustion emissions is ample (Ottawa Fire Services, 2015). This study employed analyses of personal air samples collected during fire suppression and paired urine and wipe samples collected before and after each event to examine exposures during on-shift fire suppression. Analyses of PAHs in personal air and wipe samples provide an indication of environmental contamination surface deposition; analyses of urine provide an indication of internal dose. The results provide an improved understanding of occupational exposures of

municipal firefighters to PAHs and other organic mutagens that can contribute to improved policies and procedures designed to reduce potentially harmful exposures.

3.3. Methods

3.3.1. Study Design and Sample Collection

Ethical clearance for all aspects of the study 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).

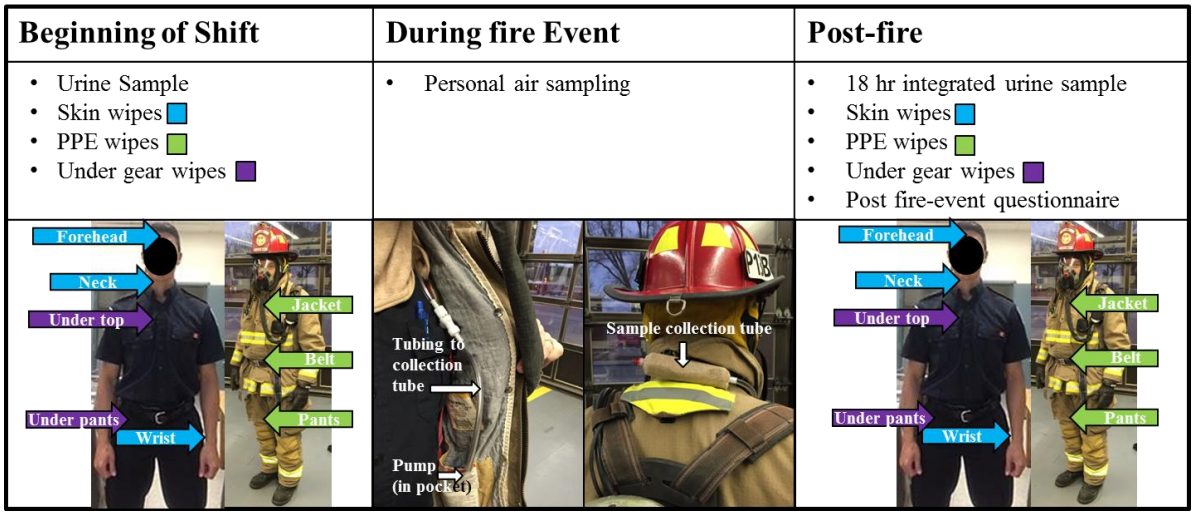

Figure 1 Illustration showing sample collection procedures for firefighter participants. The first and last panels show the location of wipe sample collections. For the personal air samples, the white arrows in the center panel indicate the location of the pump, the connecting tubing, and the sample collection tube. Particulate material was collected on a quartz filter; volatiles and semi-volatiles were collected on polyurethane foam (PUF) plugs. Photos courtesy of D. Matschke and A. Wu, used with permission.

Ottawa Fire Service (OFS) stations were selected based on the historical frequency of firefighting activities; the selected locations were both urban and suburban. Study participants only qualified if they did not smoke, did not live with smokers, and agreed not to consume charbroiled foods or be exposed to non-occupational combustion sources for the duration of the study enrolment. Participants were recruited for study blocks of five consecutive 24hr shifts, which usually spanned 12 days. The purpose of the study was explained to each participant, and after answering any questions, each participant signed a written consent form. Subsequently, 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 that was used to label all samples. These forms can be found in Appendix I – Firefighter Study Documents An overview of the sample collection strategy is illustrated in Figure 1. At the beginning of each shift, pre-exposure samples were collected, including a urine sample in a 120mL polypropylene container (FisherScientific Ltd, ON, Canada) and wipe samples of the skin (i.e., forehead, neck, and wrist), under-gear clothing, and personal protective equipment (PPE) (i.e., Bunker gear jacket and pants, and self-contained breathing apparatus (SCBA) belt). Wipe samples were collected using AlphaWipes® (Texwipe Inc, Kernersville, NC). A personal air sampler was attached to Bunker gear at the start of each shift (Figure 1). This consisted of a GilAir® Plus Personal Air Sampling Pump (Sensidyne, St. Petersburg, FL) operating at 2.5 L/min with a polyurethane foam (PUF) cartridge and a 25 mm quartz filter. The sampling pump was placed in the inside pocket of the Bunker gear coat and polypropylene tubing, run along the inside of the coat, was used to connect the pump to the sample collection cartridge that was attached to the back of the neck with Velcro (Figure 1). Following a fire suppression activity, an 18 hr integrated urine sample (i.e., all urine for 18

hours) was collected using 500mL high density polyethylene (HDPE) containers (FisherScientific Ltd, Ottawa, ON, Canada). If the end of the 18 hour period fell after the 24 hour shift was completed, a kit was provided for continued sample collection. Subjects stored the samples in a freezer until return to the fire station at the start of the next shift. Wipe samples were collected in the same manner as those collected before an event. All samples were stored in a locked -20°C freezer located at the fire station until collection by university research staff. Spot urine, skin wipe samples, and lifestyle questionnaires were also collected from OFS office workers.

3.3.2. Urine Samples

To create integrated 18 hr post-fire urine samples, urine in separate containers were thawed and pooled in 2L HDPE beakers (Sigma Aldrich, Oakville, ON, Canada). To prevent contamination HDPE beakers were soaked in 10% nitric acid for 24 hrs 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 aliquotted into 15 mL presterilized polypropylene tubes (VWR International, PA, USA) labeled with ID codes to maintain anonymity, and stored at -20°C until analysis. Samples for biomarker analyses were stored at -80°C to prevent degradation of CC16 and 8-iso-$PGF_{2\alpha}$.

Aliquots of urine were sent 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, QC, Canada) for analysis of urinary PAH metabolites via GC-MS/MS by as previously described (Gaudreau et al., 2016). Briefly, urinary metabolites were deconjugated using β-glucuronidase enzyme and 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 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 (a national biomonitoring program in Canada) were determined and expressed as µg/g creatinine.

Urinary mutagenicity was measured using the Ames/Salmonella Reverse Mutation assay (i.e. Ames test) with *Salmonella typhimurium* strain YG1041 (from Dr. Takehiko Nohmi, NIHS, Tokyo) in the presence of an exogenous metabolic activation mixture containing Aroclor-induced rat liver S9 (i.e., Molecular Toxicology Inc., Boone, NC). In preparation for the Ames test, samples were filtered, enzymatically deconjugated with pH 5.0 acetate buffer, β-glucuronidase solution and sulfatase solution (Sigma Aldrich, Oakville, ON, Canada), and concentrated using C18 solid phase extraction (VWR International, PA, USA) with methanol elution. An initial range-finding test found that many samples are 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-equivalent per plate. All doses were tested in triplicate with the exception of some limited samples. 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 hrs before the frequency of revertent (rev) colonies was scored using a ProtoCol automated colony counter (Synbiosis Corporation, Exton, PA, USA). The mean positive control 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 linear portion of the concentration-response function and expressed as rev/µmol creatinine.

Two urinary biomarkers (i.e. CC16 and 8-iso-$PGF_{2\alpha}$) were measured to assess pulmonary injury and oxidative stress, respectively. Analyses employed using ELISA kits (BioVendor

R&D, Asheville, USA and Oxford Biomedical, Abingdon, UK) and procedures followed the manufacturer's instructions. Creatinine was used to correct for urinary dilution; urinary creatinine was measured using a colorimetric assay (Oxford Biomedical, Abingdon, UK).

3.3.3. *Skin wipe and personal air analysis*

As part of a companion project skin wipe and personal air samples were analysed separately for 16 priority PAHs (see Table). Briefly, samples were spiked with ^{13}C-labelled standards of the US EPA Priority 16 PAHs (Cambridge Isotope Laboratories Inc., Andover, MA, USA), extracted using accelerated solvent extraction (ASE-350, Dionex Corporation, Sunnyvale, CA, USA) following the method outlined in the US EPA Method 3640A (U.S. Environmental Protection Agency, 1994). Wipe samples required an additional liquid-liquid extraction step before PAHs from both wipe and personal air samples were isolated via clean-up on two solid-phase extraction (SPE) cartridges (VWR, Radnor, PA, USA) first alumina then silica. Samples were concentrated to approximately 1 mL before being spiked with an internal standard (i.e., p-terphenyl-d14, Cambridge Isotope Laboratories, Tewksbury, MA 01876) and analysed an HP 6890 GC coupled with a HP 5973N mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Using single ion monitoring and the extraction efficiency of ^{13}C labeled PAHs, the concentrations of 16 priority PAHs were determined.

Table 1 PAHs measured in personal air and wipe samples, and their respective urinary metabolite(s) measured in urine.

Parent PAHs measured in personal air & wipe samples	**Urinary PAH Metabolite(s)**
Naphthalene	1-Hydroxynaphthalene, 2-Hydroxynaphthalene
Fluorene	2-Hydroxyfluorene, 3-Hydroxyfluorene, 9-Hydroxyfluorene
Phenanthrene	1-Hydroxyphenanthrene, 2-Hydroxyphenanthrene, 3-Hydroxyphenanthrene, 4-Hydroxyphenanthrene, 9-Hydroxyphenanthrene
Fluoranthene	3-Hydroxyfluoranthene
Pyrene	1-Hydroxypyrene
Benz(a)anthracene	1-Hydroxybenz(*a*)anthracene, 3-Hydroxybenz(*a*)anthrancene
Chrysene	2-Hydroxychrysene, 3-Hydroxychrysene, 4-Hydroxychrysene, 6-hydroxychrysene
Benzo(a)pyrene	3-Hydroxybenzo(*a*)pyrene
Acenaphthylene	
Acenaphthene	
Anthracene	
Benzo(b)fluoranthene	
Benzo(k)fluoranthene	
Indeno(1,2,3-cd)pyrene	
Dibenz(a,h)anthracene	
Benzo(g,h,i)perylene	

3.3.4. Statistical analyses

Statistical analyses were conducted using SAS v9.2 for Windows (SAS Institute, Cary, NC, USA) and Microsoft Excel. Where necessary values were log transformed to equalize the variance across groups, and variance homogeneity was examined using the Bartlett test. Mutagenic potency values were determined using ordinary least squares linear regression analysis, and studentised deleted residuals were examined to objectively identify concentration-response outliers. A paired (dependent) *t*-test was used to evaluate the effect of firefighting activities (i.e., pre- versus post-exposure) on creatinine-adjusted urinary PAH metabolites, creatinine-adjusted urinary mutagenicity, creatinine-adjusted urinary CC16, and creatinine-adjusted urinary 8-iso-$PGF_{2\alpha}$. The test assumes that the dependant variable (e.g., mutagenic potency) is continuous, that the subjects are matched, and the differences between the pre- and post-exposure measurements are independent and normally distributed. Single-factor analysis of variance (ANOVA) was also employed to examine differences in creatinine-adjusted PAH metabolite concentrations in firefighters' urine before and after a fire, in OFS office workers who do not participate in fire suppression, and in non-smoking Canadian males aged 25-62. The latter results were obtained from the Canadian Health Measures Survey (CHMS). The same was done for creatinine-adjusted urinary mutagenicity, creatinine-adjusted urinary CC16, and creatinine-adjusted urinary 8-iso-$PGF_{2\alpha}$ levels, but without comparisons with CHMS data due to lack of availability. Least squares linear regression was employed to investigate empirical relationships between creatinine-adjusted urinary PAH metabolites or creatinine-adjusted urinary mutagenic potency and variables that reflect the magnitude of the exposure (e.g. time at fire event, concentration of PAHs in personal air samples, changes in PAH concentrations on skin wipes).

3.4. Results & Discussion

3.4.1. Participants

Samples were collected from 27 firefighters and 20 office worker volunteers between January 2015 and April 2016. Because all firefighter participants were male and significant gender-specific differences in urinary creatinine excretion have been previously noted, the female office workers were omitted from analyses (Barr et al., 2005). 21 office worker samples were collected from 3 office locations. Values from three individuals who provided two samples within 72 hrs of each other thus were averaged to avoid overlap, leaving a total of 18 office worker samples from 17 individuals.

All fire event samples were collected between January and October of 2015. 27 male firefighters participated in one or more 5-shift study blocks over the 10 month period. 31 paired samples were collected from 16 individuals who collectively attended 19 fires, with several instances of multiple participants present at the same fire. 29 of the 31 paired urine samples had matching wipe (i.e., PPE, clothing, and skin) and personal air samples. All wipe and air samples were collected at structural fires (i.e., fires involving structural components of residential or commercial buildings). There were three instances of multiple fires occurring over one 24-hour shift, and one incident involved participant(s) attending two fire events before collecting post-fire samples. Another incident involved participant(s) attending a second fire after starting post-fire sampling from a first fire. A third incident involved two participants attending a fire, completing the collection of post-fire event samples, including the 18 hour urine sampling period, then attending a second fire before their 24-shift was completed. This was treated as two separate fire events.

Details regarding age, weight, and overall health of the subjects are shown in Table 7. All

participants (i.e., office workers and firefighters) indicted that their health and fitness levels are fair to excellent; one office worker considered indicated a “poor” fitness level. Office workers were significantly older ($p<0.05$) and the majority reported being “overweight”. The majority of firefighters reported their body weight as “just about right”. These differences may cause an overestimation of metabolite concentrations because older and less fit individuals have been shown to excrete less creatinine (Barr et al., 2005). However, the effects of creatinine level on office worker and CHMS control values are not cause for concern since these values can be regarded as additional controls over and above those afforded by the study’s repeated measure design.

Table 2 Description of self-reported study participant ages and health metrics.

			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	0	10	3	2
Firefighters	16	34 (25-50)	0	0	8	6	2	1	11	4	0	1	5	8	2

3.4.2. *Urinary PAH metabolites*

Samples below the detection limit were assigned a value of detection limit divided by the square root of 2. For urinary PAH metabolite concentrations, method blanks were subtracted from each value. For all samples, 1-hydroxybenz(*a*)anthracene, 2-hydroxychrysene, 3-hydroxybenz(*a*)anthrancene, 3-hydroxybenzo(*a*)pyrene, 3-hydroxyfluoranthene, 6-hydroxychrysene, 3-hydroxychrysene, and 4-hydroxychrysene were below the limit of detection or could not be measured due to technical difficulties, interference, or poor recovery, and values for these metabolites were omitted from the analysis. Remaining metabolites were grouped by their parent PAH (i.e., pyrene is metabolized to 1-hydroxypyrene (1-OHP), naphthalene to 1-hydroxynaphthalene or 2-hydroxynaphthalene (ΣOH-Nap), fluorene to 2-hydroxyfluorene, 3-hydroxyfluorene, or 9-hydroxyfluorene (ΣOH-Fluo), and phenanthrene to 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, or 9-hydroxyphenanthrene (ΣOH-Phen)), and the results expressed as the sum of the metabolites for each PAH. Urinary 1-OHP concentration results for nine samples (5 office workers, 4 firefighters), as well as two for urinary hydroxyphenanthrenes (one office worker, one firefighter), were unavailable due to technical difficulties. These samples were omitted from the analyses due to incomplete metabolite sums.

The results, which are displayed in Table 3 and Figure 2, show significant differences between the two control groups and firefighters both pre- and post- fire, with varying patterns depending on the PAH metabolite. OFS office workers and urinary PAH metabolite data from the Canadian Health Measures Survey (CHMS) for individuals of the same demographic as the participants in this study (i.e., non-smoking males aged 25-62) were used as controls and points of reference over and beyond that afforded by the study's repeated measure design. Compared to

both reference groups (i.e., CHMS and OFS office workers), firefighters had significantly higher 1-OHP both before and after a fire but were not significantly different before a fire for ΣOH-Phen. OFS office workers and firefighters before a fire event had significantly higher ΣOH-Fluo and ΣOH-Nap compared to the non-smoking Canadian males (i.e., CHMS data). Because office workers were significantly older ($p<0.05$) and the majority reported being "overweight", their creatinine levels were likely lower than the firefighter population thus underestimating the differences between the two groups. Significant post-fire increases in urinary concentrations of metabolites for all four PAHs were observed (i.e., compared to pre-event, $p<0.0001$, Figure 2). Post-event levels of 1-OHP increased an average of 3.7-fold with values ranging from no increase (NI) to 38.9-fold increase. Similarly, post-event levels of metabolites of phenanthrene (ΣOH-Phen) increased by an average of 5.3-fold (NI-63.4-fold), naphthalene (ΣOH-Nap) by an average of 2.9-fold (NI-12.2-fold), and fluorene (ΣOH-Fluo) by an average of 3.9-fold (NI-33.2-fold)

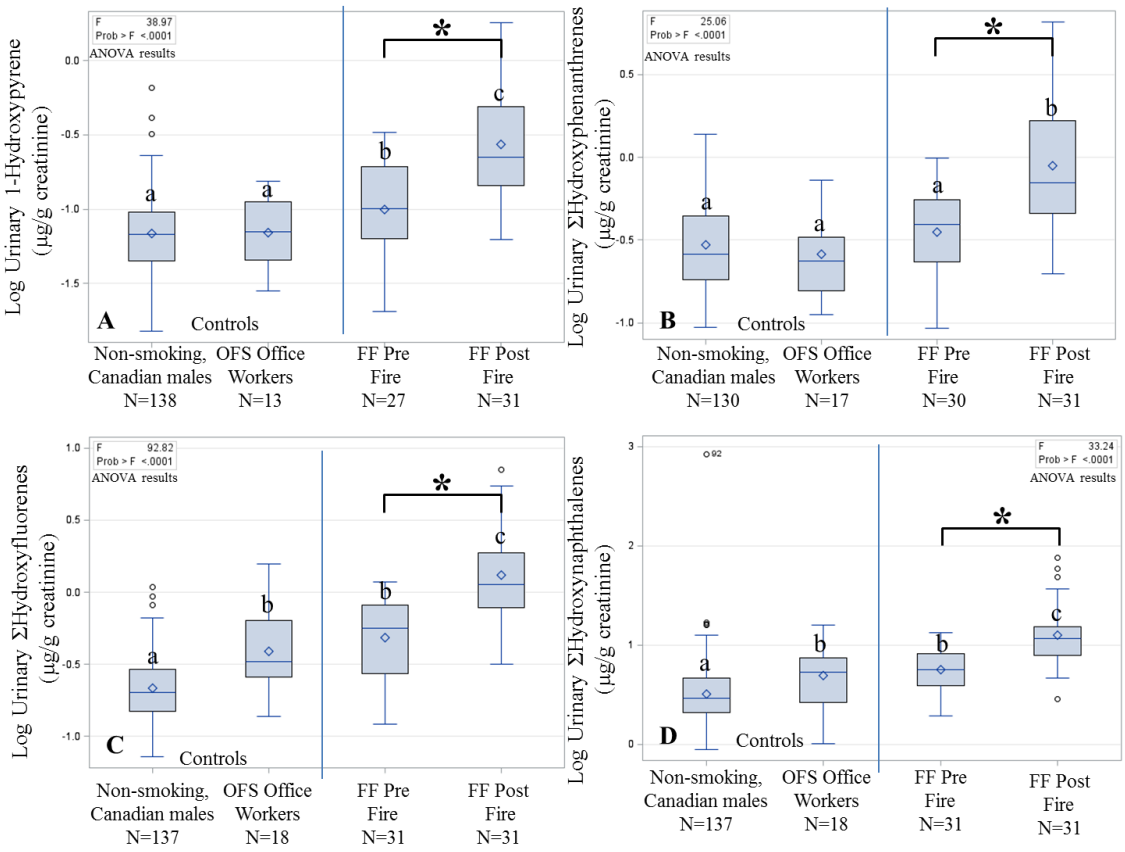

Figure 2 Boxplots showing differences in urinary concentrations of (A) 1-Hydroxypyrene, (B) ΣHydroxyphenanthrenes, (C)ΣHydroxyfluorenes, and (D) ΣHydroxynaphthalenes in non-smoking males aged 25-62 from the Canadian Health Measures Survey (CHMS), OFS office workers (OW), firefighters before a fire event (FF Pre Fire) and firefighters after a fire event (FF Post Fire). Values were log transformed to equalize variance across the groups. Boxes with the same letter are not significantly different at $p<0.05$. A paired t-test was employed to compare before and after firefighter urine samples; $p<0.0001$ is signified by an asterisk. The box represents the interquartile range, the diamond represents the mean value, the solid line represents the group median, and the whiskers extend to the 5th and 95th percentiles. Dots show outliers.

Table 3 Summary of creatinine-adjusted urinary PAH metabolite concentrations for office worker controls and firefighter (FF) subjects (i.e., both pre- and post-fire event).

	Urinary PAH metabolites (μg/g creatinine)											
	1-hydroxypyrene			Σhydroxyphenanthrenes			Σhydroxyfluorenes			Σhydroxynaphthalenes		
	N	Range	GM (+SE)	N	Range	GM (+SE)	N	Range	GM (+SE)	N	Range	GM (+SE)
Office workers	13[a]	0.03-0.16	0.07 (0.01)	17[c]	0.12-0.73	0.26 (0.02)	18	0.14-1.58	0.39 (0.02)	18	1.01-15.99	4.92 (0.37)
FF pre-fire	27[b]	0.02-0.33	0.10 (0.01)	30[d]	0.09-0.98	0.35 (0.02)	31	0.12-1.17	0.48 (0.02)	31	1.94-13.30	5.59 (0.21)
FF post-fire	31	0.06-1.81	**0.27 (0.02)**	31	0.20-6.56	**0.89 (0.06)**	31	0.32-7.09	**1.31 (0.07)**	31	2.83-75.79	**12.52 (0.72)**
Fold change[e]	27	NI-38.9	**4.0**	30	NI-63.4	**5.3**	31	NI-33.2	**3.9**	31	NI-12.2	**2.9**

SE, standard error; NI, no increase; GM, geometric mean.

A one-way ANOVA was employed to compare the three groups and are bolded when significantly different from office workers and firefighters at the start of shift ($p<0.0001$).
A paired t-test was employed to compare paired pre- and post-fire event samples and are bolded when significantly different at $p<0.0001$.

[a]Five samples were removed from this group due to technical difficulties and/or interference.

[b]Four samples were removed from this group due to interference (i.e., overlapping peaks in the chromatograph).

[c]One sample was removed from this group due to technical difficulties.

[d]One sample was removed from this group due to technical difficulties.

[e]Average individual's fold change of post-fire compared to pre-fire (i.e., not overall means)

Significant increases in urinary PAH metabolites after fire suppression have been previous reported. Fernando et al. (2016) noted significant increases in total urinary PAH metabolites after training fires, with an average fold-increase of 3.1 (Britz-McKibbin et al., 2016). The only other study of urinary PAH metabolites after live fire events compared post-event samples with reference samples collected several days after the event (Caux et al., 2002). Nevertheless, significant increases in urinary OHP were still observed. To the best of our knowledge, this is the first study to compare post-event urinary PAH levels from real firefighting scenarios with levels in urine collected prior to the same shift; and moreover, office workers from the same fire department and values from the general population. These comparisons further illustrate the significance of the elevated exposures associated with on-shift fire suppression activities and the need for additional studies to assess the efficacy of standard PPE at avoiding such exposures.

3.4.3. Relationship between urinary PAH metabolites and air and skin samples

The data were employed to examine empirical relationships between urinary metabolite concentrations and external exposure. The personal air and wipe samples clearly indicate elevated PAH exposure, as noted by urinary PAH metabolites, is associated with fire suppression and dermal exposure.

Personal air samples provide a snapshot of the environment to which a firefighter is exposed. Thus, a relationship between personal air samples and urinary output of PAH metabolites indicates that the PAH exposure by firefighters is a result of a specific, emergency fire suppression event. The results show a weak but significant positive correlation between the total urinary PAH metabolite concentration and the PAH concentration of the parent compounds

in personal air samples collected at the scene (Figure 3). This supports the contention that occupational exposure at on-shift fire events increases the firefighters' internal dose of PAHs.

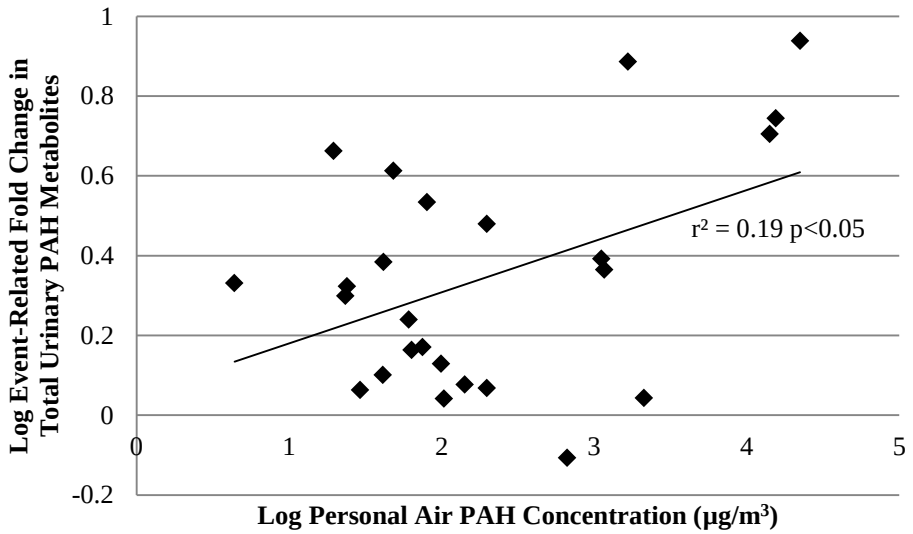

Figure 3 The relationship between log event-related fold change of total concentration of urinary PAH metabolites (i.e., sum of metabolites of pyrene, phenanthrene, naphthalene, and fluorene) and PAH concentration in personal air samples collected at the scene (i.e., sum of pyrene, phenanthrene, naphthalene, and fluorene, including both particular and volatile phases). These PAHs were chosen as they were the parent PAHs of the detectable PAH metabolites. Four samples were omitted due to incomplete sums of urinary metabolites (i.e., one or more metabolite was unable to be measured due to technical difficulties) leaving n=25.

The relationships between event related changes in PAH concentrations in skin wipes samples and their respective urinary metabolite(s) were also examined. No significant relationships were seen for total PAH concentration or low molecular weight PAHs (i.e., 2 or 3 rings) concentrations in skin wipes (data not shown). However, changes in concentrations of high molecular weight (MW) PAHs (i.e., four or more rings) from skin wipes compared to total urinary PAH metabolites showed a significant relationship ($p<0.01$) as illustrated in Figure 11. This is particularly important since high MW PAHs are the only PAHs to be classified as known, probable, or possible carcinogens (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010) Because these are usually excreted through the feces, relationships with the metabolites of lower MW PAHs in the urine may infer exposure to the total mixture of combustion emissions (Ramesh et al., 2004). Furthermore, the results showed significant relationships between event-related fold changes in urinary metabolites of individual PAHs (i.e., phenanthrene, fluorene, and naphthalene) and corresponding event-related increases in PAH levels on skin ($p<0.05$), and are shown in Figure 5. 1-OHP did not show a statistically significant relationship with high MW PAH skin concentrations but followed a similar trend as the other PAHs with a positive correlation between the two metrics (Figure 5A). This shows the usefulness of urinary PAH metabolite monitoring to investigate various routes of exposure and how metabolites apart from the traditionally used 1-OHP may provide additional information.

These results suggest that dermal contact may be an important route of firefighter PAH exposures. (Fent et al., 2014) found similar results, noting matched ranking between PAH concentrations on the neck and urinary PAH metabolite levels. The recognition of dermal contact as a noteworthy route of exposure has led to the suggestion of using wipes to remove post-fire dermal contaminants (Baxter et al., 2014; Britz-McKibbin et al., 2016; Fent & Evans, 2011;

Firefighter Cancer Support Network, 2013). Future studies should investigate the effectiveness of both dermal contact prevention (e.g., better fitting PPE, sealing gaps in PPE, utilization of gloves when touching contaminated gear and tools) and post-exposure decontamination procedures (e.g., on scene dermal wipes, timely showers).

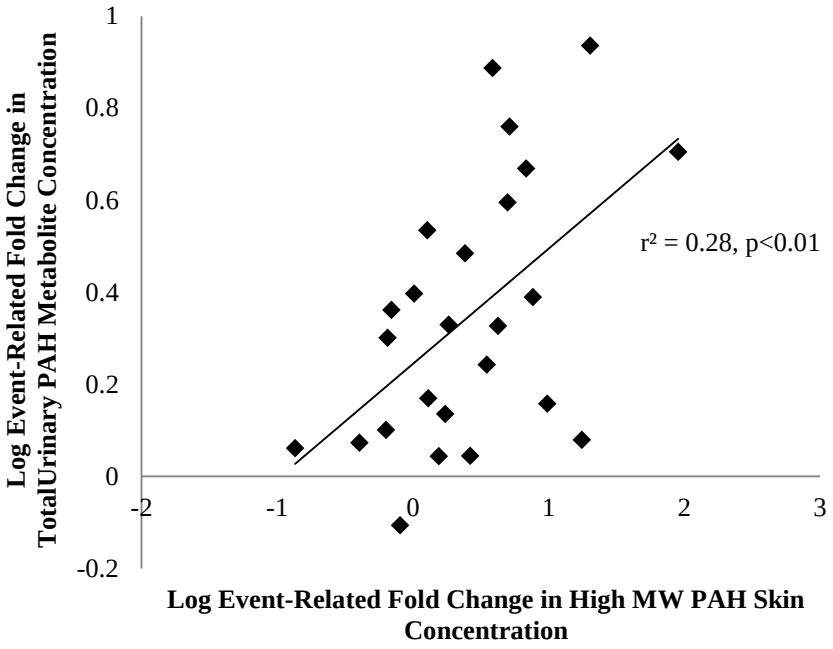

Figure 4 The relationships between event-related fold changes in total concentrations of urinary PAH metabolites and fold changes of high molecular weight PAHs (i.e. 4 or more aromatic rings) in skin wipe samples. Four lacked complete PAH metabolite data (i.e., one or more metabolite was unable to be measured due to technical difficulties) thus were omitted and resulting in n=25.

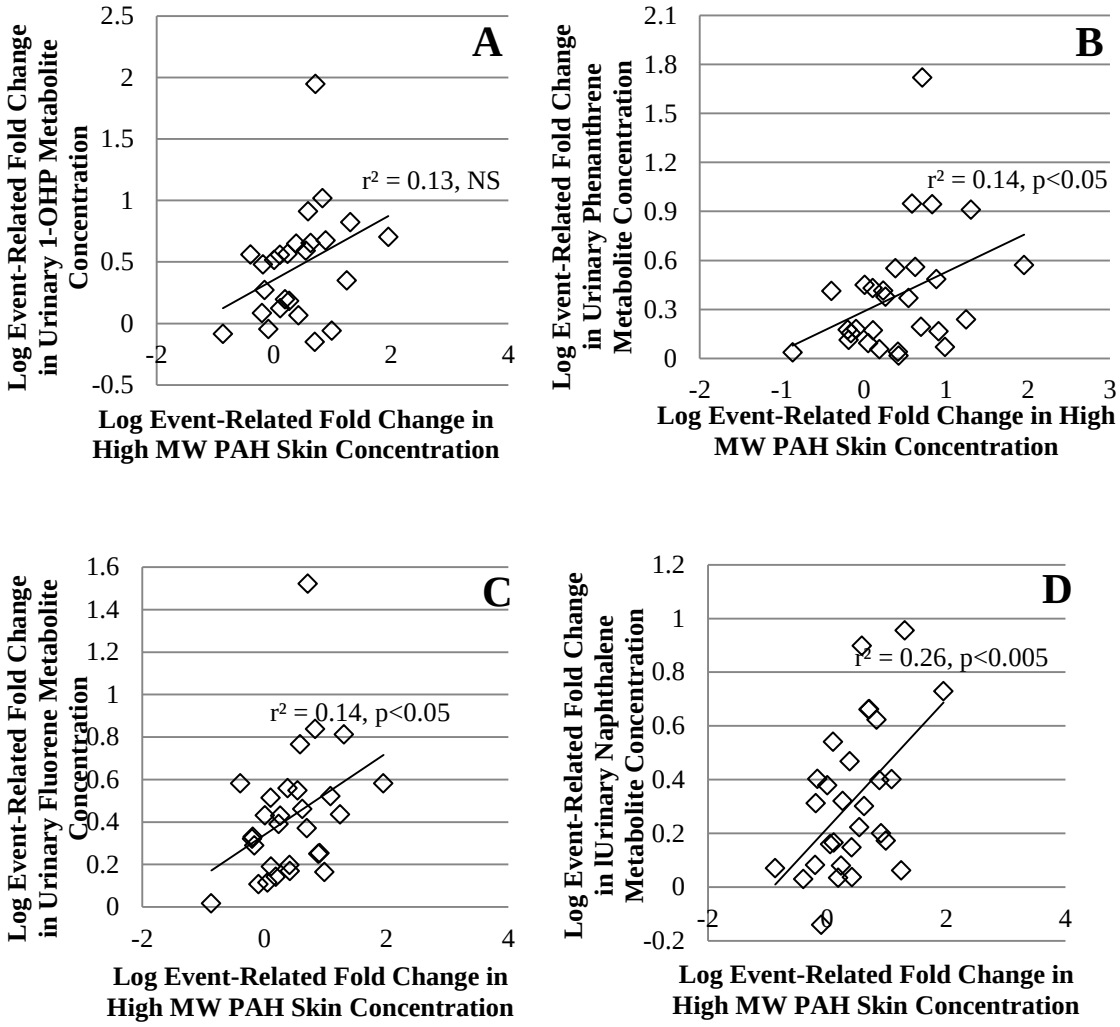

Figure 5 The relationships between event-related fold changes in (A) urinary 1-OHP concentrations; (B) Urinary ΣOH-Phen concentrations; (C) Urinary ΣOH-Fluo concentrations; and (D) Urinary ΣOH-Nap concentrations and fold changes of high molecular weight PAHs (i.e. 4 or more aromatic rings) in skin wipe samples. Four and one individual(s) lacked PAH metabolite data (i.e., one or more metabolite was unable to be measured due to technical difficulties) for 1-OHP and ΣOH-Phen, respectively, thus were omitted and resulting in n=25 and 28, respectively. The other two PAH metabolite groups (i.e., ΣOH-Fluo and ΣOH-Nap) had complete numbers thus n=29.

3.4.4. Urinary mutagenic potency

Mutagenic potency assesses overall exposure to mutagens. Post-fire urinary mutagenic potencies showed a significant average increase of 4.3-fold ($p<0.001$) relative to pre-event, ranging from no increase (NI) to 74.7-fold. No significant differences were seen between office workers and firefighters before a fire event ($p>0.05$) (Figure 6, Table 9). Long et al. (2015) was the only other study that used *Salmonella typhimurium* strain YG1041 to assess mutagenicity in paired urine samples from before and after exposure to combustion emissions. That study examined individuals who used traditional steam baths heated by wood fires. The steam baths, called Temescales, have little ventilation and high levels of wood smoke exposure are common. Although firefighters are equipped with personal protective equipment (PPE), the urinary mutagenicity results still showed a mean post-exposure increase in mutagenic potency that is 2.5-fold greater than that observed in the Long et al. (2015) study where no PPE was used. Relative differences in the mutagenic potency of the combustion emissions, and the intensity of the exposure (i.e., air concentration and exposure duration), likely account for the observed difference between firefighters and the subjects examined in the Long et al.. study. This further illustrates the need for studies of firefighters' exposures during live fire events, as opposed to studies conducted at training centres where fires only use organic fuels such as untreated wood and straw. Moreover, there is a need for studies that investigate differences between the mutagenic potency of different combustion emissions, and, similar to what was stated regarding PAH exposures, the efficacy of standard PPE to prevent occupational exposures during fire suppression.

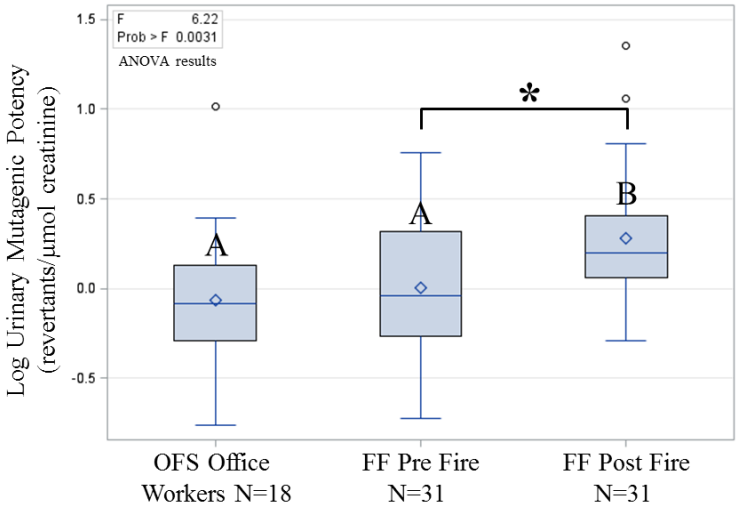

Figure 6 Boxplot showing differences between urinary mutagenic potency of OFS office workers, firefighters before a fire event (FF Pre Fire), and firefighters after a fire event (FF Post Fire). Values were log transformed to equalize the variance across the groups. A paired t-test was employed to compare matched before and after firefighter values; significance at $p<0.0001$ is signified by an asterisk. The box represents the interquartile range (i.e., 25^{th} to 75^{th} percentile), the diamond represents the mean value, the solid line represents the group median, and the whiskers extend to 5^{th} and 95^{th} percentiles. Circles represent outliers.

Table 4 Summary of creatinine-adjusted mutagenic potencies for office worker controls and firefighter (FF) subjects (i.e., both pre- and post-fire event).

	Urinary Mutagenicity (revertants/μmol creatinine)		
	N	Range	GM (+SE)
Office workers	18	0.17-10.35	0.87 (0.08)
FF pre-fire	31	0.19-5.76	1.01 (0.07)
FF post-fire	31	0.51-22.68	**1.90 (0.12)**
Fold change[a]	31	NI-74.7	**4.32**

SE, standard error; NI, no increase; GM, geometric mean.

A one-way ANOVA was employed to compare the three groups and are bolded when significantly different from office workers and firefighters at the start of shift ($p<0.0001$).
A paired t-test was employed to compare paired pre- and post-fire event samples and are bolded when significantly different at $p<0.0001$.

[a]Average individual's fold change of post-fire compared to pre-fire (i.e., not overall means)

3.4.5. Urinary biomarkers

Comparative analysis of two urinary biomarkers was used to determine if firefighters are subjected to oxidative stress or lung injury. Urinary 8-iso-$PGF_{2\alpha}$ concentrations were used to assess oxidative stress. OFS office workers were found to have significantly higher urinary 8-iso-$PGF_{2\alpha}$ concentrations compared to firefighters both before and after a fire ($p<0.05$, Figure 7). This may be due to office workers being significantly older than the firefighter participants and significant age-related increases in urinary 8-iso-$PGF_{2\alpha}$ concentrations for both firefighters and office workers ($p<0.05$). This contradicts previous studies that noted significant decreases in rat and human urinary isoprostane with increasing age (Andziak & Buffenstein, 2006; Keaney et al., 2003). Other health factors related to age and body weight, including as blood cholesterol, blood glucose, and cardiovascular disease, have also been associated with higher urinary 8-iso-$PGF_{2\alpha}$ (Keaney et al., 2003). Because the majority of office workers considered themselves "overweight", and reported poor health and fitness scores compared to firefighters (Table 2), significantly elevated concentrations of 8-iso-$PGF_{2\alpha}$ relative to firefighters is not surprising. In terms of changes in oxidative stress levels in firefighters from attending fire events, a paired *t*-test showed no significant event-related differences in urinary 8-iso-$PGF_{2\alpha}$ concentrations. This is surprising because increases in 8-iso-$PGF_{2\alpha}$ have been noted in individuals exposed to combustion emissions such as in occupational settings with welders, women exposed to woodsmoke from cookstoves, and most relatedly to these results, wildland firefighters in the field and in smoke simulators during breathing apparatus training (Commodore et al., 2013; Ferguson, Semmens, Dumke, Quindry, & Ward, 2016; Gaughan et al., 2014; Nuernberg et al., 2008). However, other studies have found similar results to the ones presented here where no significant changes in urinary 8-iso-$PGF_{2\alpha}$ occurred after exposure to combustion emissions

including short-term chamber exposure to wood smoke and wildland firefighters attending prescribed burns (Adetona et al., 2013; Stockfelt, Sallsten, Almerud, Basu, & Barregard, 2013). The lack of event-related increases in urinary 8-iso-$PGF_{2\alpha}$ may be due to hyperoxic conditions resulting from SCBA use. Oxygen is known to interfere with the formation 8-iso-$PGF_{2\alpha}$ and situations with high oxygen concentrations have been associated with low urinary 8-iso-$PGF_{2\alpha}$ (Fessel, Porter, Moore, Sheller, & Roberts, 2002). Firefighters routinely use their SCBA, and SCBA use has been shown to create a systemic hyperoxic condition by increasing oxygen consumption by 83% (Louhevaara, Smolander, Tuomi, Korhonen, & Jaakkola, 1985). Moving forward, 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 in teasing out less pronounced changes (Dorjgochoo et al., 2012). 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 (e.g., urinary 8-hydroxy-2′-deoxyguanosine or malondialdehyde) (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003; Jovanovic, Clements, & MacLeod, 1998).

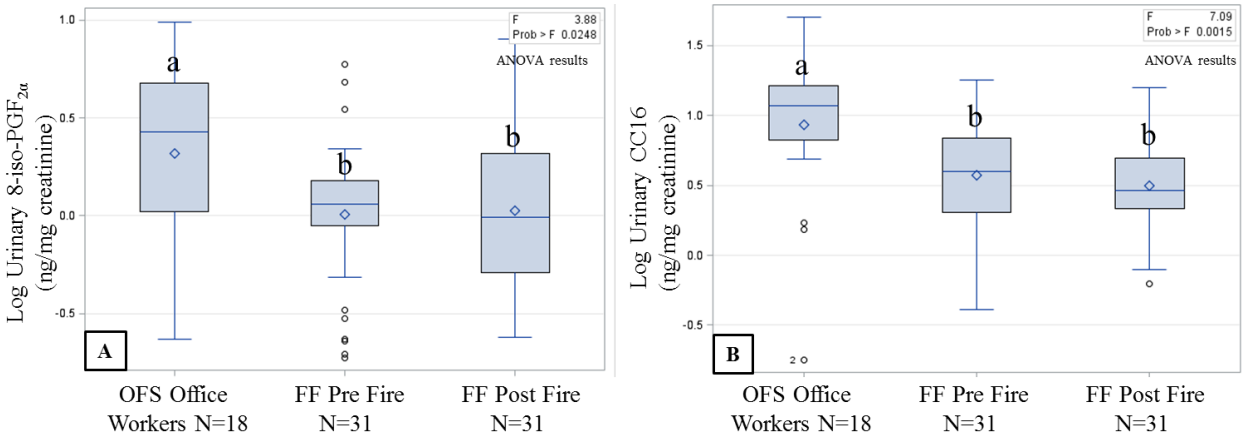

Figure 7 Boxplots showing differences between urinary (A) 8-iso-$PGF_{2\alpha}$ and (B) CC16 of OFS office workers, firefighters before a fire event (FF Pre Fire), and firefighters after a fire event (FF Post Fire). Values were log transformed to equalize variance across the groups. A paired t-test was employed to compare matched before and after firefighter values and no significant differences were seen. The box represents the interquartile range (i.e. 25^{th} to 75^{th} percentile), the diamond represents the mean value, the solid line represents the group median, and the whiskers extend to 5^{th} and 95^{th} percentiles. Circles represent outliers.

Table 5 Summary of creatinine-adjusted urinary biomarker concentrations for office worker controls and firefighter (FF) subjects (i.e., both pre- and post-fire event).

	Urinary Biomarkers (ng/mg creatinine)					
	8-iso-$PGF_{2\alpha}$			Clara Cell 16		
	N	Range	GM (+SE)	N	Range	GM (+SE)
Office workers	18	0.2-9.8	2.1 (0.2)	18	0.2-50.6	8.5 (1.1)
FF pre-fire	31	0.2-5.9	1.0 (0.4)	31	0.4-17.8	3.8 (0.2)
FF post-fire	31	0.2-8.0	1.1 (0.1)	31	0.6-15.8	3.2 (1.0)
Fold change[a]	31	NI-13.2	1.8	31	NI-3.8	1.2

SE, standard error; NI, no increase; GM, geometric mean.

[a]Average individual's fold change of post-fire compared to pre-fire (i.e., not overall means)

The urinary biomarker for lung injury (i.e., CC16) showed similar results to 8-iso-$PGF_{2\alpha}$. OFS office workers were found to have significantly higher urinary concentrations of CC16 compared to firefighters both before and after a fire ($p<0.05$, Figure 7); moreover, age was again found to be significantly related to increases in urinary CC16 concentrations for both firefighter and office workers ($p<0.01$). When compared to 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 examined by (Bolger et al., 2011; St Helen et al., 2013) (0.65 and 0.68 ng/mg creatinine), but also well below another control group of 8 males between the ages of 21-37 years old examined by (Bolger et al., 2011; St Helen et al., 2013) (27.5 ng/mg creatinine). Similar to the 8-iso-$PGF_{2\alpha}$ results, paired analyses of the results 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. The aforementioned relationship between PAH concentrations in skin wipe samples and urinary PAH metabolite concentrations supports this contention by suggesting that the primary route of exposure is dermal. However, this, in addition to the office workers having significantly higher CC16 concentrations, contradicts previous studies of firefighters that found significant increases in serum CC16 concentrations after exposure to combustion by-products during overhaul, and 328% increases in serum after a polypropylene fire (Bernard et al., 1997; Burgess et al., 2001). However, these studies examined CC16 in serum and the difference in analytical matrix may be the source of the discrepancy. Investigation into alternative biomarkers of lung injury, which were previously used to monitor lung injury in 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 (Bernard et al., 1997; Weiden et al., 2013).

3.4.6. Empirical relationships between urinary metrics and firefighting activities

3.4.6.1. Multiple fires in one shift

The results show incremental increases in urinary mutagenicity and urinary PAH metabolite levels, and are shown in Figure 8 and Figure 9, respectively. This is not surprising because, although post-event urine was collected for 18hrs, the reported urinary elimination half-lives for orally or dermally delivered PAHs is between 2.5 to 15 hours (Brzeźnicki, Jakubowski, & Czerski, 1997; Buckley & Lioy, 1992; Li et al., 2012; St. Helen et al., 2012; Viau, Carrier, Vyskočil, & Dodd, 1995; Viau & Vyskočil, 1995). More specifically, Brzeźnicki, S., Jakubowski, M. & Czerski (1997) showed the half-life of 1-OHP to be 6.0–9.0 hours for inhalation, 4.4–12 hours for ingestion, and 11.5–15 hours for dermal.(Brzeźnicki et al., 1997) The urinary elimination half-life of fluorene is also in this range (i.e., 4.1-8.2 hours) as is phenanthrene (i.e., 3.5-5.1) and naphthalene (i.e., 2.5-4.3) (Li et al.., 2012; St. Helen et al.., 2012). In this case the second event occurred slightly more than one half life after the first event, thus contributing to incrementally higher levels of urinary metabolites.

Although both fires occurred in two floor, single family residences over ~30 years old, and size of incremental differences in urinary metabolite levels shown in Figure 9 appear to be related to fire intensity and the participants' role in suppression. The first fire was reported to be less intense, spreading to objects throughout a kitchen and producing a light haze. The participants (i.e., C10 and C12) arrived while the fire was decaying (i.e., no visible flames), and worked on overhaul for approximately 30 minutes. The second fire was reported to have spread to multiple floors with smoke throughout the entire structure; it was fully developed with flames pushing from the structure at the time of arrival. C10 and C12 worked on internal attack and overhaul for approximately 10 and 80 minutes, respectively.

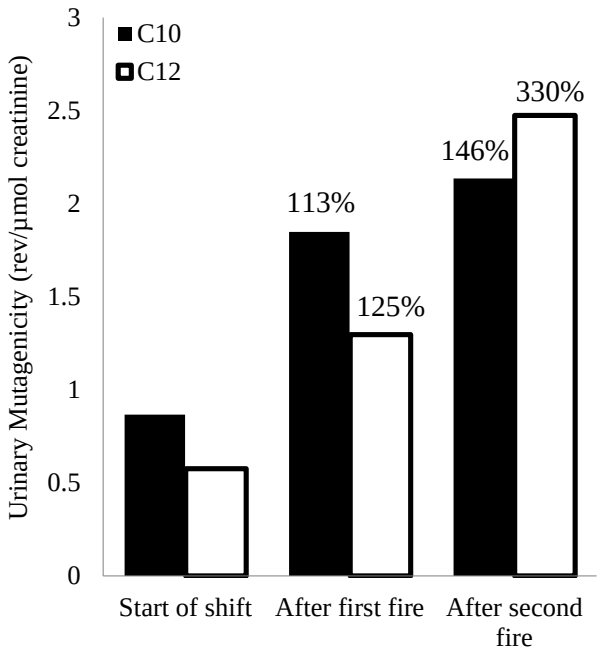

Figure 8 Incremental increases in urinary mutagenicity after two fires during the same shift separated by 18 hrs. Subjects C10 and C12 are firefighters from the same station and platoon who attended the same two fires during the same shift. Percentages indicate percent increase from start of shift value.

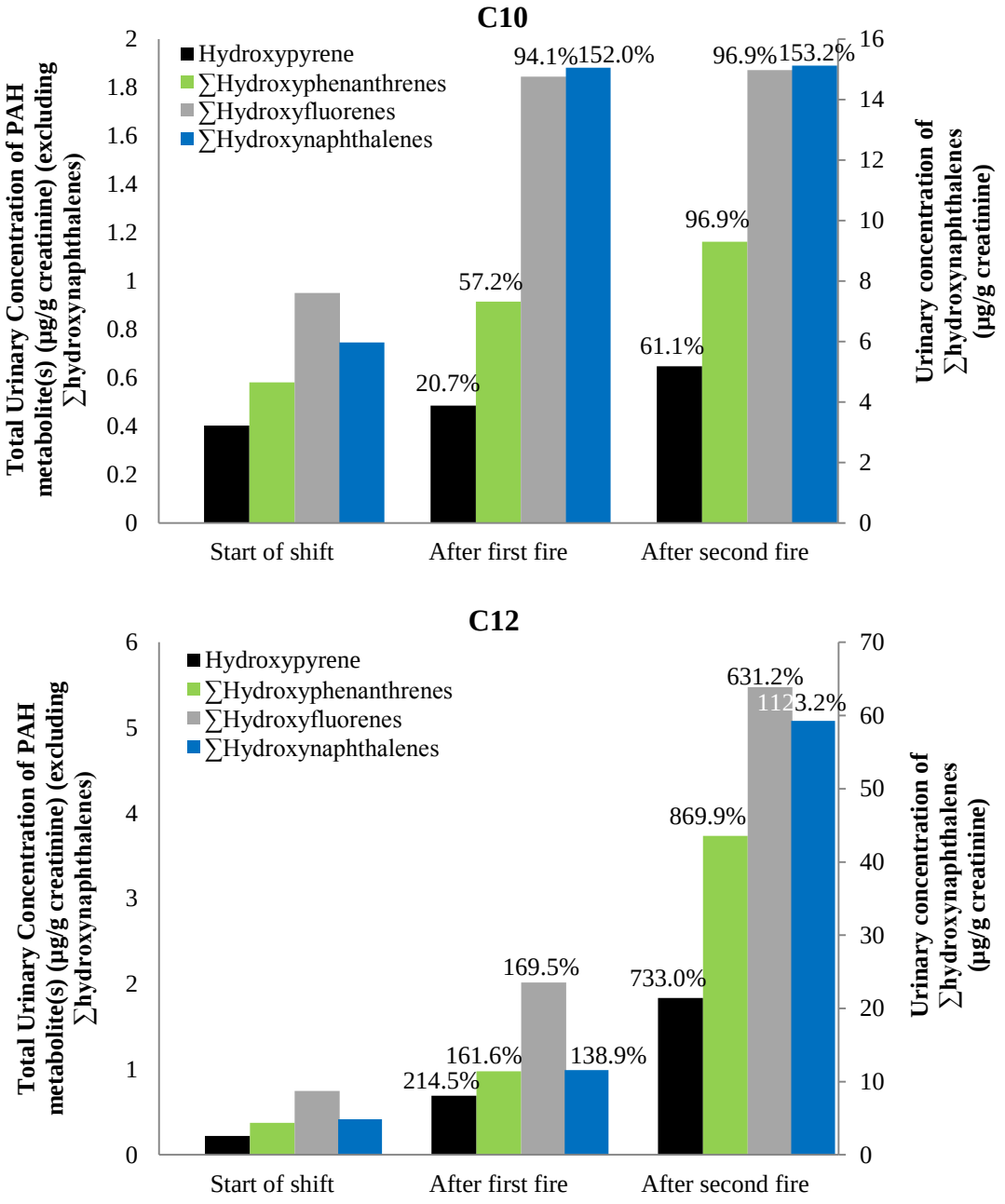

Figure 9 Incremental increases in urinary PAH metabolite concentrations after two fires during the same shift separated by 18 hrs. Subjects C10 and C12 are firefighters from the same station and platoon who attended the same fires during the same shift. Percentages indicate percent increase from start of shift value.

Quantity and/or severity of fire suppression activities could be driving this incremental increase. Urinary excretion of combustion byproduct metabolites from the first exposure may be seen in the post-fire results from the second fire. What is more likely is that the higher intensity of the second fire produced a higher increase in urinary mutagenicity and PAH metabolites. Ultimately, this shows the high variability of urinary chemistry in firefighters throughout a shift. It may also further suggest that the use of urinary biomarkers is in fact indicative of the nature of the fire event.

3.4.6.2. Duration of firefighting activity

Duration of fire suppression was found to be empirically related to event-related changes in urinary concentrations of pyrene, phenthanthrene, and fluorene metabolites (r^2=0.49, 0.48, 0.46, respectively, $p<0.0001$, Figure 10). Significance is maintained for metabolites of pyrene and phenanthrene after the outliers enclosed in Cluster E are removed (r^2=0.15 and 0.16, receptively, $p\leq0.05$, data not shown). Fold change in urinary metabolites of naphthalene was not significantly related to duration of fire suppression, and was therefore omitted from the figure. As suggested by Campo et al.. (2006), naphthalene, which is the most volatile PAH, may be a less reliable marker of exposure. More specifically, when duration of shift-specific fire suppression time is compared to event-related fold changes in urinary PAH metabolite concentrations, several clusters of data points show elevated levels of urinary metabolites (see Figure 10).

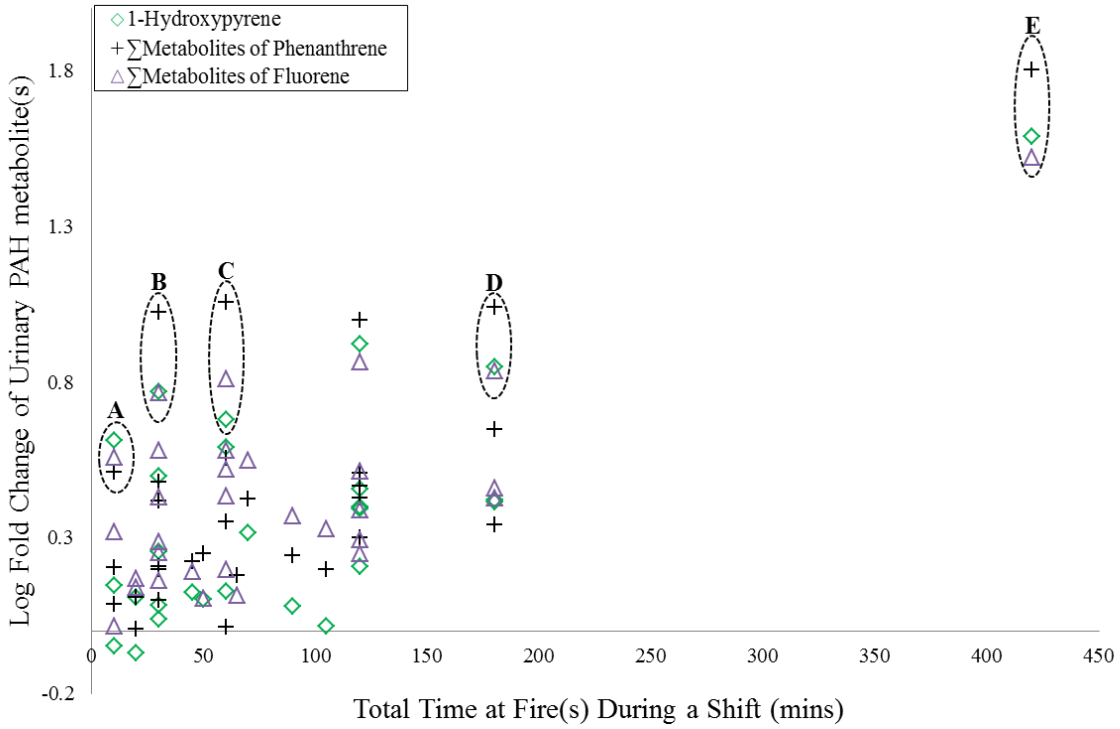

Figure 10 Event-related fold change in urinary concentrations of pyrene, phenanthrene, and fluorene metabolites versus the reported fire suppression time (min). Metabolites of naphthalene were omitted as they did not show a significant relationship with total time at fire(s) during a shift. Dotted circles labelled A-E highlight clusters of points coinciding with an individual at a particular fire showing above average fold changes in the concentrations of urinary PAH metabolite.

Figure 11 A firefighter performing vertical ventilation, a fire suppression tactic, during a training fire. Photo courtesy of Scott Stilborn©, used with permission.

Figure 12 An Ottawa firefighter in full Bunker gear with red arrow indicating where a flash hood is located. Photo courtesy of D. Matschke, used with permission.

Interestingly, several clusters of elevated values, highlighted in Figure 10, may anecdotally be related to fire suppression role and/or proper use of PPE. Each cluster was found to be connected to one individual at a specific fire. Using information from their fire event questionnaires, it is possible to suggest reasons underlying these individuals' elevated exposures and fold changes in urinary PAH metabolite concentrations. Cluster A and B relate to two of the three individuals in the study who reported performing vertical ventilation as a fire suppression technique. Vertical ventilation is a fire suppression tactic that utilizes the creation of openings in the roof and elsewhere in the lower structure to encourage smoke, heat, and gases to move up and out of the structure. This confers improved victim survivability, more effective fire attack, and a safer environment for the fire suppression crew. However, once the roof opening is created, the roof crew can become engulfed in a column of superheated and pressurized smoke as shown in a training scenario in Figure 11. This can be expected to cause above average exposure to combustion emissions. The individual in Cluster C failed to wear a flash hood (shown in Figure 13) due to time constraints. This is particularly important since previous studies have shown that a firefighters' neck is particularly prone to dermal exposure.(Fent et al.., 2014) Moreover, the lack of protection may have further increased the surface skin temperature, which has been shown to increase surface blood flow and PAH absorption (i.e., 400% increase for every 5° increase in skin temperature) (Firefighter Cancer Support Network, 2013; Jones, Cocker, Dodd, & Fraser, 2003). Cluster D is of an individual who attended a container fire three hours into the 18-hour post-fire sampling period from a fire he attended earlier in the day. Because half-lives for urinary PAH metabolites fall within this time window, this repeated exposure likely caused elevated levels during the remaining 15 hours of sample collection. The fire suppression duration for the individual in Cluster E is the longest in the study (420 min).

This individual was involved in the suppression of a multiunit, commercial fire, and reported "multicolored smoke". The fire event was the largest fire in terms of square footage and third largest in terms of number of trucks and firefighters (i.e., 26 trucks and 100 firefighters). The duration of exposure and/or the intensity/magnitude of the fire may explain this individual's high fold change in urinary PAH metabolites. Although the discussions of the clusters indicated in Figure 10 are anecdotal, the noted associations between post-event urinary metabolite levels and the nature of the fire suppression activities highlight the importance of fire type and firefighter activities in determining exposure. The associations permit the formulation of hypotheses regarding factors that augment exposures; however, additional studies are required to rigorously examine the effects of firefighter role and fire type in the determination of combustion emissions exposure.

3.5. Conclusions

The results confirm that involvement in on-shift fire suppression activities significantly increases urinary excretion of PAH metabolites and organic mutagens (i.e., PAH metabolites and mutagenicity). Empirical relationships between the levels of urinary combustion emission biomarkers, and the concentrations of PAHs in personal air collected at the scene, supports the assertion that fire suppression results in significant exposures. The empirical relationship between urinary PAH metabolite concentrations and duration of fire suppression reinforces the validity of this assertion. Lack of changes in urinary CC16, a biomarker of pulmonary lung damage, and empirical relationships between urinary PAH metabolite concentrations and dermal PAH concentrations suggest that dermal contact is an important route of exposure to combustion emissions. Scrutiny regarding the influence of fire type and fire suppression role indicates that an individuals' level of exposure is determined by complex dynamic interplay of variables relating

to fire intensity, time at the scene, role in fire suppression, and appropriate use of PPE. Moving forward, it will prove interesting to investigate the efficacy of interventions (e.g., more effective PPE, decontamination) in reducing firefighters' exposures to combustion emissions. Given the hypothesized importance of dermal contact, the ability of post-fire skin decontamination to reduce exposure should be investigated. It seems reasonable to hypothesize that adequate skin and PPE decontamination will effectively reduce exposures and associated health risks.

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Chapter 4. Conclusions

The results presented in this thesis identify determinants of PAH exposures through the use of monitoring PAH metabolites in the urine, first in the general population and then in a very specific occupational cohort that frequently encounter combustion emissions. This was completed through analysis of population biomonitoring data (i.e., Chapter 2) and a more targeted study of occupational exposures to combustion emissions with firefighters (i.e. Chapter 3). These chapters addressed the thesis objectives outlined in the Introduction (i.e., Chapter 1). More specifically, the thesis:

(a) analysed population biomonitoring data to investigate the influence of occupational, demographic, and lifestyles factors on urinary levels of PAH metabolites in Canadians (Chapter 2); and

(b) used urinary monitoring of PAHs and other organic mutagens to assess the influence of fire suppression activities (i.e., firefighting) on firefighters' exposures to combustion emissions.

4.1. Study outcomes and evaluations of stated hypotheses

Chapter 2 addressed the first hypothesis that in reference to the typical Canadian, exposure to tobacco smoke, age, sex, occupation, and home characteristics significantly influence urinary concentrations of PAH metabolites. Population biomonitoring data provide baseline information about contaminant exposures in the overall population; by pairing biomonitoring metrics with questionnaire data (e.g., demographic and lifestyle factors) it is possible to investigate the determinants of exposure. This study used a variety of statistical methods to analyse data from Cycle 2 of the Canadian Health Measure Survey. More specifically, the analyses examined the empirical relationships between urinary concentrations of

various PAH metabolites and various demographic and lifestyle variables. Secondly, the analyses evaluated the relative utility of different PAH metabolites (e.g., 1-OHP, ΣOH-Fluorenes, etc.) to assess the influence of various exposure determinants (e.g., smoking, age, occupation, household income, etc.). With respect to the first objective, it was found that individuals who are current smokers, work in places with limited smoking restrictions, live in households making less than $35,000 a year, live in older homes, and work in Blue Collar occupations tended to have higher urinary levels of PAH metabolites. Iterative model selection procedures (i.e., forward, backward and stepwise) permitted identification of the most parsimonious model and identification of variables that have the strongest relative ability to explain variations in urinary PAH metabolite concentration. Analyses of urinary concentrations of pyrene, phenanthrene, fluorene, and naphthalene metabolites revealed that smoking, sex, and age are the most important determinants of metabolite levels. Metabolites of fluorene were also significantly affected by home age, and metabolites of naphthalene were also significantly related to occupation and workplace smoking restrictions. With respect to the second objective, smoking status, income, home age and occupation were found to be more strongly related to metabolites of fluorene, whereas urinary concentration of 1-OHP, the more traditional PAH biomonitoring metric, was strongly related to age and workplace smoking restrictions. Therefore it is clear that measuring multiple PAH metabolites allows for not only assessment of variables relating to urinary PAH metabolite levels, and associatively, PAH exposure, but also the utility of different metabolites to assess potential sources of exposure. Overall, the empirical analyses succeeded in identifying the variables that were most predictive of urinary PAH metabolite levels in Canadians (i.e., smoking, age, and sex). Furthermore, the results obtained illustrate the ability to employ metabolites of PAHs other than pyrene (i.e., hydroxyfluorenes) to assess

exposures to combustion-derived materials such as tobacco smoke. Although causation cannot be determined from the types of empirical relationships investigated herein, they are useful in identifying the factors that contribute to PAH exposures, thus pinpointing important avenues for further investigation to aid in risk management from such exposures. Follow-up studies should further examine other population biomonitoring data (i.e., both in Canada and abroad) to compare to these results as well as utilize focussed cohort studies to specifically investigate the causes underlying the observed effects, particularly the ones that are not easily explained (e.g., sex).

Chapter 3 investigated the hypotheses that fire suppression is associated with significant increases in urinary biomarkers of combustion emission exposure and that post-suppression levels of urinary biomarkers are determined by a firefighter's role in fire suppression, his/her duration at the scene, and the nature of the fire itself. This study constitutes the first attempt to analyse paired urine and skin wipe samples collected before and after on-shift fire suppression (i.e., not during training exercises), as well as personal air samples collected at the scene. The results obtained confirmed that involvement in on-shift fire suppression is associated with statistically significant increases in exposures to both PAHs and organic mutagens. Empirical relationships between urinary PAH metabolite concentrations and both duration of fire suppression event and concentrations of PAHs in personal air collected at the scene, strengthens the assertion that on-shift firefighting increases an individual's exposure to combustion-derived PAHs at the scene. In addition, the lack of change in urinary CC16, a biomarker of pulmonary lung damage, and empirical relationships between urinary PAH metabolite concentrations and dermal PAH contamination, suggest that dermal contact is a major route of PAH exposure. Lastly, by examining the nature of the fire event and an individual's role in fire suppression, the

results revealed that an individuals' exposure level is determined by a series of dynamic variables related to time at the scene, fire suppression tactics, and PPE use. Future work should investigate the efficacy of interventions (e.g., more effective or stricter use of PPE, decontamination protocols) in reducing firefighters' exposures to combustion emissions. Given the suggested importance of dermal routes of exposure, prevention of dermal contamination and improved protocols for decontamination should be investigated. Thus, this chapter permitted rejection of the null hypothesis that on-shift fire suppression does not alter firefighters' exposures to PAHs and other organic mutagens. On the contrary, participation in fire suppression is associated with statistically significant increases in urinary biomarkers of combustion emission exposure (i.e., PAH metabolites and urinary mutagenicity). Moreover, levels of exposure biomarkers are significantly influenced by role in fire suppression, duration at the scene, and the nature of the fire itself.

4.2. Concluding Remarks

Populations are exposed to combustion emissions on a daily basis, including carcinogenic, mutagenic, and teratogenic PAHs. Although often unavoidable, some individuals in a population experience above average exposures to PAHs via contact with a variety of contaminated environmental media (i.e., air, water, food, soil). Quantifying the internal dose of PAHs can be difficult and invasive (e.g., blood sampling); however, non-invasive collection and analysis of urinary PAH metabolites can provide information on exposure and internal dose. Furthermore, metabolites of different PAHs may provide insight into different routes and/or sources of exposure.

Moving forward, it should prove interesting to apply the methods presented in Chapter 2 to other national collections of biomonitoring data to investigate the determinants of metabolite

levels (e.g., NHANES from the United States or the BIOAMBIENT.ES project in Spain). In addition, where available, other variables could be investigated including genetic susceptibility, gender roles, age-related behaviour differences (e.g., crawling on the floor), geographical location, home heating sources, and dietary traditions. For example, there is strong tradition of cooking meat over an open flame in Argentina thus warrants investigation into the effects this may have on this population's dietary PAH exposure and metabolite levels. Multiple biomarkers of combustion emissions and demographic and lifestyle variables should continue to be scrutinized to better streamline research that permits the identification of intervention strategies to reduce exposure and risk.

Although not detected in the analyses of Canadian population data presented herein, earlier cohort studies have clearly documented increased PAH exposures for individuals involved in occupations that employ high temperature combustion (e.g., metal refining and founding, coke production). Such exposures can be significant since a substantial portion of time is generally spent at the workplace. Indeed, the results presented in Chapter 3 indicate that fire suppression significantly increases exposures to PAH-containing combustion emissions. Thus, firefighting is an occupation associated with higher PAH exposures, and presumably, elevated risks for diseases associated with PAH exposures. The hypothesised importance of dermal contact as the major route of firefighters' PAH exposures suggests the need for strategies to reduce skin contamination by prevention of exposure and/or reduction of contamination after contact. The former may include improvements to PPE design and efficacy (e.g., leak avoidance), and implementation of strictly enforced policies regarding PPE use. The latter may include decontamination protocols to reduce dermal contamination and concomitant internal dose. For example, some firefighting organizations are currently exploring the utility of on-scene

skin wipes to remove dermal contamination, thereby reducing the risk of internal exposure. Better understanding of the factors that contribute to firefighters' PAH exposures will enable the design and implementation of effective exposure reduction techniques that will ultimately reduce the risks of PAH-related health risks.

PAH exposure assessment via non-invasive urinary biomonitoring permits identification of exposure determinants and subsequent formulation of risk management tools and strategies. Moreover, empirical analyses of relationship between urinary metabolite levels and a variety of demographic and lifestyle variables permits the formulation of hypotheses regarding the precise sources and causes of exposure levels. Identification of exposure determinants, and a clear understanding of the mechanisms underlying their influence on exposure, permits rational, effective determination of interventions to reduce exposures and the risk of adverse health effects.

Appendix I – Firefighter Study Documents

Consent form (firefighter participants - English)

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Consent Form (Firefighters)

Title of the study: Occupational exposure to metals and polycyclic aromatic hydrocarbons by firefighters

Name of researchers:

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David Matschke, City of Ottawa Fire Services
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Hugo Lemieux, Vice President Research Office, University of Ottawa
Email: hlemieux@uottawa.ca

Invitation to Participate: I am invited to participate in the above-mentioned research study conducted by Dr. Jules Blais, Dr. Laurie Chan, Dr. Paul White, Dr. Tracy Kirkham, Mr. David Matchke, and Dr. Hugo Lemieux, and funded by the Ontario Ministry of Labour.

Purpose of the Study: The purpose of the study is to determine how firefighters are exposed to chemicals produced during combustion (e.g., polycyclic aromatic hydrocarbons, antimony, lead, and cadmium) while fighting fires and living in fire halls. An understanding of how firefighters are exposed to these chemicals will help in developing strategies to reduce the likelihood of exposure in the future. The project is supported by Ottawa Fire Service senior management (e.g., Chief and Deputy Chiefs), the Ottawa Fire Service Health and Safety Committee, and the Ottawa Professional Firefighters Association.

Participation: By participating in this study, I acknowledge that I am not a smoker, nor live with smokers, and I will avoid open fires and char-broiled (i.e., barbequed) foods. My participation will consist of providing urine samples and wipes of skin, personal protective equipment (PPE), and clothing before and after firefighting events. My participation will encompass five (5) 24-hr shifts spread over approximately twelve (12) days. I will also provide wipes of PPE following decontamination procedures. During fire fighting activities, I will wear a small personal air sampler. My responsibilities and time commitments are outlined in the table below (i.e., Table

1). All of my samples will be stored in a locked freezer, provided by the university and located at the fire hall, until pick up by university staff. Urine samples will be analysed for selected metals, polycyclic aromatic hydrocarbon metabolites, isoprostane and CC16 (protein markers of genetic and cellular damage), and urinary mutagenicity, as outlined in Table 2. I will participate in answering questions in one questionnaire about my work and home environments, and my health, habits and hobbies, and another questionnaire about my activities during each fire event over the duration of my participation in the study. I understand that sample collections will take place following firefighting events, which will happen at times that are impossible to predict. I understand that a member of the research team may also contact me to collect the following samples from my workplace for metal and aromatic hydrocarbon content: air samples, swabs from clothing and equipment. All sample collection following a fire event should take about 30 to 60 minutes.

Table 1. Breakdown of participant's activities and estimated time commitments.

Start of Study Block - Day 1	**Time**		**Days 2-5**	**Time**		**After Firefighting Event**	**Time**		**After Decontamination**	**Time**
Consent Form	20 mins		Urine Sample	5 mins		Skin Wipes (6)	15 mins		PPE Wipes (6)	15 mins
General Questionnaire	20 mins		Skin Wipes (6)	15 mins		PPE Wipes (6)	15 mins			
Personal air sampler setup	15 mins		PPE Wipes (6)	15 mins		Clothing Wipes (4)	15 mins			
Urine Sample	5 mins		Clothing Wipes (4)	15 mins		Store air sampler	3 mins			
Skin Wipes (6)	15 mins					Fire Event Questionnaire	20 mins			
PPE Wipes (6)	15 mins					Urine Sample (total for 18 hrs)	18 hrs			
Clothing Wipes (4)	15 mins									

Table 2. Summary of Analyses to be Conducted

	Wipes of skin, clothing, PPE, and PPE after decontamination	Air	Urine
Antimony	√	√	√
Cadmium	√	√	√
Lead	√	√	√
PAHs	√	√	
PAH Metabolites			√
CC16			√
Isoprostane			√
Mutagenicity			√

Risks: There are no foreseeable risks associated with the sample collections planned for this study. Wearing the equipment for personal air sampling may cause minor inconvenience, but the risk of obstruction during a fire is minimal and acceptable. All sampling procedures were reviewed by the Ottawa Fire Service senior management and the Division Chief-Safety and Innovation. In addition, all sampling procedures have been reviewed and approved by the University of Ottawa and Health Canada Research Ethics Boards. Analyses performed on samples I provide will not include any information that can be used to assess my health status. If I am concerned about my study results, and/or my occupational exposures to chemicals in general, I can consult the Canadian Centre for Occupational Health and Safety (www.ccohs.ca/oshanswers/occup_workplace/firefighter.html), or the Occupational Health and Safety Branch of the Ontario Ministry of Labour (www.labour.gov.on.ca/english/hs/). In addition, I can consult with the project investigators and/or the project occupational hygienist who can provide an opinion regarding my levels. Finally, I can consult my family physician for an opinion regarding potential health effects.

Benefits: My participation in this study will help determine whether firefighters are exposed to hazardous substances; and moreover, will help occupational hygienists develop strategies to reduce exposures in my workplace. There will be no remuneration, payment or reward for participating in the study. Since, to our knowledge, this is the first study of career firefighters' exposures to hazardous substances, both before and during firefighting activities, it is difficult to predict the utility of the study results. It may be necessary to design and execute a follow-up study to address issues/questions raised in this study.

Confidentiality and anonymity: I have received assurance from the researchers that the information I share will remain strictly confidential. I understand that my study results will be used only for the development of intervention plans to reduce exposures in the workplace and for research. My confidentiality will be protected by virtue of the fact that any linkage of my identity to the study results will only be known by the Principal Investigators. All the other researchers will only work with files containing coded identities.

Anonymity
Personal information will be protected in the following manner: (1) all participants will be assigned a randomized ID code and only the Principal Investigators will have the key to the coding, (2) only group results will be published in research reports describing the study results, and (3) no individual data or personal identity will be revealed in any publications describing the study results.

Conservation of data: The collected information, including both hard copies of the questionnaires and electronic data, will be kept in a secured room at the Department of Biology of the University of Ottawa for a period of five (5) years after which it will be destroyed. The key to the ID coding system will be kept by the Principal Investigators in a secure office at the University of Ottawa.

Voluntary Participation: I am under no obligation to participate and if I choose to participate I can withdraw from the study at any time and/or refuse to answer any questions without suffering any negative consequences. If I choose to withdraw, all data gathered until the time of

withdrawal will be destroyed. Although the project is endorsed by the Ottawa Fire Service, there is no obligation for me to participate.

Acceptance: I, _____ (Name of Participant), agree to participate in the above-described research study conducted by Drs. Jules Blais, Laurie Chan, and Paul White of the Department of Biology, Faculty of Science, University of Ottawa.

If I have any questions about the study, I may contact Dr. Jules Blais by phone: (613) 562-5800 Ext. 6650, Fax: (613) 562-5486 or email: jules.blais@uottawa.ca

If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5Tel.: (613) 562-5387 Email: ethics@uottawa.ca

There are two copies of the consent form, one of which is mine to keep.

_____
Participant ID

_____ _____
Participant's signature Date (dd/mm/yyyy)

_____ _____
Witness on behalf of the Researcher's signature Date (dd/mm/yyyy)

Consent form (firefighter participants - French)

Université d'Ottawa | University of Ottawa

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Formulaire de consentement (pompiers)

Titre de l'étude: Exposition professionnelle des pompiers aux métaux ainsi qu'aux hydrocarbures aromatiques polycycliques.

Nom des chercheurs:

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Hugo Lemieux, Cabinet de la vice-rectrice à la recherche, Université d'Ottawa
Courriel: hlemieux@uottawa.ca

Invitation à participer: Je suis invité à participer a l'étude mentionnée ci-dessus menée par Dr. Jules Blais, Dr. Laurie Chan, Dr. Paul White, Dr. Tracy Kirkham, Mr. David Matchke et Dr. Hugo Lemieux financée par le ministère du travail de l'Ontario.

But de l'étude: Le but de cette étude est de déterminer comment les pompiers sont exposés aux composés chimiques produits par une matière en combustion (incluant les hydrocarbures aromatiques polycycliques, l'antimoine, le plomb et le cadmium) en combattant des incendies et en résidant à la caserne de pompiers. La compréhension du mécanisme d'exposition aux composés chimiques aidera à développer des stratégies pour diminuer les risques d'expositions dans le futur. Le projet à l'appui de la Direction senior du service des incendies d'Ottawa (e.g., chef et chefs adjoints), le Comité de santé et sécurité au travail du service des incendies d'Ottawa et l'Association professionnelle des pompiers d'Ottawa.

Participation: En participant à cette étude, je confirme que je suis un non-fumeur, ne réside pas avec des fumeurs, j'éviterai les feux à ciel ouvert et la nourriture cuite sur le grill (i.e., nourriture cuite sur le barbecue). Ma participation consistera à donner un échantillon d'urine, des échantillons-lingettes frottés sur la peau, équipement de protection personnelle (EPP) et vêtements avant et après avoir combattu un incendie. Ma participation se déroulera durant 5

quarts de travails de 24 heures et s'étalera sur approximativement 12 jours. Je fournirai également des échantillons-lingettes frottés après les procédures de décontamination. Durant les combats d'incendies, je porterai un petit échantillonneur d'air personnel. Mes responsabilités et temps réservés sont énumérés dans le tableau ci-dessous (i.e., Tableau 1). Tous mes échantillons seront entreposés dans un congélateur barré, fourni par l'université et situé à la caserne de pompiers jusqu'à ce qu'ils soient ramassés par un employé de l'université. Les échantillons d'urines seront analysés pour les métaux sélectionnés, les métabolites des HAPs, l'isoprostane, la CC16 (marqueur (protéine) de dommages génétiques et cellulaires) et la mutagénicité de l'urine tel qu'énuméré dans le tableau 2. Je vais participer en répondant au questionnaire à propos de mon travail, résidence, santé, habitudes comportementales et passe-temps ainsi qu'un autre questionnaire à propos de mes activités et démarches pendant chaque combat d'incendies effectués durant le cours de cette étude. Je comprends que les échantillons seront prélevés après les combats d'incendies, donc auront lieu selon un horaire imprévisible. Je comprends qu'un membre de l'équipe de recherche pourra me contacter afin de recueillir les échantillons à mon lieu de travail pour la teneur en métaux ainsi que les hydrocarbures aromatiques polycycliques: échantillons d'air, échantillons-lingettes frottés aux vêtements ainsi qu'à l'équipement. Tous échantillonnages effectués après un combat d'incendie devrait durer de 30 minutes à une heure.

Table 1. Décomposition des activités du participant et temps estimés engagés

début du bloc d'étude - Jour 1	**temps**		**jours 2-5**	**temps**		**après le combat d'incendies**	**temps**		**après la décontamination**	**temps**
Formulaire de consentement	20 mins		Échantillon d'urine	5 mins		Échantillons-lingettes frottés de la peau (6)	15 mins		Échantillons-lingettes frottés de l'EPP (6)	15 mins
Questionnaire général	20 mins		Échantillons-lingettes frottés de la peau (6)	15 mins		Échantillons-lingettes frottés de l'EPP (6)	15 mins			
Installation de l'échantilloneur d'air personnel	15 mins		Échantillons-lingettes frottés de l'EPP (6)	15 mins		Échantillons-lingettes frottés des vêtements (4)	15 mins			
Échantillon d'urine	5 mins		Échantillons-lingettes frottés des vêtements(4)	15 mins		Remisage de l'échantilloneur d'air personnel	3 mins			
Échantillons-lingettes frottés de la peau (6)	15 mins					Questionnaire du combat d'incendie	20 mins			
Échantillons-lingettes frottés de l'EPP(6)	15 mins					Échantillon d'urine (total pour 18 heures)	18 hrs			
Échantillons-lingettes frottés des vêtements (4)	15 mins									

Table 2. Résumé des analyses qui seront effectués

	échantillons-lingettes frottés de la peau, EPP et EPP après décontamination	Air	Urine
Antimoine	√	√	√

Cadmium	√	√	√
Plomb	√	√	√
HAPs	√	√	
Métabolites des HAPs			√
CC16			√
Isoprostane			√
Mutagénicité			√

Risques: Il n'y a pas de risques prévisibles associés à la prise d'échantillons prévu par cette étude. Le port de l'équipement d'échantillonneur d'air personnel peut causer des désagréments, mais le risque d'obstructions lors d'un feu est minime et acceptable. Tous les protocoles d'échantillonnages ont été revus par la Direction senior du service d'incendies d'Ottawa, chefs adjoints et innovation. De plus tous les protocoles d'échantillonnages ont été revus et approuvés par le Comité d'éthique en recherche de l'Université d'Ottawa ainsi que de Santé Canada. Les analyses effectués sur les échantillons que je fournirai ne contiendront aucune information permettant d'évaluer mon état de santé. Si je suis préoccupé par mes résultats de cette étude, ou mon exposition en milieu de travail aux composés chimiques en général, je peux consulter le Centre canadien d'hygiène et de sécurité au travail (http://www.cchst.ca/oshanswers/occup_workplace/firefighter.html), le Département de sécurité et santé au travail du Ministère du travail de l'Ontario(http://www.labour.gov.on.ca/french/hs/index.php). De plus, je peux consulter les chercheurs du projet et/ou l'hygiéniste du travail du projet qui peut fournir un avis sur mes niveaux. Enfin, je peux consulter mon médecin de famille pour un avis sur les effets potentiels sur la santé.

Bienfaits: Ma participation à cette étude aidera à déterminer si les pompiers sont exposés à des substances nocives. De plus, ma participation aidera également l'hygiéniste en milieu de travail à développer un plan pour réduire l'exposition aux composés chimiques dans mon milieu de travail. Il n'y aura aucune rémunération, paiement ou récompenses pour avoir participé à cette étude. Puisque, selon nos connaissances, ceci constitue la première étude de l'exposition des pompiers de carrières aux substances nocives, avant et durant les combats d'incendies, il est difficile de prévoir l'utilité des résultats de cette étude. Il pourrait être nécessaire de concevoir et d'effectuer une étude supplémentaire pour répondre aux questions et points litigieux engendrés par la présente étude.

Confidentialité et anonymat: J'ai reçu l'assurance des chercheurs que l'information que je vais partager demeurera strictement confidentielle. Je comprends que le contenu sera utilisé seulement pour développer un plan d'intervention et pour buts de recherches. Aussi, ma confidentialité sera protégée par le fait que le lien entre mon identité et les données sera uniquement connu par les chercheurs principaux. Les autres chercheurs travailleront seulement sur des fichiers dont l'identité des sujets est encodée.

Anonymat:
L'information personnelle sera protégée de la façon suivante: (1) tous les participants recevront un code d'identité aléatoire et seul le chercheur principal aura la clef de codage, (2) seuls les

résultats de groupes seront publiés dans les rapports de recherche, (3) Aucunes données individuelles ou d'identité personnelle ne seront dévoilés lors de publications.
Conservation des données: L'information amassée (copie papier du questionnaire ainsi que les données électroniques des paramètres d'échantillonnage) sera conservée dans une salle sécurisée au Département de Biologie de l'Université d'Ottawa pour une durée de 5 ans et sera ensuite détruite. La clef du system d'encodage aléatoire d'identité sera gardée par le chercheur principal à son bureau situé à l'Université d'Ottawa.

Participation volontaire: Je suis soumis à aucune obligation de participer, et si je choisis de participer, je peux me retirer de l'étude en tout temps et/ou refuser de répondre à n'importe quelle question sans que j'aille a subir de conséquences négatives. Si je décide de me retirer, toutes les données amassées jusqu'à mon retrait seront détruites. Même si le projet est endossé par le Service des incendies d'Ottawa, il n'y a aucune obligation pour moi d'y participer.

Acceptation: Je, _____ (nom du participant), accepte de participer a l'étude de recherche mentionnée ci-dessus menée par Dr. Jules Blais, Dr. Laurie Chan et Dr. Paul White du Département de Biologie, Faculté des Sciences, Université d'Ottawa.

Si j'ai quelconque question à propos de cette étude, je peux contacter Dr. Jules Blais par téléphone: (613) 562-5800 Ext. 6650 Fax: (613) 562-5486 ou par courriel: jules.blais@uottawa.ca

Si j'ai des questions au sujet de la conduite éthique de cette étude, je peux contacter le responsable d'éthique en recherche à l'Université d'Ottawa, pavillon Hall, 550 rue Cumberland, salle 154, Ottawa, ON K1N 6N5 Tél.: (613) 562-5387 courriel: ethique@uottawa.ca

Il y a deux copies du formulaire de consentement, l'une d'elle est pour moi.

_____
ID du participant

_____ _____
Signature du participant Date (jj/mm/aaaa)

_____ _____
Témoin, représentant au nom de la signature du chercheur. Date (jj/mm/aaaa)

Consent form (office worker participants - English)

Université d'Ottawa | University of Ottawa

Faculté des sciences | Faculty of Science
Département de biologie | Department of Biology
Pavillon Gendron Hall
30 Marie-Curie Ottawa ON Canada K1N 6N5
☏ 613-562-5718 🖷 613-562-5486 bio@uOttawa.ca

Consent Form (Office Workers)

Title of the study: Occupational exposure to metals and polycyclic aromatic hydrocarbons by firefighters

Name of researcher:

Jules Blais, Professor, Department of Biology, University of Ottawa
Tel: 613-562-5800 ext 6650. Email: jules.blais@uottawa.ca

Laurie Chan, Professor, Department of Biology, University of Ottawa
Tel: 613-562-5800 ext. 6349. Email: laurie.chan@uottawa.ca

Paul White, Adjunct Professor, Department of Biology, University of Ottawa
Tel: 613-941-7373. Email: paul.white@hc-sc.gc.ca

Tracy Lea Kirkham, Assistant Professor, Dalla Lana School of Public Health, University of Toronto; Tel: 416-978-6239. Email: tracy.kirkham@utoronto.ca

David Matschke, City of Ottawa Fire Services
Email: Dave.Matschke@ottawa.ca

Hugo Lemieux, Vice President Research Office, University of Ottawa
Email: hlemieux@uottawa.ca

Invitation to Participate: I am invited to participate in the abovementioned research study conducted by Dr. Jules Blais, Dr. Laurie Chan, Dr. Paul White, Dr. Tracy Kirkham, Mr. David Matchke, and Dr. Hugo Lemieux, and funded by the Ontario Ministry of Labour.

Purpose of the Study: The purpose of the study is to determine how firefighters are exposed to chemicals produced during combustion (e.g., polycyclic aromatic hydrocarbons, antimony, lead, and cadmium) while fighting fires and living in fire halls. An understanding of how firefighters are exposed to these chemicals will help in developing strategies to reduce the risk of exposure in the future. The project is supported by Ottawa Fire Service senior management (e.g., Chief and Deputy Chiefs), the Ottawa Fire Service Health and Safety Committee, and the Ottawa Professional Firefighters Association.

Participation: By participating in this study, I acknowledge that I am not a smoker nor do I live with smokers. My participation will consist of providing urine samples on two occasions over the course of the study. The urine samples will be analyzed for metals, polycyclic aromatic hydrocarbon metabolites, isoprostane and CC16 (i.e., protein markers of genetic and cellular damage), and urinary mutagenicity. I will participate in answering a questionnaire about my work and home environments, and my health, habits and hobbies. I understand that a member of

the research team may also contact me to collect the following samples from my workplace for metal and polycyclic aromatic hydrocarbon content: air samples, swabs from clothing and skin. Collection of all samples should take no more than 30 minutes.

Risks: There are no foreseeable risks associated with the sample collections planned for this study. All sampling procedures have been reviewed and approved by the University of Ottawa and Health Canada Research Ethics Boards, as well as the Ottawa Fire Service. Analyses performed on samples I provide will not include any information that can be used to assess my health status. If I am concerned about my study results and/or my exposures to chemicals, I can consult with the Canadian Centre for Occupational Health and Safety (www.ccohs.ca/oshanswers/occup_workplace/firefighter.html), or the Occupational Health and Safety Branch of the Ontario Ministry of Labour (www.labour.gov.on.ca/english/hs/). In addition, I can consult with the project investigators and/or the project occupational hygienist who can provide an opinion regarding my levels. Finally, I can consult my family physician for an opinion regarding potential health effects.

Benefits: My participation in this study will help determine whether firefighters are exposed to hazardous substances; and moreover, will help occupational hygienists develop strategies to reduce exposures in my workplace. There will be no remuneration, payment or reward for participating in the study. Since, to our knowledge, this is the first study of career firefighters' exposures to hazardous substances, both before and during firefighting activities, it is difficult to predict the utility of the study results. It may be necessary to design and execute a follow-up study to address issues/questions raised in this study.

Confidentiality and anonymity: I have received assurance from the researchers that the information I share will remain strictly confidential. I understand that my study results will be used only for the development of intervention plans to reduce exposures in the workplace and for research. My confidentiality will be protected by virtue of the fact that any linkage of my identity to the study results will only be known by the Principal Investigators. All the other researchers will only work with files containing coded identities.

Anonymity
Personal information will be protected in the following manner: (1) all participants will be assigned a randomized ID code and only the Principal Investigators will have the key to the coding, (2) only group results will be published in research reports describing the study results, and (3) no individual data or personal identity will be revealed in any publications describing the study results.

Conservation of data: The collected information, including both hard copies of the questionnaires and electronic data, will be kept in a secured room at the Department of Biology of the University of Ottawa for a period of five (5) years after which it will be destroyed. The key to the ID coding system will be kept by the Principal Investigators in a secure office at the University of Ottawa.

Voluntary Participation: I am under no obligation to participate and if I choose to participate I can withdraw from the study at any time and/or refuse to answer any questions without suffering any negative consequences. If I choose to withdraw, all data gathered until the time of withdrawal will be destroyed. Although the project is endorsed by the Ottawa Fire Service, there is no obligation for me to participate.

Acceptance: I, _____ (Name of Participant), agree to participate in the above research study led by Drs. Jules Blais, Laurie Chan, and Paul White of the Department of Biology, Faculty of Science, University of Ottawa.

If I have any questions about the study, I may contact Dr. Jules Blais by phone: (613) 562-5800 Ext. 6650, Fax: (613) 562-5486 or email: jules.blais@uottawa.ca

If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5Tel.: (613) 562-5387 Email: ethics@uottawa.ca

There are two copies of the consent form, one of which is mine to keep.

_____
Participant ID

_____ _____
Participant's signature Date (dd/mm/yyyy)

_____ _____
Witness on behalf of the Researcher's signature Date (dd/mm/yyyy)

Consent form (office worker participants - French)

Université d'Ottawa | University of Ottawa

Faculté des sciences | Faculty of Science
Département de biologie | Department of Biology
Pavillon Gendron Hall
30 Marie-Curie Ottawa ON Canada K1N 6N5
☏ 613-562-5718 🖷 613-562-5486 bio@uOttawa.ca

Formulaire de consentement (employés de bureau)

Titre de l'étude: Exposition professionnelle des pompiers aux métaux ainsi qu'aux hydrocarbures aromatiques polycycliques.

Nom des chercheurs:

Jules Blais, Professeur, Département de Biologie, Université d'Ottawa,
Tél: 613-562-5800 ext 6650. Courriel: jules.blais@uottawa.ca

Laurie Chan, Professeur, Département de Biologie, Université d'Ottawa
Tél: 613-562-5800 ext. 6349 Courriel: laurie.chan@uottawa.ca

Paul White, Professeur Adjoint, Département de Biologie, Université d'Ottawa
Tél: 613-941-7373 Courriel: paul.white@hc-sc.gc.ca

Tracy Lea Kirkham, Professeur Adjoint, École de santé publique Dalla Lana, Université de Toronto Tél: 416-978-6239 Courriel: tracy.kirkham@utoronto.ca

David Matschke, Services des incendies d'Ottawa
Courriel: Dave.Matschke@ottawa.ca

Hugo Lemieux, Cabinet de la vice-rectrice à la recherche, Université d'Ottawa
Courriel: hlemieux@uottawa.ca

Invitation à participer: Je suis invité à participer a l'étude mentionnée ci-dessus menée par Dr. Jules Blais, Dr. Laurie Chan, Dr. Paul White, Dr. Tracy Kirkham, Mr. David Matchke et Dr. Hugo Lemieux financée par le ministère du travail de l'Ontario.

But de l'étude: Le but de cette étude est de déterminer comment les pompiers sont exposés aux composés chimiques produits par une matière en combustion (incluant les hydrocarbures aromatiques polycycliques, l'antimoine, le plomb et le cadmium) en combattant des incendies et en résidant à la caserne de pompiers. La compréhension du mécanisme d'exposition aux composés chimiques aidera à développer des stratégies pour diminuer les risques d'expositions dans le futur. Le projet à l'appui de la Direction senior du service des incendies d'Ottawa (e.g., chef et chefs adjoints), le Comité de santé et sécurité au travail du service des incendies d'Ottawa et l'Association professionnelle des pompiers d'Ottawa.

Participation: En participant à cette étude, je confirme que je suis un non-fumeur et ne réside pas avec des fumeurs. Ma participation consistera à donner un échantillon d'urine à deux reprises durant cette étude. Les échantillons d'urines seront analysés pour les métaux sélectionnés, les métabolites d'hydrocarbures aromatiques polycycliques(HAPs), l'isoprostane, la CC16 (marqueur (protéine) de dommages génétiques et cellulaires) et la mutagénicité de l'urine. Je vais participer en répondant au questionnaire à propos de mon travail, résidence, santé, habitudes et

passes temps. Je comprends qu'un membre de l'équipe de recherche pourra me contacter afin de recueillir les échantillons à mon lieu de travail pour la teneur en métaux ainsi que les hydrocarbures aromatiques polycycliques: échantillons d'air, lingettes frottés aux vêtements ainsi qu'à la peau. Tous échantillonnages ne devraient durer plus de 30 minutes.

Risques: Il n'y a pas de risques prévisibles associés à la prise d'échantillons prévu par cette étude. Tous les protocoles d'échantillonnages ont été revus et approuvés par le service d'incendies d'Ottawa, le Comité d'éthique en recherche de l'Université d'Ottawa ainsi que de Santé Canada. Les analyses effectués sur les échantillons que je fournirai ne contiendront aucune information permettant d'évaluer mon état de santé. Si je suis préoccupé par mes résultats de cette étude, ou mon exposition en milieu de travail aux composés chimiques en général, je peux consulter le Centre canadien d'hygiène et de sécurité au travail (http://www.cchst.ca/oshanswers/occup_workplace/firefighter.html), le Département de sécurité et santé au travail du Ministère du travail de l'Ontario (http://www.labour.gov.on.ca/french/hs/index.php). De plus, je peux consulter les chercheurs du projet et/ou l'hygiéniste du travail du projet qui peut fournir un avis sur mes niveaux. Enfin, je peux consulter mon médecin de famille pour un avis sur les effets potentiels sur la santé.

Bienfaits: Ma participation à cette étude aidera à déterminer si les pompiers sont exposés à des substances nocives. De plus, ma participation aidera également l'hygiéniste en milieu de travail à développer un plan pour réduire l'exposition aux composés chimiques dans mon milieu de travail. Il n'y aura aucune rémunération, paiement ou récompenses pour avoir participé à cette étude. Puisque, selon nos connaissances, ceci constitue la première étude de l'exposition des pompiers de carrières aux substances nocives, avant et durant les combats d'incendies, il est difficile de prévoir l'utilité des résultats de cette étude. Il pourrait être nécessaire de concevoir et d'effectuer une étude supplémentaire pour répondre aux questions et points litigieux engendrés par la présente étude.

Confidentialité et anonymat: J'ai reçu l'assurance des chercheurs que l'information que je vais partager demeurera strictement confidentielle. Je comprends que le contenu sera utilisé seulement pour développer un plan d'intervention et pour buts de recherches. Aussi, ma confidentialité sera protégée par le fait que le lien entre mon identité et les données sera uniquement connu par le chercheur principal. Les autres chercheurs travailleront seulement sur des fichiers dont l'identité des sujets est encodée.

Anonymat:
L'information personnelle sera protégée de la façon suivante: (1) tous les participants recevront un code d'identité aléatoire et seul le chercheur principal aura la clef de codage, (2) seuls les résultats de groupes seront publiés dans les rapports de recherche, (3) Aucunes données individuelles ou d'identité personnelle ne seront dévoilés lors de publications.

Conservation des données: L'information amassée (copie papier du questionnaire ainsi que les données électroniques des paramètres d'échantillonnage) sera conservée dans une salle sécurisée au Département de Biologie de l'Université d'Ottawa pour une durée de 5 ans et sera ensuite détruite. La clef du system d'encodage aléatoire d'identité sera gardée par le chercheur principal à son bureau situé à l'Université d'Ottawa.

Participation volontaire: Je suis soumis à aucune obligation de participer, et si je choisis de participer, je peux me retirer de l'étude en tout temps et/ou refuser de répondre à n'importe quelle question sans que j'aille a subir de conséquences négatives. Si je décide de me retirer, toutes les données amassées jusqu'à mon retrait seront détruites. Même si le projet est endossé par le Service des incendies d'Ottawa, il n'y a aucune obligation pour moi d'y participer.

Acceptation: Je, _____ (nom du participant), accepte de participer a l'étude de recherche mentionnée ci-dessus menée par Dr. Jules Blais, Dr. Laurie Chan et Dr. Paul White du Département de Biologie, Faculté des Sciences, Université d'Ottawa.

Si j'ai quelconque question à propos de cette étude, je peux contacter Dr. Jules Blais par téléphone: (613) 562-5800 Ext. 6650 Fax: (613) 562-5486 ou par courriel: jules.blais@uottawa.ca

Si j'ai des questions au sujet de la conduite éthique de cette étude, je peux contacter le responsable d'éthique en recherche à l'Université d'Ottawa, pavillon Hall, 550 rue Cumberland, salle 154, Ottawa, ON K1N 6N5 Tél.: (613) 562-5387 courriel: ethique@uottawa.ca

Il y a deux copies du formulaire de consentement, l'une d'elle est pour moi.

_____
ID du participant

_____ _____
Signature du participant Date (jj/mm/aaaa)

_____ _____
Témoin, représentant au nom de la signature du chercheur. Date (jj/mm/aaaa)

QUESTIONNAIRE #1

GENERAL QUESTIONNAIRE FOR STUDY PARTICIPANTS

Université d'Ottawa | University of Ottawa

Faculté des sciences | Faculty of Science
Département de biologie | Department of Biology
Pavillon Gendron Hall
30 Marie-Curie Ottawa ON Canada K1N 6N5
☏ 613-562-5718 🖷 613-562-5486 bio@uOttawa.ca

Fire Fighters' Study: General Questionnaire

Study ID# _____

Section A. You and Your Work Environment (This section is to be completed if you are a firefighter. If you are not a firefighter, please proceed to Section B)

We'd like to begin by asking you a few questions about your job and work environment.

A.1. What is your current job title? Please specify: _____

A.2. How long have you held that title? _____ Years

A.3. How old were you when you started firefighting? _____ Years

A.4. On average, how many hours including overtime do you work each month? _____ hrs

A.5. Are you currently a member of a specialty team?

☐ No

☐ Yes. Please specify: _____

A.6. What will be your primary role as firefighter during the course of this study?

_____

A.7. Will your work require you to operate pumps on the fire truck?

☐ No

☐ Yes

A.8. How many times per month do you typically wear the self-contained breathing apparatus?
_____ times/month

A.9. Do you currently have a second/part time paid job in addition to your firefighting job?

☐ No

☐ Yes

If Yes,

A.9.a. How many hours a week on average do you work at your secondary employment?

_____ hours/week

A.9.b. What is the primary job title of your secondary employment?

_____

A.9.c. Are you exposed to any of the following smoke/fires/fumes/tasks in your second job?

Diesel exhaust/fumes	□ No	□ Yes
Gas exhaust/fumes	□ No	□ Yes
BBQ's	□ No	□ Yes
Food smokers (ex. for fish)	□ No	□ Yes
Fried foods	□ No	□ Yes
Incense/burning candles	□ No	□ Yes
Broilers or other industrial burners/incinerators	□ No	□ Yes

A.9.c.1. if yes, specify: _____

Coal tar or coal tar creosote	□ No	□ Yes
Asphalt, roofing tar, or driveway sealant	□ No	□ Yes
Other open fires/flames,	□ No	□ Yes

A.9.c.2. if yes, specify: _____

Battery production	□ No	□ Yes
Smelting/founding,	□ No	□ Yes

A.9.c.3. if yes, specify: _____

Coke production	□ No	□ Yes
Dyes or pigments	□ No	□ Yes
Electroplating	□ No	□ Yes
Glass (including decorative) production	□ No	□ Yes
Welding	□ No	□ Yes

A.9.c.4. if yes, specify: _____

Painting or paint removal	□ No	□ Yes

A.9.c.5. if yes, specify: _____

Radiator repair	□ No	□ Yes
Demolition	□ No	□ Yes
Firearm operation	□ No	□ Yes

A.9.d. How often are you exposed to smoke or open fires at this second job?

☐	Never	☐	Once a week
☐	Less than once a month	☐	2 to 3 times a week
☐	Once a month	☐	4 to 6 times a week
☐	2 to 3 times a month	☐	Every day

A.10. Which term best describes the location of your place of work (i.e., fire-hall)?

- ☐ urban
- ☐ sub-urban
- ☐ rural

A.11. How far is your residence from your place of work (i.e., fire-hall)?

- ☐ less than 5 km
- ☐ 5-10 km
- ☐ 10-15 km
- ☐ 15-20 km
- ☐ more than 20 km

A.12. How do you commute to work on a regular basis?

- ☐ drive in gasoline vehicle
- ☐ drive in diesel vehicle
- ☐ drive in hybrid/electric vehicle
- ☐ public transportation
- ☐ walk
- ☐ bicycle
- ☐ other _____

A.13. If you selected bicycle or walking, does the frequency vary with season?

- ☐ No
- ☐ Yes

A.14. If yes, how many weeks per year is cycling or walking used exclusively?
_____ weeks/year

A.15. Which alternative mode of transportation is most commonly used during the winter months?
_____

Section B. Health and Diet Information

We would like to know a few things about your health and diet that may assist in interpreting the results of this study.

Exercise and Fitness

A.16. In general, would you say your fitness level is?
☐ Poor ☐ Fair ☐ Good ☐ Very Good ☐ Excellent

A.17. On average, how many times a week do you exercise outside of work for 30 minutes or more of continuous activity?

☐ <1 ☐ 1-2 ☐ 2-3 ☐ 3-4 ☐ >5

A.18. In general, would you say your health is:

☐ Poor ☐ Fair ☐ Good ☐ Very Good ☐ Excellent

A.19. Do you consider yourself:

☐ Overweight
☐ Underweight
☐ Just about right

Diet and Cooking Practices

A.20. During the past 12 months, how often did you drink alcoholic beverages?

☐ Less than once a month
☐ Once a month
☐ 2 to 3 times a month
☐ Once a week
☐ 2 to 3 times a week
☐ 4 to 6 times a week
☐ Every day

A.21. During the past 12 months, how often did you drink caffeinated coffee, tea, and soda?

☐ Less than once a week
☐ Once a week
☐ 2 to 3 times a week
☐ Once a day
☐ 2 to 3 times a day
☐ 4 to 6 times a day

A.22. Are you a vegetarian?

☐ No
☐ Yes

If yes,

A.22.a. Do you consume any animal products (e.g., eggs, fish and shellfish, etc.)?

☐ No
☐ Yes, specify _____

A.23. When NOT consuming Bar-B-Q'd meats, which cooking method(s) do you employ most frequently? Indicate the top three with adjacent numbers (i.e., 1, 2 or 3).

☐ pan frying (includes stir frying)
☐ deep frying
☐ oven broiling
☐ oven baking
☐ boiling or stewing
☐ microwaving
☐

A.24. Please elaborate on your diet by completing the following table.

Food Type	Cooking Method	Frequency of Cooking	Doneness Level of Meat	Comments /

		1/month	2-3/month	1/week	2-3/week	daily	Rare	Medium Rare	Medium	Medium Well	Well	Very Well	Notes
Chicken	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	
Pork	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	
Beef / Lamb	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	
Fish	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	
Shellfish	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	
Vegetables	□ pan frying □ bake □ broil □ deep frying □ BBQ □ other*	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	□ □ □ □ □ □	

*__Other cooking methods__ include boiling, stewing, pouching, steaming, and microwaving.
Please use the Comments/Notes column to provide any details on other cooking methods (i.e. specific method) or to provide any information you think is important and missed.

Illness and Medications

A.25. Have you ever been diagnosed with a skin condition such as eczema or psoriasis?

☐ No

☐ Yes
☐ Don't Know / Not Sure

A.26. Do you use steroid based creams for skin conditions?
☐ No
☐ Yes
☐ Don't Know / Not Sure

A.27. Do you ever use shampoos or topical skin treatments that may contain coal tar products for treatment of dermal conditions such as eczema, psoriasis or dandruff?
☐ No
☐ Yes
☐ Don't Know / Not Sure

If don't know/not sure for question B11 OR B12:
A.27.a. If unsure, please list skin and scalp treatment products you use here.
_____
_____

A.28. Are you currently using these shampoos or topical skin treatments that may contain coal tar products?
☐ No
☐ Yes
☐ Not applicable

B.14. Do you currently have or have ever been diagnosed with any chronic illnesses (e.g., asthma, cancer, heart disease, arthritis)? If yes, please specify.
☐ No
☐ Yes, Specify _____
☐ Don't Know / Not sure

B 15. Have you recently (i.e., past 2 months) had any illness (e.g., common cold, influenza, etc.). If yes, please specify.
☐ No
☐ Yes, Specify _____
☐ Don't know / Not sure

B.16. Have you recently (i.e., past 2 months) been exposed to X-rays during a medical or dental procedure (e.g., dental X-ray, skeletal X-ray, CT scan)? If yes, please specify.
A.28.a. No
A.28.b. Yes, Specify _____
A.28.c. Don't know / Not sure

B. 17. Are you currently taking any prescribed medications? If yes, please specify type and daily dosage.

☐ No
☐ Yes. Specify: _____
☐ Don't Know / Not Sure

B. 18. Are you currently taking any nutritional health products (e.g., vitamin supplements, mineral supplments, herbal remedies, fish oil, flax oil, probiotics, etc.)? If yes, please specify type and daily dosage. For multivitamin and mineral supplments, simply indicate type and dosage (e.g., Jamieson Power Vitamns for Men, one per day).

☐ No
☐ Yes. Specify: _____
☐ Don't Know / Not Sure

Section C. You and Your Home Environment

We are now going to ask you some questions about you and your home situation to better understand the people included in the study.

General Background Information

A.2. What is your gender? ☐ Male ☐ Female

A.3. What is your date of birth? (i.e. dd/mm/yyyy) __ __ / __ __ / __ __ __ __

A.4. People living in Canada come from many different backgrounds. What country or countries reflect your heritage best? Please specify:

_____

_____

A.5. What is your current marital status?

☐ Single, never married
☐ Living Common Law. Number of years lived together: _____yrs
☐ Married. Number of years married: _____yrs
☐ Divorced/Separated. Number of years divorced/separated: _____yrs
☐ Widowed. Number of years widowed: _____yrs
☐ Other. Please specify: _____

A.6. Do you have children?

☐ No
☐ Yes

If yes,

☐ Do your children live with you?

☐ Yes, Full time
☐ Yes, Part-time
☐ No

Housing Information

A.7. Which term best describes the property where you live?

☐ Urban house (single or multi-family building)

- ☐ Urban apartment or condominium
- ☐ Sub-urban house (single or multi-family building)
- ☐ Sub-urban apartment or condominium
- ☐ Rural house or apartment
- ☐ Working farm

A.8. What is the approximate age of your home (years)

- ☐ <10 years
- ☐ 10-20 years
- ☐ 20-30 years
- ☐ 30-50 years
- ☐ 50-100 years
- ☐ >100 years

A.9. How often do you vacuum and/or dust your home?

- ☐ More than once per week
- ☐ Once per week
- ☐ Once every 2 weeks
- ☐ Less than once every 2 weeks

A.10. How is your home heated?

- ☐ Oil
- ☐ Gas
- ☐ Electric
- ☐ Wood stove

A.11. Do you have a fireplace?

- ☐ Gas
- ☐ Electric
- ☐ Wood
- ☐ No fireplace

If yes,

- ☐ How often do you use your fireplace in the winter?
  - ☐ More than twice a week
  - ☐ Once a week
  - ☐ 1-3 times per month
  - ☐ Less than once per month

A.12. Does your home have an attached garage?

- ☐ No
- ☐ Yes

If yes,

- ☐ Do you regularly park your vehicle in the attached garage?
  - ☐ No
  - ☐ Yes

- ☐ Which of the following items do you regularly store in your attached garage? (Check all that apply.)
  - ☐ Gasoline
  - ☐ Used engine oil (e.g., automobile, snow-blower, lawn-mower)
  - ☐ Diesel fuel and/or kerosene
  - ☐ Paints, wood stains, varnishes, paint thinners and/or solvents
  - ☐ Pesticides (i.e., herbicides, insecticides, fungicides, rodenticides, etc)

A.13. Do you have any oil lamps or burners?

- ☐ No
- ☐ Yes

If yes,

- ☐ How many hours per month do you use oil lamps or burners?

  _____ hrs/month

Neighbourhood Information

A.14. Do you live near an incinerator?

- ☐ No
- ☐ Yes

A.15. Do you live near a major industry?

- ☐ No
- ☐ Yes

A.16. Are any of the following activities performed near your home? (Check all that apply.)

- ☐ Agriculture burning
- ☐ Crematorium burning
- ☐ Recreational fires/burning

Activity and Hobby Information

A.17. Do you engage in bonfires or similar activities?

- ☐ No
- ☐ Yes

If yes,

- ☐ How frequently do you engage in these activities?
  - ☐ once per week
  - ☐ twice per month
  - ☐ once per month
  - ☐ less than once per month

A.18. Are you regularly (i.e., more than once per month) exposed to any of the following smoke/fires/fumes/activities in your home environment?

☐ Food smokers (ex. for fish)	□ No □ Yes
☐ Incense/burning candles	□ No □ Yes
☐ Asphalt, roofing tar or driveway sealant	□ No □ Yes
☐ Welding	□ No □ Yes, specify: _____
☐ Painting or paint removal	□ No □ Yes, specify: _____
☐ Firearm operation	□ No □ Yes

A.19. Which of the following gasoline-powered devices do you use on a regular basis (i.e., more than once per month)

- ☐ Lawn-mower or lawn tractor
- ☐ Edge trimmers (i.e., "weed-whacker")
- ☐ Chainsaw
- ☐ Electric generator
- ☐ Leaf blower

A.20. Do you engage in any of the following activities (indicate by check mark)?

- ☐ Welding
- ☐ Carpentry and joinery
- ☐ Off-road motorised summer sports (i.e., all-terrain vehicles, dirt bike, etc.)
- ☐ Off-road motorised winter sports (i.e., snow-mobiling)
- ☐ Power boating and related activities, including jet skiing

Section D. Smoking Information

Lastly we are going to ask you about your smoking history. Smoking is known to be a carcinogenic risk factor and affect exposure to metals and PAHs.

A.21. In your lifetime, have you smoked a total of 100 or more cigarettes (about 4 packs)?

- ☐ Yes
- ☐ No

A.22. Have you ever smoked a similar quantity of any other tobacco products besides cigarettes?

- ☐ Yes
- ☐ No

A.23. At the present time, do you smoke cigarettes daily, occasionally or not at all?

- ☐ Daily
- ☐ Occasionally
- ☐ Not at all

A.24. At present, do you smoke any other tobacco products daily, occasionally, or not at all?

- ☐ Daily
- ☐ Occasionally
- ☐ Not at all

A.25. Have you ever smoked a whole cigarette?

- ☐ Yes
- ☐ No

A.26. Have you ever smoked cigarettes daily?

- ☐ Yes
- ☐ No

A.27. Have you ever smoked a whole cigar or a pipe with pipe tobacco?

- ☐ Yes
- ☐ No

A.28. Do you currently smoke cigar or pipe tobacco?

- ☐ Yes
- ☐ No

If Yes,

☐ Which type do you currently smoke (check all that apply)?

- ☐ Cigar
- ☐ Pipe tobacco

☐ How frequently do you smoke cigars/pipe tobacco?

- ☐ Daily
- ☐ Weekly
- ☐ Monthly
- ☐ Occasionally, less than once a month

A.29. Do you use nicotine containing smoking cessation aides?

- ☐ Yes
- ☐ No

Fire Event Questionnaire

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FIRE EVENT QUESTIONNAIRE

INCIDENT NUMBER _____

SUBJECT ID _____

DATE : _____

1. FIRE EVENT TYPE

1.1. What type of fire was the event?

- ☐ Structure fire (Section 2A)
- ☐ Container fire (Section 2B)
- ☐ Transportation fire (Section 2C)
- ☐ Brush/ forest fire (Section 2D)
- ☐ Other (Section 2E)

Please fill out the appropriate section pertaining to the type of fire indicated next to the fire type selected **and** the OTHER INFORMATION (2E) section. All other questions are to be filled out in the questionnaire unless otherwise noted.

2. FIRE INFORMATION

2A: STRUCTURE FIRE INFORMATION

2.1. What was the structure made of?

- ☐ Brick
- ☐ Stone
- ☐ Cement
- ☐ Wood/timber
- ☐ Steel/metal
- ☐ Combination, specify: _____

2.2. What was the primary purpose of the structure?

- ☐ Single family residence
- ☐ Multi-family residence
- ☐ Commercial
- ☐ Industrial
- ☐ Agriculture
- ☐ Other: _____

2.3. What is the approximate size of the structure?

2.3..1. Number of floors _____

2.3..2. Square footage/meters _____

2.4. What is the age of the structure? _____ years

2.5. What components were impacted by the fire?

- ☐ Structure
- ☐ Content
- ☐ Both structure and content

2.6. Where was the fire location within the building? (ex. Center of building, 4th floor)

_____

_____

2.7. How wide spread was the fire?

- ☐ Limited to object of origin
- ☐ Spread to multiple objects in room of origin
- ☐ Structure in room of origin
- ☐ Spread to structure on floor of room of origin
- ☐ Spread to multiple floors of structure
- ☐ Spread to entire structure

2.8. How much smoke was in the structure?

- ☐ Light haze
- ☐ Room(s) half full
- ☐ Room(s) totally obscured
- ☐ Entire floor obscured
- ☐ Entire structure obscured
- ☐ Structure and surrounding obscured

2B: CONTAINER FIRE INFORMATION

2.9. What is the approximate size of the container?

2.9..1. Meters cubed volume _____m3

2.9..2. Container dimensions _____

2.10. Where was the container located?

- ☐ Commercial area (retail)
- ☐ Commercial area (food)
- ☐ Industrial
- ☐ Residential (multi-family)
- ☐ Other, specify: _____

2C: TRANSPORT FIRE INFORMATION

2.11. What was the type of vehicle on fire?

- ☐ Passenger car/ pickup
- ☐ Motorcycle
- ☐ Cube van/ Delivery truck
- ☐ Motorhome/trailer
- ☐ Bus
- ☐ Semi-truck
- ☐ Train
- ☐ Industrial vehicle, specify: _____

2.12. Where was the fire confined to?

- ☐ Engine only
- ☐ Cab only
- ☐ Container/hauled contents
- ☐ Engine and cab
- ☐ Container and cab
- ☐ All areas

2.13. What was the fuel type of the vehicle?

- ☐ Propane
- ☐ Gas
- ☐ Diesel
- ☐ Electric
- ☐ Combination, specify: _____

2D: BRUSH/FOREST FIRE INFORMATION

2.14. What was the approximate size of the area on fire? _____ m^3

2.15. Please describe the land use (Ex. Park) and type of vegetation on fire.

_____

_____

2D: OVERALL FIRE INFORMATION (FILLED OUT FOR ALL FIRE TYPES)

2.16. How many fire trucks/apparatus responded to the call? _____

2.17. How long were you at the fire in total? _____ mins

2.18. Was it raining/snowing during the fire?

- ☐ No
- ☐ Yes

2.19. Was it windy at the fire site?

- ☐ No
- ☐ Yes

2.20. Upon arrival to the scene, the fire was in what stage?

- ☐ Incipient
- ☐ Growing
- ☐ Fully developed
- ☐ Oxygen limited
- ☐ Flashover
- ☐ Decay

2.21. How intense was the fire?

- ☐ Smouldering/smoke with no visible flames
- ☐ Smoke with minimal flames
- ☐ Smoke with flames
- ☐ Heavy smoke with flames pushing from structure/object

2.22. How much smoke was coming from the fire location?

- ☐ Light haze/limited smoke
- ☐ Medium haze/smoke
- ☐ Heavy haze/smoke
- ☐ Very heavy haze smoke

2.23. What color was the smoke at the fire?

- ☐ White
- ☐ Light grey
- ☐ Grey
- ☐ Black/brown
- ☐ Dark black

2.24. Please describe in detail what the main substance(s) burning during the fire.

_____

_____

_____

2.25. Please use this space to explain any specific details about the fire you feel are important and were not addressed above (ex. use of structure, known/unusual chemicals or objects in space, unique features):

_____

_____

_____

_____

_____

3. ROLE/TASK INFORMATION

We are interested in obtaining information about what your role and what tasks you performed during the fire call. Your role and tasks may be important in explaining your exposures.

3.1. What was your main role during the event?

_____

_____

3.2. Where were you in relation to fire/smoke for the majority of the event?

- ☐ Upwind/within smoke filled area
- ☐ Downwind
- ☐ Equal time up and downwind
- ☐ Inside the structure

3.3. How far away from the fire were you located for the majority the time?

_____

_____

3.4. Did you enter the burning object (i.e. building/structure, container, vehicle) at any time?

☐ No

☐ Yes, during knockdown

If yes,

3.6..1. How long were you inside the object? _____

3.6..2. Were you wearing SCBA? □Yes □No

☐ Yes, during overhaul

If yes,

3.6..3. How long were you inside the object? _____

3.6..4. Were you wearing SCBA? □Yes □No

3.5. What tasks did you perform during this event? Please select all that apply/applicable.

Task(s)	**Task performed?**	**Duration of task (minutes)**
Search and rescue:		
On floor below fire	□Yes □No	_____
On fire floor	□Yes □No	_____
On floor above fire	□Yes □No	_____
Other, search and rescue entry (ex. vehicle, container)	□Yes □No	_____
Suppression:		_____
Internal attack – below fire floor	□Yes □No	_____
Internal attack – on fire floor	□Yes □No	_____
Internal attack – above fire floor	□Yes □No	_____
Internal attack - other	□Yes □No	_____
External attack	□Yes □No	_____
Exposure protection/ventilation	□Yes □No	_____
Vertical ventilation	□Yes □No	_____
Horizontal ventilation	□Yes □No	_____
Forcible entry	□Yes □No	_____
Pump operations	□Yes □No	_____
Water supply	□Yes □No	_____
Accountability	□Yes □No	_____
Overhaul	□Yes □No	_____
Command	□Yes □No	_____
Support	□Yes □No	_____
	_____	_____
Other, please specify	_____	_____

3.6. Did you use manual (i.e. non-motorized) equipment during the fire, including axes, hooks, poke poles, sledgehammers, crowbars, ropes, etc.?

☐ No
☐ Yes

3.7. Were you near the fire trucks/apparatus during the event?

☐ No
☐ Yes

If yes:

3.6..5. How long were you near them? _____
3.6..6. How far away were you on average? _____

3.7. Did you operate motorized equipment? (Tick all that apply)

Equipment Type	**Exposed**	**If Yes:**		
		Duration in minutes	**Were you wearing SCBA?**	**Were you wearing gloves?**
Hydraulic spreaders/lifts/rams	□Yes □No	_____	□Yes □No	□Yes □No
Fire apparatus pump	□Yes □No	_____	□Yes □No	□Yes □No
Chain saws	□Yes □No	_____	□Yes □No	□Yes □No
Ventilation fans	□Yes □No	_____	□Yes □No	□Yes □No
Cutters	□Yes □No	_____	□Yes □No	□Yes □No
Concrete saws	□Yes □No	_____	□Yes □No	□Yes □No
Portable pump	□Yes □No	_____	□Yes □No	□Yes □No

3.8. Did you work near motorized equipment you did not operate?

☐ No
☐ Yes

If yes:

3.8..1. How long were you working near the equipment? _____
3.8..2. Were you wearing SCBA or a N95 mask? _____
3.8..3. How far away were you from the equipment? _____

3.9. Did you handle hose during the fire?

☐ No
☐ Yes

3.10. Did you assist in reloading the hose onto the truck following the fire?

☐ No
☐ Yes

If yes:
Did you wear gloves while handling hose? □Yes □No

3.11. Did you assist in unloading the hose for cleaning upon arrival back at the fire hall following the fire?

☐ No
☐ Yes

If yes:
Did you wear gloves while handling hose? □Yes □No

4. PPE INFORMATION

We are interested in knowing what PPE you used during this fire call and how long it was used for to help explain your internal exposures. Your internal exposure will reflect exposures you breathed in and those that were absorbed by your skin.

4.8. Did you wear all of your turnout gear during the fire event (boots, pants, jacket, gloves, hat)?

☐ No, reason why not:_____
☐ Yes

If yes:
Did you remove your turnout gear during the event for any reason?

☐ No
☐ Yes

If yes, please describe why and for how long.

_____

_____

4.8. Did you wear SCBA during the fire at all?

☐ No
☐ Yes

If yes:
How many tanks did you use? _____

How long was the total duration you used SCBA? _____

4.9. Were you at any time exposed (even minimally) to smoke or other chemicals during the event when you were not protected from SCBA?

- ☐ No
- ☐ Yes

If yes,

Approximately how long were you exposed? _____ min

How strong was the exposure? _____

4.10. Did you wear a N95 particle mask?

- ☐ No
- ☐ Yes, specify when:_____

4.11. Approximately how many shifts have you worn your turnout gear since it was cleaned?

_____

_____

4.12. Was your turnout gear cleaned since the last fire you attended?

- ☐ No
- ☐ Yes

4.13. Does your turnout gear have residual evidence of smoke/fire exposure following the fire (i.e. is there visible soot/dirt)?

- ☐ No
- ☐ Yes

5. Additional Information/Comments:

Please use this space to provide any additional information you feel is important about the details of this fire call that were not addressed in the questionnaire.

_____

_____

_____

_____

_____

_____

Results Letter for Participants

uOttawa

Université d'Ottawa | University of Ottawa

Faculté des sciences | Faculty of Science
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October 31, 2016

Dear [Participant Name]:

We thank you for participating in the University of Ottawa's research project "Occupational Exposures to Metals, Polycyclic Aromatic Hydrocarbons, and Organic Mutagens in Firefighters". This project assessed exposure of selected Ottawa Fire Service (OFS) employees (i.e., fire-fighters and office workers) to selected metals and organic compounds. I am writing at this time to provide you with a summary of the study results.

Results from the wipes, air, and urine samples you provided are enclosed. These results provide you with a snapshot of your exposure to certain chemicals both before and after firefighting activities, or in your daily office activities (i.e., in the case of office workers). It is important to understand that everyone is exposed to potentially hazardous chemicals on a daily basis; many substances are normally found in the environment and exposures are unavoidable. To assist you in evaluating your results, we are providing you with several reference values that can be used for comparison. These include: (1) average values for all firefighter participants; (2) average values for all office staff participants; and (3) values for a typical Canadian.

By participating in this research project you have permitted our research team and the funding authority (i.e., Ontario Ministry of Labour) to gain insight into occupational exposures of Ottawa firefighters to selected substances. The study results will be used to determine how firefighters are exposed to potentially harmful substances; and moreover, how they can be protected.

If you have any questions about your results, or the study results in general, please don't hesitate to contact me.

Sincerely,

Jules Blais, PhD
Professor of Biology and Environmental Toxicology
University of Ottawa
Phone: (613) 562-5800 Ext. 6650
Email: jules.blais@uottawa.ca

RESULTS OF WORKPLACE AIR MONITORING

This study assessed concentrations of polycyclic aromatic hydrocarbons (PAHs), antimony, cadmium, and lead in air samples collected in vehicle bays and fire truck cabs at three OFS fire stations. Air samples from an OFS administration office were collected as a point of reference.

Table 1 Concentrations of total PAHs in air samples from vehicle bays, fire truck cabs, and an OFS office. Results are reported in micrograms per cubic metre ($\mu g/m^3$).

	Study Average (Min. – Max.)			Reference Values	
Chemical	Vehicle Bay	Fire Truck Cab	Office (reference site)	ALEL[b]	ASEL[c]
PAHs[a]	0.11 (0.052-0.17)	2.30 (1.70-3.00)	0.0082	187.5	600
Antimony	0.0018 (0.0006-0.0037)	0.078 (0.037-0.15)	0.00031	468.7	1500
Cadmium	0.00005 (0.000033-0.000058)	0.0018 (0.00092 -0.0025)	0.000078	9.38	30
Lead	0.0024 (0.002-0.0031)	0.052 (0.0084-0.074)	0.0039	46.87	150

[a]PAHs measured include naphthalene, acenapthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, ideno(1,2,3-*cd*)pyrene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and benzo[*ghi*]perylene.

[b]Adjusted long term exposure limit (ALEL) for firefighters' extended work shift (i.e., adjusted from the typical 8- to a 24-hour shift length). Calculated using the Brief and Scala method (Government of Alberta, 2011).

[c]Allowable short term exposure limit (ASEL) – a limit at which workers are allowed to be exposed for a total of 30 minutes or less during a workday.

- Average total PAH concentrations measured in vehicle bays were **2 times higher than typical urban air values** (Dann, 1998).
- Average total PAH and antimony concentrations **inside fire truck cabs were significantly higher** than vehicle bays and the office (reference site).
- All air concentrations of total PAHs, antimony, cadmium, and lead were **well below the adjusted long term exposure limit (ALEL)** calculated based on the exposure standards set by Ontario Ministry of Labour and the Occupational Safety and Health Administration (OSHA).

Dann, T. (1998). "*Ambient Air Measurements of Polycyclic Aromatic Hydrocarbons (PAH), Polychlorinated Dibenzo-p-Dioxins (PCDD) and Polychlorinated Dibenzofurans in Canada (1987-1997).*" Report AAQD 97-3.

Government of Alberta (2011). The Effects of Unusual Work Schedules and Concurrent Exposures on Occupational Exposure Limits (OELs). https://work.alberta.ca/documents/WHS-PUB-ch055.pdf.

RESULTS FROM SAMPLES TAKEN BEFORE, DURING, AND AFTER FIRE EVENTS

This study collected personal air samples during fire events, as well as urine and wipe samples of skin, clothing, and personal protective equipment (PPE) before and after the same events. Concentrations of PAHs, antimony, cadmium, and lead were measured.

Some values are reported as "N/A" (not available or applicable). This is due to either insufficient resources to analyse certain samples (i.e., office worker wipes), inapplicability (i.e. office workers would not have post-fire samples), or a lack of supporting values in the literature (i.e. some chemicals and sample types do not have occupational exposure limits). Results for PPE "Pre-" and "Post-decontamination" samples are not available for each individual participant. Samples for decontamination investigation were randomly selected from PPE samples submitted to the OFS cleaning facility. Values indicating levels before and after decontamination are reported below.

Table 2 Summary of **PAHs** in personal air samples during live fires, wipe and urine samples taken before (i.e. Pre) and after a fire event (i.e. Post), and wipe samples taken on Personal Protective Equipment (PPE) before (i.e. Pre) and after decontamination (i.e. Post).

		Study Average (Min. – Max.)		Reference Values	
Sample Type	**Your Result**	Firefighters	Office Workers	Occupational Exposure Limit	CHMS[d]
Personal Air Sample (µg/m^3)[a]	**N/A**	2725 (5.23-28,604)	N/A[b]	600[c]	N/A
Wipe Sample (ng/cm^2)[a]					
Skin Pre	**N/A**	1.97 (0.01-14.87)	N/A	N/A	N/A
Skin Post	**N/A**	4.34 (0.08-24.98)	N/A	N/A	N/A
Clothing Pre	**N/A**	1.81 (0-9.15)	N/A	N/A	N/A
Clothing Post	**N/A**	2.98 (0.04-20.12)	N/A	N/A	N/A
PPE Pre	**N/A**	2.33 (0.05-18.81)	N/A	N/A	N/A
PPE Post	**N/A**	12.62 (0.25-128.34)	N/A	N/A	N/A
Urine Sample (µg/g creatinine)[e]					
Pyrene Pre		0.13 (0.02-0.33)	0.08	0.95[f]	0.081
Pyrene Post	**N/A**	0.27 (0.062-1.81)	N/A	0.95[f]	0.081
Phenanthrene Pre		0.42 (0.093-0.98)	0.47	N/A	0.33
Phenanthrene Post	**N/A**	1.38 (0.20-6.56)	N/A	N/A	0.33
Fluorene Pre		0.57 (0.12-1.17)	0.31	N/A	0.24
Fluorene Post	**N/A**	1.74 (0.32-7.09)	N/A	N/A	0.24
Naphthalene Pre		6.21 (1.94-13.30)	6.27	N/A	4.0
Naphthalene Post	**N/A**	16.95 (2.83-75.79)	N/A	N/A	4.0
PPE Decon. Wipes (ng/cm^2)[a]					
Pre Decontamination	N/A	8.18 (1.65-45.65)	N/A	N/A	N/A
Post Decontamination	N/A	1.6 (1.1-3.16)	N/A	N/A	N/A

[a]Parent PAHs measured are naphthalene, acenapthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene.

[b]N/A indicates not applicable or available.

[c]Allowable short term exposure limit (ASEL) - a limit at which workers are allowed to be exposed for a total of 30 minutes or less during a workday.

[d]Values based on data taken from the '2009-2011 Canadian Health Meausures Survey' for non-smoking males aged 25-62. Data report prepared by Statistics Canada and found on Health Canada's DAIS system. Responsibility for use and interpretation of these values is entirely that of the users.

[e]Concentrations are totals of the listed metabolite(s) for each PAH. Metabolite(s) of PAHs are measured and summed according to their parent PAH (i.e., original compound before metabolism): pyrene is metabolized to 1-hydroxypyrene, phenanthrene is metabolized to 1-, 2-, 3-, 4-, or 9-hydroxyphenanthrene, fluorene is metabolized to 2-, 3-, or 9- hydroxyfluorene, and naphthalene is metabolized to 1- or 2-hydroxynaphthalene.

[f]American Conference of Governmental Industrial Hygienists (ACGIH) benchmark value used to indicate occupational exposure to PAHs and identify workplaces that require improved industrial hygiene (ACGIH, 2005).

Table 3 Summary of **antimony** in personal air samples during live fires, wipe and urine samples taken before (i.e. Pre) and after a fire event (i.e. Post), and wipe samples taken on Personal Protective Equipment (PPE) before (i.e. Pre) and after decontamination (i.e. Post).

		Study Average (Min. – Max.)		Reference Values	
Sample Type	**Your Result**	Firefighters	Office Workers	Occupational Exposure Limit	CHMS[c]
Personal Air Sample (µg/m^3)	**N/A**	25.2 (0.02-168.8)	N/A[a]	1500[b]	N/A
Wipe Sample (ng/cm^2)					
Skin Pre		2.71 (0.28-10.32)	N/A	N/A	N/A
Skin Post	**N/A**	17.61 (3.14-91.17)	N/A	N/A	N/A
Clothing Pre		3.96 (0.17-16.74)	N/A	N/A	N/A
Clothing Post	**N/A**	5.97 (0.95-17.01)	N/A	N/A	N/A
PPE Pre		37.84 (0-94.71)	N/A	N/A	N/A
PPE Post	**N/A**	47.7 (0-119.34)	N/A	N/A	N/A
Urine Sample (µg/g creatinine)					
Pre		0.058 (0-0.31)	0.3	N/A	0.1
Post	**N/A**	0.12 (0-0.53)	N/A	N/A	0.1
PPE Decon. Wipes (ng/cm^2)					
Pre decontamination	N/A	70.05 (18.04-140.35)	N/A	N/A	N/A
Post decontamination	N/A	33.9 (23.6-46.22)	N/A	N/A	N/A

[a]N/A indicates not applicable or available.
[b]Allowable short term exposure limit (ASEL) - a limit at which workers are allowed to be exposed for a total of 30 minutes or less during a workday.
[c]Values based on data taken from the '2009-2011 Canadian Health Meausures Survey' for non-smoking males aged 25-62. Data report prepared by Statistics Canada and found on Health Canada's DAIS system. Responsibility for use and interpretation of these values is entirely that of the users.

Table 4 Summary of **cadmium** in personal air samples during live fires, wipe and urine samples taken before (i.e. Pre) and after a fire event (i.e. Post), and wipe samples taken on Personal Protective Equipment (PPE) before (i.e. Pre) and after decontamination (i.e. Post).

		Study Average (Min. – Max.)		Reference Values	
Sample Type	**Your Result**	Firefighters	Office Workers	Occupational Exposure Limit	CHMS[c]
Personal Air Sample (µg/m^3)	**N/A**	0.53 (0-6.85)	N/A[a]	30[b]	N/A
Wipe Sample (ng/cm^2)					
Skin Pre		0.25 (0-1.8)	N/A	N/A	N/A
Skin Post	**N/A**	0.30 (0-0.71)	N/A	N/A	N/A
Clothing Pre		0.4 (0-4.33)	N/A	N/A	N/A
Clothing Post	**N/A**	0.33 (0-1.32)	N/A	N/A	N/A
PPE Pre		0.42 (0-3.35)	N/A	N/A	N/A
PPE Post	**N/A**	0.59 (0-2.30)	N/A	N/A	N/A
Urine Sample (µg/g creatinine)					
Pre		0.13 (0-0.53)	0.26	5[d]	0.35
Post	**N/A**	0.11 (0-0.3)	N/A	5[d]	0.35
PPE Decon. Wipes (ng/cm^2)					
Pre decontamination	N/A	1.06 (0-8.12)	N/A	N/A	N/A
Post decontamination	N/A	0.01 (0-0.07)	N/A	N/A	N/A

[a]N/A indicates not applicable or available.
[b]Allowable short term exposure limit (ASEL) - a limit at which workers are allowed to be exposed for a total of 30 minutes or less during a workday.
[c]Values based on data taken from the '2009-2011 Canadian Health Meausures Survey' for non-smoking males aged 25-62. Data report prepared by Statistics Canada and found on Health Canada's DAIS system. Responsibility for use and interpretation of these values is entirely that of the users.
[d]Biological exposure index (BEI) – an exposure level that is associated with no adverse health effects (ACGIH, 2012)

Table 5 Summary of **lead** in personal air samples during live fires, wipe and urine samples taken before (i.e. Pre) and after a fire event (i.e. Post), and wipe samples taken on Personal Protective Equipment (PPE) before (i.e. Pre) and after decontamination (i.e. Post).

		Study Average (Min. – Max.)		Reference Values	
Sample Type	**Your Result**	Firefighters	Office Workers	Occupational Exposure Limit	CHMS[c]
Personal Air Sample (µg/m^3)	**N/A**	33.73 (0-665)	N/A[a]	150[b]	N/A
Wipe Sample (ng/cm^2)					
Skin Pre		1.41 (0-9.15)	N/A	N/A	N/A
Skin Post	**N/A**	23.59 (0-182.6)	N/A	N/A	N/A
Clothing Pre		2.68 (0-10.59)	N/A	N/A	N/A
Clothing Post	**N/A**	7.58 (0-47.3)	N/A	N/A	N/A
PPE Pre		21.15 (0-78.9)	N/A	N/A	N/A
PPE Post	**N/A**	338.93 (0-3,338)	N/A	N/A	N/A
Urine Sample (µg/g creatinine)					
Pre		0.33 (0-1.37)	0.39	N/A	0.36
Post	**N/A**	0.38 (0-1.88)	N/A	N/A	0.36
PPE Decon. Wipes (ng/cm^2)					
Pre decontamination	N/A	29.02 (2.63-86.84)	N/A	N/A	N/A
Post decontamination	N/A	0.43 (0.1-0.91)	N/A	N/A	N/A

[a]N/A indicates not applicable or available.
[b]Allowable short term exposure limit (ASEL) - a limit at which workers are allowed to be exposed for a total of 30 minutes or less during a workday.
[c]Values based on data taken from the '2009-2011 Canadian Health Meausures Survey' for non-smoking males aged 25-62. Data report prepared by Statistics Canada and found on Health Canada's DAIS system. Responsibility for use and interpretation of these values is entirely that of the users.

Table 6 Summary of **urinary biomarker** concentrations. 'Pre' indicates before a fire event (i.e., start of shift). 'Post' indicates after a fire event. N/A indicates not applicable or available. rev/µmol creatinine = revertants per micromole creatinine.

		Study Average (Min. – Max.)	
Sample Type	**Your Result**	Firefighters	Office Workers
Mutagenic Potency (rev/µmol creatinine)			
Pre		1.44 (0.19-5.76)	1.45
Post	**N/A**	2.97 (0.51-22.68)	N/A[a]
Clara Cell 16 (ng/mg creatinine)			
Pre		5.0 (0.4-17.8)	13.9
Post	**N/A**	4.2 (0.6-15.8)	N/A
8-Isoprostane (ng/mg creatinine)			
Pre		1.4 (0.2-5.9)	3.1
Post	**N/A**	1.6 (0.2-8.0)	N/A

Mutagenic potency measures the ability to induce genetic damage.
Clara Cell 16 is a marker of lung injury.
8-Isoprostane is a marker of cell damage.
rev/µmol creatinine = revertants per micromole creatinine
[a]N/A indicates not applicable or available

WILL THE MEASURED LEVELS AFFECT MY HEALTH? SHOULD I BE CONCERNED?

Everyone is exposed to potentially hazardous chemicals on a daily basis; many substances are naturally found in the environment, and exposures are unavoidable. For example, PAHs are commonly found in urban air and cooked foods, and everyone is exposed to some extent. Exposures can occur in the home, as well as during occupational and recreational activities outside the home.

With the exception of urinary cadmium, there are no guideline values to determine whether you should be concerned about your exposure. Therefore, there is not enough scientific information to determine what levels of exposure can result in harm.

If you are concerned about your results, and your exposures to the agents examined in this study, you may consult with the project's principal investigator and/or the project's occupational hygienist. In addition, you can consult your family doctor for a medical opinion regarding potential health risks. Information about the substances examined in this study and sources of additional information can be found in the appendices.

CONCLUSIONS AND RECOMMENDATIONS

- Some concentrations of PAHs and lead in personal air collected during fire events were above the occupational exposure limit set by the Ontario Ministry of Labor.
  → Firefighters properly wearing PPE (including SCBA) are not expected to be exposed to dangerous levels. However, improper use or premature removal of SCBA, and/or handling of contaminated PPE and equipment, may contribute to increased exposure.
- Concentrations of PAHs, antimony, and lead on skin and PPE after a fire were significantly elevated compared to levels on skin before a fire (i.e. background levels). Wipe samples of clothing collected after a fire had significantly elevated lead concentrations.
  → This illustrates the importance of showering and properly decontaminating your PPE and clothing after a fire.
- Levels of urinary PAH metabolites, mutagenic activity, and antimony were significantly elevated after a fire.
  → This suggests that contact with combustion emissions during fire suppression is contributing to elevated exposures to selected substances (i.e., chemicals entering the body). Observed relationships between PAHs on the skin and metabolites in the urine suggest that skin contact is an important route of exposure. This further illustrates the importance of properly showering and decontaminating after firefighting events.
- Each individual's exposure level will be determined by complex, dynamic interplay between duration at the fire suppression scene, proper use of PPE, the type of fire, and the role in fire suppression.
- Complete study results (i.e., report to the Ministry of Labour) will be available online and through the OFS.