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Firefighters' occupational exposure and health: Contribution from biomonitoring assays

Ana Sofia Rosa Amorim

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Resumo

Os bombeiros estão diariamente expostos a vários poluentes durante a sua atividade ocupacional, incluindo a matéria particulada (PM) e hidrocarbonetos aromáticos policíclicos (PAHs). A Agência Internacional de Investigação para o Cancro (IARC) classificou a atividade como bombeiro como carcinogénica para o Homem. Atualmente os estudos que caracterizam os bombeiros portugueses são limitados. Este trabalho caracteriza a exposição dos bombeiros a PAHs presentes na PM em ambiente de quartel: garagem de veículos, sala de armazenamento de equipamentos de proteção individual (PPE) e área comum e as concentrações de metabolitos de PAHs (OHPAHs) na urina de bombeiros após o combate a um fogo florestal controlado. Em ambiente de quartel, a concentração total de PM-PAHs foi de 61,248 ng/m^3, sendo a maior parte partículas finas (43,956 ng/m^3), seguida por ultrafinas (12,952 ng/m^3) e grossas (4,339 ng/m^3). O total de medianas dos PAHs na PM foi mais elevada na área comum (0,929 ng/m^3), seguida pela garagem (0,808 ng/m^3) e sala de armazenamento de PPE (0,444 ng/m^3). Os PAHs com 2-3 anéis aromáticos foram os predominantes (64,7-88,8% do total de HAPs), sendo o acenaftileno o PAH mais abundante (0,187-0,713 ng/m^3). Os PAHs carcinogénicos contribuíram para 14,6-48,3% do total de PAHs, sendo mais abundantes na sala de armazenamento de PPE. As concentrações de OHPAHs na urina aumentaram gradualmente após a participação dos bombeiros no fogo (1,2468 μmol/mol creatinina *versus* 1,6939 μmol/mol creatinina *versus* 2,5627 μmol/mol creatinina). Fumadores têm concentrações de OHPAHs mais elevadas do que não fumadores (2,3461 μmol/mol creatinina *versus* 1,6253 μmol/mol creatinina *versus* 3,0364 μmol/mol creatinina). 1-Hidroxinaftaleno+1-Hidroxiacenafteno e 2-Hidroxifluoreno foram os metabolitos mais abundantes.

Palavras-chave: Bombeiros; Exposição ocupacional; Qualidade do ar; Matéria particulada; Hidrocarbonetos aromáticos policíclicos

Abstract

Firefighters are daily exposed to various pollutants during their occupational activities, including particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs). The International Agency for Research on Cancer (IARC) has classified firefighting as carcinogenic to humans. Currently, studies characterizing Portuguese firefighters are limited. This work characterizes the exposure of firefighters to PAHs present in PM in different fire station environments: vehicle garage, personal protective equipment (PPE) storage rooms, and common areas, as well as the concentrations of PAHs metabolites (OHPAHs) in firefighters' urine after fighting a controlled forest fire. In the fire station environment, the total concentration of PM-PAHs was 61.248 ng/m^3, with most being fine particles (43.956 ng/m^3), followed by ultrafine (12.952 ng/m^3), and coarse (4.339 ng/m^3). The total median of PAHs in PM was highest in the common area (0.929 ng/m^3), followed by the garage (0.808 ng/m^3) and PPE's storage room (0.444 ng/m^3). PAHs with 2-3 aromatic rings were predominant (64.7-88.8% of total PAHs), with acenaphthylene being the most abundant PAH (0.187-0.713 ng/m^3). Carcinogenic PAHs accounted for 14.6-48.3% of the total PAHs, being most abundant in the PPE's storage room. OHPAHs concentrations in urine gradually increased after firefighters participated in firefighting (1.2468 μmol/mol creatinine *versus* 1.6939 μmol/mol creatinine *versus* 2.5627 μmol/mol creatinine). Smokers had higher OHPAHs concentrations than non-smokers (2.3461 μmol/mol creatinine versus 1.6253 μmol/mol creatinine versus 3.0364 μmol/mol creatinine). 1-Hydroxynaphthalene+1-Hydroxyacenaphthene and 2-Hydroxyfluorene were the most abundant metabolites.

Keywords: Firefighters; Occupational exposure; Air quality; Particulate matter (PM); Polycyclic aromatic hydrocarbons (PAHs)

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List of abbreviations

1OHAce – 1-Hydroxyacenaphtene

1OHNaph – 1-Hydroxynaphthalene

1OHPhe 1-Hydroxyphenanthrene

1OHPyr – 1-Hydroxypyrene

2OHFlu – 2-Hydroxyfluorene

3OHB[a]P – 3-Hydroxybenzo[a]pyrene

Ace – Acenaphthene

Acy – Acenaphthylene

Ant – Anthracene

APF – Assigned protection factor

APRs – Air-purifying respirators

B[a]A – Benz[a]anthracene

B[a]P – Benzo[a]pyrene

B[b+j]F – Benzo[b+j]fluoranthene

B[g,h,i]P – Benzo[g,h,i]perylene

B[k]F – Benzo[k]fluoranthene

BMI – Body mass index

Chry – Chrysene

CVD – Cardiovascular diseases

CYP – Cytochrome P450 oxidase system

D[a,h]A – Dibenz[a,h]anthracene

D[a,l]Pyr – Dibenzo[a,l]pyrene

Dp – Cut-off diameter

Fln – Fluoranthene

Flu – Fluorene

HBM – Human biomonitoring

HMW – High molecular weight

IARC – International Agency for Research on Cancer

Ind – Indeno[1,2,3-c,d]pyrene

LMW – Low molecular weight

LOD – Limit of detection

LOQ – Limit of quantification

Naph – Naphthalene

NIOSH – National Institute for Occupational Safety and Health

OHPAHs – Monohydroxylated polycyclic aromatic hydrocarbons

OSHA – Occupational Safety and Health Organization

PAHs – Polycyclic aromatic hydrocarbons

Phe – Phenanthrene

PM – Particulate matter

PPE – Personal protective equipment

PTSD – Post-traumatic stress disorder

Pyr – Pyrene

SCBA – Self-contained breathing apparatus

USEPA – United States Environmental Protection Agency

WHO – World Health Organization

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1. Introduction

In general, the mission of fire departments includes several important aspects including the protection of citizens' lives and property. These tasks involve firefighting, rescue of populations in fires, floods, building collapses, and in any other kind of accidents. Fire departments are involved in search and rescue operations, which includes salvage of shipwrecks and other underwater operations. Firefighters reply to emergencies that involve transportation of sick and injured persons, providing pre-hospital emergency care to the civil population. Fire departments are also involved in civil protection functions that include training and information campaigns, focusing on the prevention of fires and accidents among populations (Decreto-Lei n.o 247/2007, 2007).

Firefighters are a group of workers that are regularly exposed to various types of risks due to their work environment, encountered through the different work tasks. Firefighters are exposed to fire emissions released during firefighting activities of structure, wildland, and vehicle fires as well as exposure to risks related to other non-fire events, including medical emergencies and pre-hospital medical care, handling hazardous materials, toxic material spills, and building collapses. The occupation as a firefighter exposes these subjects to different biological, chemical, physical (such as extreme temperatures, reduced humidity, and noise), and psychological (work at critical and traumatic events) (Figure 1).

Figure 1. Types of risks associated with firefighting: a) example of biological risk, b) example of chemical risk, c) example of physical risk and d) example of psychological risk.

Credits: Cláudia Esteves and Daniela Mendes (Bombeiros Voluntários de Barcelos).

1.1. Firefighters' occupational health

Firefighters often experience stressing experiences in the line of duty, such as witnessing the death of an adult or child, people with severe injuries, and attending to burned victims, which may disturb the psychological well-being and impact their mental health (Regambal et al., 2015). These types of stimuli can lead to the development of several mental disorders, such as sleep deprivation, negatively impacting the cognitive ability of firefighters as well as their physical and psychological health (Frost et al., 2021). Depression and post-traumatic stress disorder (PTSD) are identified as the leading mental health diseases that affect firefighters, which symptoms are likely aggravated when exposed to other experiences, raising a high possibility of substance abuse. In a study with 735 active and retired full-time firefighters from Australia, 13% showed PTSD symptoms and 11% demonstrated depression signs on moderate levels, with 6% reporting heavy drinking and 24% involved in unsafe drinking (Harvey et al., 2016). Another study reunited data from 1027 volunteers and full-time firefighters, including retired firefighters, and concluded that suicide thoughts rates and behaviors throughout firefighters' careers are much higher than in the general population, with 46.8% of firefighters reporting suicide ideation when compared to 5.6-14.3% of the general population. It was specified that 15.5% of the firefighters attempted suicide at least one time (Stanley et al., 2015).

Different authors reported a high incidence of obesity among firefighters, a condition associated with increasing incidence of cardiovascular diseases (CVD) (Kales et al., 2007; Soteriades et al., 2005). CVD is a cause of death among firefighters, mostly during fire combat due to the higher level of physical demand (Jeung et al., 2022).

PPE also compromises individuals' water intake due to the possibility of dehydration during high-stress conditions and increases in the cardiovascular strain. There is also a higher risk of CVD with exposure to pollution and environmental conditions related to firefighting (Smith et al., 2013).

The grade of evidence associated with the occupational activity as a firefighter is classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) since sufficient evidence for the development of cancer was demonstrated (IARC, 2023).

The occupational activity as a firefighter is genotoxic, induces oxidative stress, epigenetic alterations, chronic inflammation, and modulation of receptor-mediated effects, showing

typical traces of carcinogenic activity (IARC, 2016). This evidence was supported by different studies performed with firefighters including those that include firefighting on various types of fires (wildland, structure and controlled fires) (IARC, 2023).

Occupational activity as a firefighter causes mesothelioma and bladder cancer, with increased incidence rates when compared to the general population (Daniels et al., 2014; DeBono et al., 2023). However, the causes for these types of cancer might be associated with firefighters' exposure to different known/probable/possible carcinogenic agents, including asbestos, polycyclic aromatic hydrocarbons (PAHs), ammonia, carbon monoxide, nitrogen oxides, formaldehyde, metals, engine exhausts (diesel and gasoline), radioactivity, ultraviolet radiation, viruses and many others (IARC, 2023).

The development of prostate, colon, and testicular cancers, as well as melanoma and non-Hodgkin lymphoma, are positively associated with occupational exposure of firefighters. Although the IARC working group found limited evidence to conclude this relation, studies in firefighters attested an increased incidence of these types of cancer in firefighters (DeBono et al., 2023; Harrison et al., 2022; IARC, 2023; LeMasters et al., 2006).

Firefighters identify the risk of cancer associated with their profession into two categories, direct factor and indirect factors. The direct factors resulting from occupational exposure during work, which include exposure to emissions and particles inhalation, contact with contaminated PPE that may not be adequately cleaned in-between calls, exhaust from vehicles, contact with hazardous substances, toxic spills, contaminated patients in medical emergencies, or any harmful materials or situations that firefighters need to physically handle. The indirect factors englobe the type of diet, the work-related stress, and the sleep disruptions during night shifts, among other factors (Anderson et al., 2017).

1.2. Firefighters´ occupational exposure

During fires, a heterogeneity mixture of gaseous and particulate pollutants is released, including particulate matter (PM), carbon monoxide, volatile and semi-volatile organic compounds, PAHs, among others (IARC, 1984; Teixeira et al., 2024). The emissions from wildland fires depend on their duration, which may vary from some hours up to consecutive days or even weeks, and the fuel nature and quantity. The weather conditions, particularly temperature, relative humidity, and wind intensity/direction might also affect the intensity, progression, and composition of fire emissions (Ferreira De Castro et al., 2006; Teixeira et

al., 2024). Nonetheless, the contact with fire emissions also occurs inside fire stations due to the direct contact with vehicles and machinery used in firefighting activities (Oliveira, et al., 2017; Sparer et al., 2017).

In non-fire occasions, firefighters are also exposed to other chemical hazards such as asbestos, firefighting foams containing perfluorinated and polyfluorinated substances, diesel fuel fumes and other toxic materials (IARC, 2023). Breathing apparatus protection index is measured in an assigned protection factor (APF) that is the workplace level of respirator protection. During structure and vehicle fires, firefighters wear a self-contained breathing apparatus (SCBA) that has an APF of 10,000, making it the safest respirator type. Although SCBA is employed in structure and vehicle fires, in wildland fires it is not often used as wildland fires sometimes can last several days, making SCBA not as suitable, since it is heavy, bulky and can reduce firefighters' movements. During wildfires, firefighters either use bandanas, N95 masks or air-purifying respirators (APRs), which have an APF between 10 and 1,000, meaning that these types of respirators do not have as much protection, making wildland fires an important source of fire-related pollutants (*e.g.*, PM and PAHs) in the occupational exposure of firefighters (NUSTL, 2022; OSHA, 2017).

Figure 2. Firefighter during forest fire in Portugal.

Credits: Cláudia Esteves (Bombeiros Voluntários de Barcelos).

1.2.1. Particulate Matter

PM is a complex and heterogeneous mixture of particles suspended in the air that vary in composition, size, and origin (IARC, 2023; Lawal, 2017; Ravindra et al., 2008). PM is

classified according to its aerodynamic diameter as coarse (PM > 2.5 μm of aerodynamic diameter), fine (PM ≤ 2.5 μm) and ultrafine (PM ≤ 0.1 μm) particles. PM is generated indoors by various sources such as cooking, cleaning, combustion activities (*e.g.* use of fireplaces and burning candles), mold and pests as well as outdoors that includes industrial emissions, car exhaust, and fires (USEPA, 2024c, 2024e).

According to IARC, outdoor PM is carcinogenic to humans (Group 1) (IARC, 2023). Exposure to PM can harm human health by promoting CVD, hypertension, cancer, obesity, diabetes, and overall mortality (Addis Alemayehu et al., 2020; Teixeira, Oliveira, et al., 2023). Different authors suggest a positive relation between short-term exposure to wildfire-specific PM and respiratory morbidity, especially for fine particles ($PM_{2.5}$), establishing that wildfire smoke negatively affects respiratory health, suggesting that $PM_{2.5}$ from wildfires might cause more impact on human health when compared to ambient $PM_{2.5}$ (Jiao et al., 2024; Xu et al., 2023).

Firefighters frequently spend a lot of time at fire stations. The concentrations of PM found inside fire stations depend on the station layout, the effectiveness of cleaning routines, and the maintenance practices, meaning that PM levels concentration vary, and even increase, through various sources such as tools, vehicle emissions, and PPE contaminated with particles from fires. PM can be transported throughout the fire station by firefighters. Consequently, PM including particles originated from fires, remains in the fire station environment, leading to prolonged exposure periods (Teixeira, Oliveira, et al., 2023).

The World Health Organization (WHO) updated the ambient air guidelines for PM_{10} and $PM_{2.5}$ for short- (45 and 15 $\mu g/m^3$, respectively) and long-term exposure (15 and 5 $\mu g/m^3$, respectively) (WHO, 2021). The Occupational Safety and Health Organization (OSHA) defined an occupational exposure limit of 5.0 mg/m^3 for respirable dust (OSHA, 2009).

1.2.2. Polycyclic aromatic hydrocarbons

PAHs are a large group of semi-volatile and volatile organic compounds consisting of carbon and hydrogen, arranged in a fused ring structure containing more than one benzene ring in their molecular structure (Lawal, 2017; Ravindra et al., 2008). United States Environmental Protection Agency (USEPA) has labelled 16 PAHs as priority pollutants (USEPA, 2014) (Figure 3). PAHs are produced during the incomplete combustion of organic matter, principally at high temperatures and under oxygen-deficient conditions, either by

biological processes or by combustion sources. PAHs are originated from both natural (*e.g.*, volcanic activity and fires) and anthropogenic (*e.g.*, industrial fumes and vehicle exhaust) sources (Abdel-Shafy & Mansour, 2016; Ravindra et al., 2008). The human exposure to PAHs can occur through several pathways, being inhalation an important route with sources such as cigarette smoke, industrial emissions, automobile exhaust, hazardous waste, tobacco smoke, and fires (Lawal, 2017). Moreover, human exposure by the dermal contact assumes an important route in occupational settings (Abdel-Shafy & Mansour, 2016; Lawal, 2017).

PAHs can be classified in low molecular weight (LMW), which contain 2 or 3 benzenic rings in their structure (such as naphthalene (Naph), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene (Phe), and anthracene (Ant)), and high molecular weight (HMW), with 4 to 6 rings (including fluoranthene (Fln), pyrene (Pyr), benz[a]anthracene (B[a]A), chrysene (Chry), benzo[b+j]fluoranthene (B[b+j]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), dibenzo[a,l]pyrene (D[a,l]Pyr), dibenzo[a,h]anthracene (D[a,h]A), benzo[g,h,i]perylene (B[g,h,i]P), and indeno[1,2,3-c,d]pyrene (Ind)). These compounds are graded in different subgroups by IARC, according to their carcinogenic properties. B[a]P (5 rings) is the only compound classified as carcinogenic to humans (Group 1), while other PAHs were classified as probable carcinogens (Group 2A), including D[a,h]A and D[a,l]Pyr, and as possible carcinogenic (Group 2B), such as Ant, Naph, B[a]A, Chry, Ind and benzofluoranthene isomers. Ace, Flu, Phe, Fln, Pyr, and B[g,h,i]P are not classified as carcinogenic to humans (Group 3) (IARC, 2010).

After exposure, PAHs undergo metabolization in the human body through bioactivation mediated by the cytochrome P450 oxidase system (CYP), which is associated with oxidation and hydroxylation in the liver. PAHs are converted into unstable and reactive metabolites by CYP, with the assistance of epoxide hydrolase, being CYP1 family responsible for the metabolic activation of these compounds (Shimada, 2006; Shimada & Fujii-Kuriyama, 2004; von Koschembahr et al., 2020). PAHs are known for their potential to cause cancer, mainly because of these reactive intermediates that make adducts with DNA (Kamal et al., 2015). This DNA-binding capability is responsible for the carcinogenic effects that cause mutations. Additionally, PAHs exhibit genotoxic, teratogenic, and mutagenic mechanisms that raise cancer susceptibility (CCME, 2010; Silva Junior et al., 2021).

The National Institute for Occupational Safety and Health (NIOSH) defined 100 µg/m^3 has the limit for occupational exposure for PAHs, and OSHA has established the limit of 200 µg/m^3 (NIOSH, 2019; OSHA, 2009).

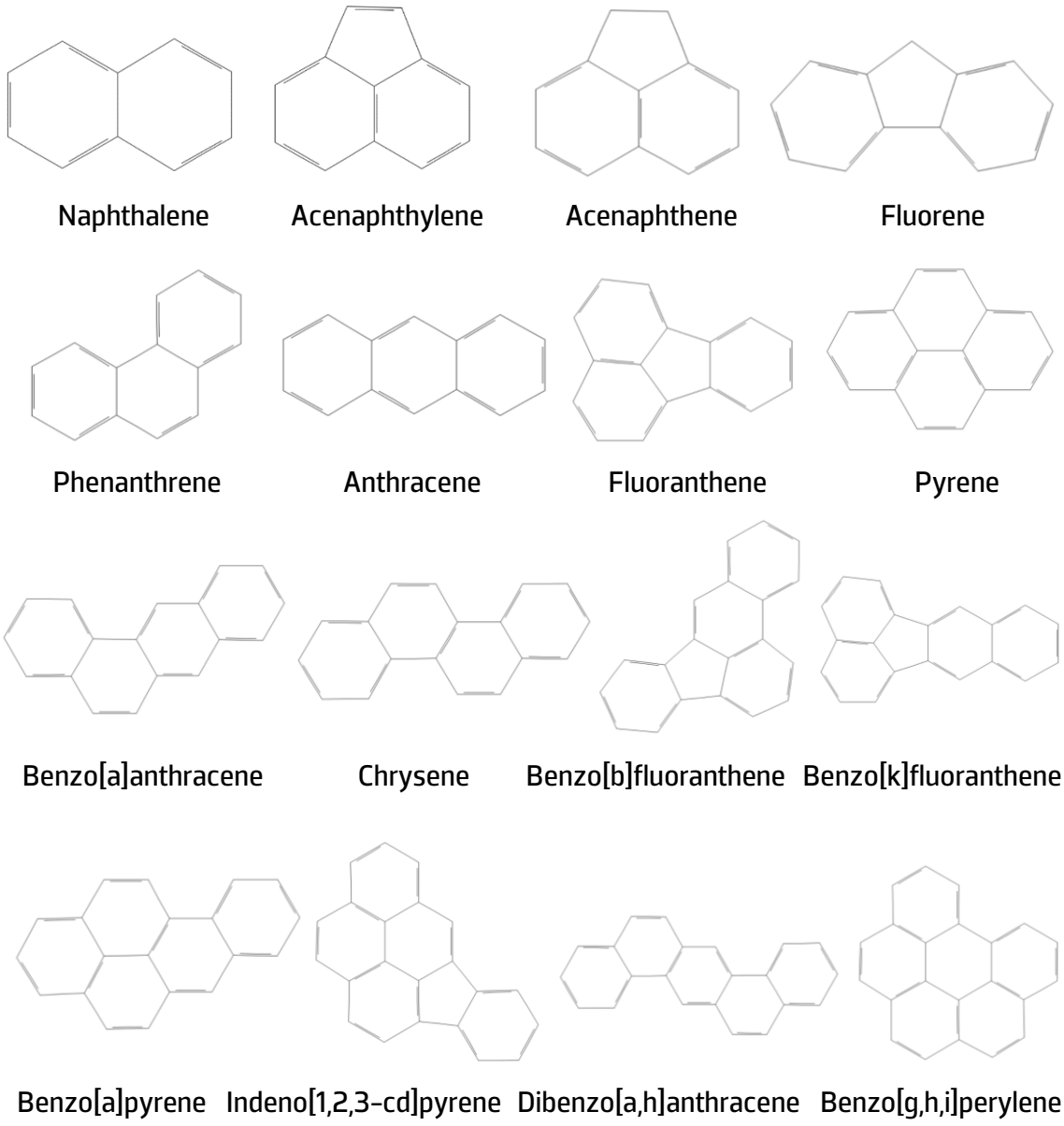


Figure 3. Structure of PAHs on the USEPA priority list (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo [k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene and Benzo[g,h,i]perylene).

1.3. Biomonitoring studies

Human biomonitoring (HBM) is a surveillance tool to determine biomarkers in biological samples including fluids and tissues to determine short- and/or long-term exposures and changes in biological markers (Zare Jeddi et al., 2022). HBM allows the identification of environmental/occupational risks, including in vulnerable groups, and with the objective to identify changes in biomarkers and the prevention of adverse health effects (Angerer et al., 2007; Ladeira & Viegas, 2016).

HBM biomarkers are categorized into three distinct types: biomarkers of exposure, which represent a change in biomolecules and/or processes of the system due to a recent exposure to xenobiotic agents in the organism (biological markers); biomarkers of effect, which represents changes in biomolecules and/or biological response systems related to the functional capacity of the biological system that is altered on the presence of an impairment or a disease early-stage; biomarkers of susceptibility, which is an indication of the susceptibility of a person and its biological system to develop a specific disease.

As firefighters are exposed to PAHs, their exposure can be assessed through biomonitoring of biological samples. Unmetabolized PAHs can be detected in blood and saliva, while metabolized PAHs are primarily excreted through urine. These can serve as biomarkers of exposure, facilitating a comprehensive assessment of the internalized dose of PAHs during various firefighting tasks, as PAHs generally show higher concentrations following exposure. Additionally, other biomarkers can be utilized to measure firefighters' exposure to different fire scenarios. These biomarkers can be detected not only in blood, urine, and saliva but also in exhaled breath (Barros et al., 2023). Biomarkers of effect can be assessed in various biological samples such as blood, urine, saliva, exhaled breath condensate and sputum/bronchoalveolar lavage fluid. These allow for the analysis of health impacts of occupational exposure of firefighters such as oxidative stress, protein damage, epigenetic changes, stress hormone levels, inflammation, lung injuries, vascular injuries, and liver function (Barros et al., 2021).

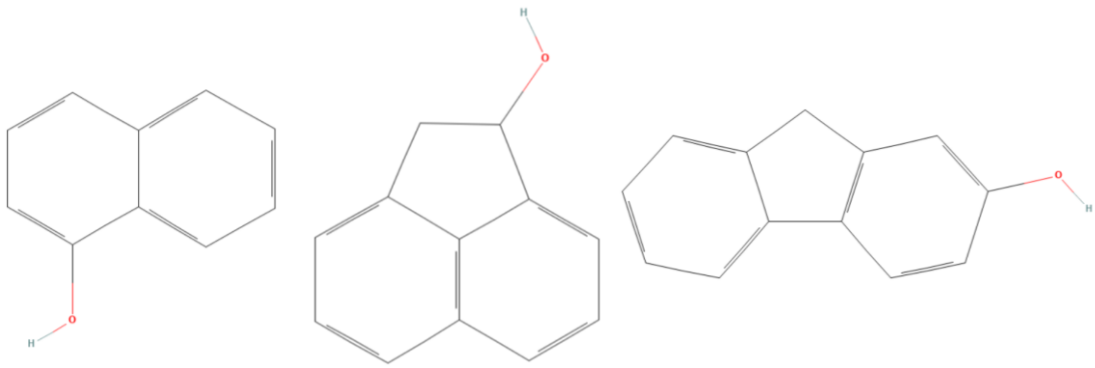
1.3.1. Urinary monohydroxylated PAHs

After being absorbed by the human organism and bioactivated by CYP, some reactive intermediates are biologically transformed into PAH metabolites and eliminated after conjunction with water-soluble groups, while others can reach biomolecules and cells.

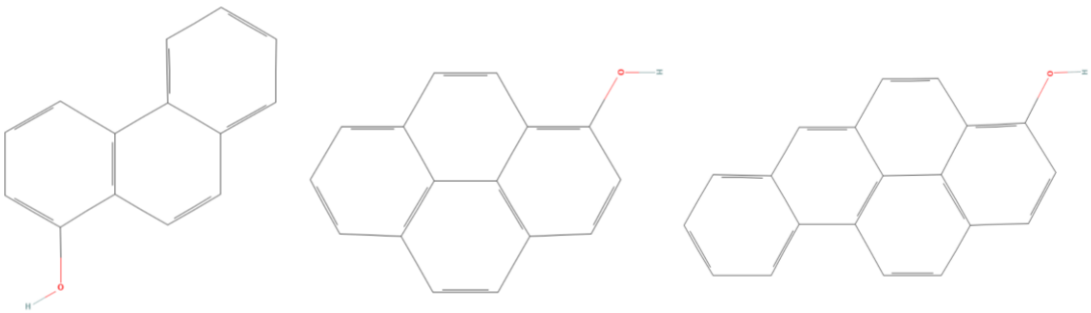
These non-eliminated reactive intermediates can bind to DNA, forming stable DNA adducts, increasing the toxicity in the body and the cancer risk (Kamal et al., 2015; von Koschembahr et al., 2020).

Excreted PAH metabolites are frequently used as biomarkers of exposure to PAHs and can be accessed via biological fluids such as urine, blood, and fecal matter, providing the total exposure to PAHs (Abdel-Shafy & Mansour, 2016; Hwang et al., 2022).

Biomonitoring monohydroxyl-PAHs (OHPAHs) indicate the total internal concentration of PAHs. The most frequently characterized OHPAHs biomarkers are 1-hydroxynaphthalene (1OHNaph), the metabolite of the parent compound Naph, 1-hydroxyacenaphtene (1OHAce) from Ace, 2-hydroxyfluorene (2OHFlu) from Flu, 1-hydroxyphenanthrene (1OHPhe) from Phe, 1-hydroxypyrene (1OHPyr) from Pyr, and 3-hydroxybenzo[a]pyrene (3OHB[a]P) from B[a]P (IARC, 2023; Louro et al., 2022) (Figure 4). Since B[a]P is classified as carcinogenic to humans, its urinary metabolite is used as a biomarker of exposure to carcinogenic PAHs (Louro et al., 2022; Oliveira M et al., 2015).



1-Hydroxynaphthalene 1-Hydroxyacenaphthene 2-Hydroxyfluorene



1-Hydroxyphenanthrene 1-Hydroxypyrene 3-Hydroxybenzo(a)pyrene

Figure 4. Structure of monohydroxylated PAHs used in biomonitoring studies (1-Hydroxynaphthalene, 1-Hydroxyacenaphthene, 2-Hydroxyfluorene, 1-Hydroxyphenanthrene, 1-Hydroxypyrene and 3-Hydroxybenzo(a)pyrene).

2. Objetives

The main objective of this study is to evaluate the firefighters' occupational exposure to PAHs while working at fire stations and during firefighting activities.

The specific objectives of this work are:

1) To assess the exposure to coarse, fine and ultrafine PM and its composition in PAHs via inhalation inside the fire station and during a controlled forest fire;

2) To evaluate the total exposure to PAHs through determination of urinary monohydroxylated PAHs during regular working activities at the fire station and during firefighting activities;

3) To compare the firefighters' occupational exposure with available guidelines and recommendations as well as with related literature.

3. Materials and methods

3.1. Population study

A total of 15 healthy male firefighters from two fire stations serving the cities of Marco de Canaveses (FS1) and Amarante (FS2), district of Porto (North of Portugal) were enrolled in this study. All participants signed the informed consent forms previously approved by the Ethic Committee of the University of Porto (Report 105/CEUP/2020). A questionnaire was used to collect the sociodemographic and professional information of each participant, including age, sex, heigh, weight, diagnostic of chronic diseases, use of regular medication, smoking habits, years of service as firefighter and recent participation in fire events.

3.2. Sampling campaigns

3.2.1. Collection of air samples

Air sampling was conducted in the winter season at the fire stations. Samples were collected on regular working days from several specific locations within the fire station, including the PPE's storage room (where firefighting equipment is kept on clothing hanger) that is next to the garage without physical separation; the garage, where fire trucks are parked and various types of cleaning and firefighting materials are stored; and the common area that connects other areas of the fire station and that is physically separated from the PPE's storage room, the garage, and the outside by a wooden door. Table 1 characterizes the sampled spaces inside FS1 and FS2.

Table 1. Characterization of sampled microenvironments inside fire stations.

Location of fire stations	– City center on a road with high daily road; – Proximity to a commercial area.
PPE's storage room	– Adjacent room without physical separation with the garage; – PPE at clothes hanger; – Wood floor and cement walls.
Garage	– Open on one side; – No physical separation between garage and headquarters; – Ten heavy vehicles; – Cement floor and walls.
Common area	– Physical separation from the garage and PPE's storage room with a closed wood door; – Physical separation from the outside with a closed wood door; – Tiles floor and cement walls.

Air samples were collected with a Dekati Low-Pressure Impactor (DLPI+, Dekati®, Finland), coupled with a vacuum pump (Leybold, Sogevac, Germany) working at a constant flow rate of 9.96 L/min. The impactor collects 14 fractions of PM with a specific cut-off diameter (Dp), separating the fractions in different stages, ultrafine particles (stage 1 [0.015 μm], stage 2 [0.031μm], stage 3 [0.054 μm], stage 4 [0.095 μm]), fine particles (stage 5 [0.156 μm], stage 6 [0.256 μm], stage 7 [0.382 μm], stage 8 [0.603 μm], stage 9 [0.947 μm], stage

10 [1.63 μm], stage 11 [2.47 μm], and coarse particles (stage 12 [3.65 μm], stage 13 [5.36 μm] and stage 14 [9.88 μm]).

The equipment was positioned at 1.5 m above ground level to simulate the regular breathing zone of firefighters and avoiding interference from windows and doors. Air samples were collected on the surface of aluminum filters (Ø25 mm; Dekati®, Finland), previously weighted at controlled temperature and humidity, and coated with a grease substrate (Apiezon®-L, Sigma-Aldrich) to avoid loss of particles. The collected filters were conditioned for 24h at controlled temperature and moisture, weighted, and stored in a freezer at -20 °C before chemical analysis.

3.2.2. Collection of urine samples

Firefighters were exposed to controlled forest fires. Controlled fires are often conducted to reduce forest fuel and create firebreaks, preventing the spread of wildfires and protecting nearby communities. As a precaution, two controlled fires were organized by local authorities, and firefighters ignited dried vegetation with a gasoline-diesel mixture during the cold season in Marco de Canaveses and Amarante. These controlled fires burned about 2000 m², lasting 6 hours in Marco de Canaveses and 7 hours in Amarante. Spot urine samples from the firefighters that participated in the controlled fires were collected in sterilized polycarbonate containers before exposure (t=0), immediately after exposure (t=1), the morning after, and 24 hours after exposure (t=2). After collecting, all samples were stored in a -20 °C freezer until analysis.

3.3. Extraction methods

3.3.1. Extraction of PAHs

To extract PAHs from air samples, each filter was dissolved with 10 mL of acetonitrile in an ultrasonic bath (Sonorex Digital 10, Bandelin, Germany) for 20 minutes, at room temperature (Figure 5).

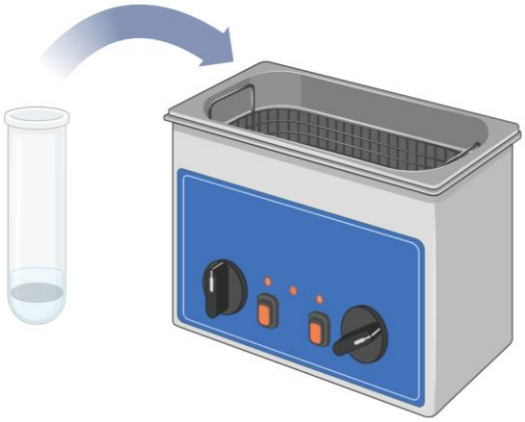


Figure 5. Representation of extraction of PAHs from filters on ultrasonic bath.

Extracts were evaporated at room temperature in a rotatory evaporator (Rotavapor R-200, Buchi Labortechinik AG, Switzerland) until dry. It was used 300 μL of acetonitrile to reconstitute extracts, filtering with a polytetrafluoroethylene filter, before chromatographic analysis. The extracted samples were stored at -20 °C.

3.3.2. Extraction of monohydroxyl-PAHs

Extraction of urinary OHPAHs method was adapted from (Oliveira et al., 2016). An aliquot of 10 mL of urine sample was buffered at pH 5.0 with 20 mL of acetate buffer, purged under nitrogen (30 minutes) and incubated at 37 °C for 120 minutes, with 80 μL of β-glucuronidase/arylsulfatase from *Helix pomatia* (EC 3.2.1.31/EC3.1.6.1; 5.5/2.6 U/ml) purchased from Roche Diagnostics (Indianapolis, USA) and 150 μL of tert-Butylhydroquinone (20 g/L; EC 2177522; ≥98%) from Fluka® Analytical, Sigma-Aldrich Chemie GmbH (Germany). The hydrolyzed sampled urine mixtures were extracted with a Sep-Pak® Light Plus C18 (Waters; Sigma-Aldrich, Steinheim, Germany), which was preconditioned with 5.0 mL of methanol and 10.0 mL of ultra-pure water. After elution of the hydrolyzed sample, cartridges were sequentially cleaned with 10.0 mL of water, and 10.0 mL of methanol/water (20:80; *v/v*). The C18 cartridges were completely dried under nitrogen flow and eluted with 20.0 mL of methanol/ethyl acetate (10:90; *v/v*). Solid phase extraction of the urine mixture is represented in Figure 6.

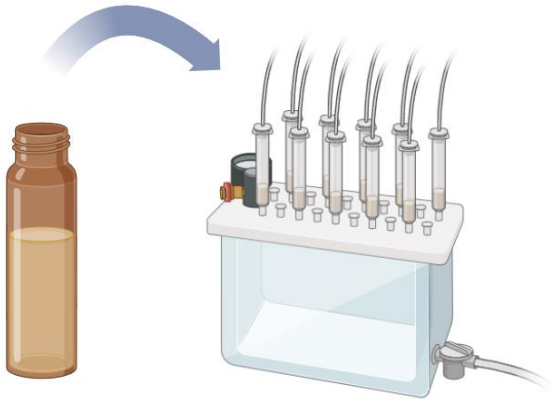


Figure 6. Representation of solid phase extraction of the urine mixture.

Extracts were evaporated until dry at room temperature in a rotatory evaporator (Rotavapor R-200, Buchi Labortechinik AG, Switzerland) and redissolved with 500 μL of methanol. Samples were frozen (-20 °C) until chromatographic analysis.

3.4. Chromatographic analysis

3.4.1. Quantification of PAHs

Extracts were analyzed using the methodology described by Castro *et al.* (Castro et al., 2009). Briefly, a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump, DGU-20AS degasser and photodiode array SPD M20A (PAD) and fluorescence RF-10AXL (FLD) detectors was employed. The compound separation was performed in a C18 column (CC 150/4 Nucleosil 100-5C18 PAH, 150 x 4.0 mm; 5 μm particle size; Macherey-Nagel, Duren, Germany) maintaining at a temperature of 25 ± 1 °C. The mobile phase was a mixture of water and acetonitrile, which followed gradient conditions, starting at 50% ultra-pure water and 50% acetonitrile, with a of linear increase up to 100% acetonitrile in 15 minutes, with a final hold of 13 minutes. The initial conditions were reached in 1 minute and maintained for 6 minutes before the next run. The total run time was 40 minutes with a flow rate of 0.8 mL/min. The limit of detection (LOD) and the limit of quantification (LOQ), defined as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, ranged, respectively, 0.07 (B[a]A) to 37.2 (Ace) and 0.22 (B[a]A) to 124.1 (Ace). Calibration was daily checked with standards. All extracts were analyzed in triplicate.

3.4.2. Quantification of monohydroxyl-PAHs

Extracts were analyzed following the chromatographic methodology described by Oliveira *et al.* (Oliveira et al., 2016) using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump, DGU-20AS degasser, and photodiode array SPD-M20A and fluorescence RF-10AXL detector. The separation of PAH metabolites was performed in a C18 column (CC 150/4 Nucleosil 100-5C18 PAH, 150 x 4.0 mm; 5 μm particle size; Macherey-Nagel, Duren, Germany) maintaining at a temperature of 25 ± 1 °C. The mobile phase was a mixture of water and methanol, which followed gradient conditions, starting at 50% ultra-pure water and 50% acetonitrile, with a 3-minute linear increase up to 70% methanol and 30% ultra-pure water, holding those conditions for 7 minutes, then a linear gradient to 100% of methanol in 6 minutes, with a hold of 5 minutes. The total run time was 30 minutes with a flow rate of 1.0 mL/min. Compounds were detected at optimum excitation/emission wavelength pair for each metabolite: 232/337 nm (1OHNaph and 1OHAce), 265/335 nm (2OHFlu), 263/363 nm (1OHPhen), 242/388 nm (1OHPy), and 308/432 nm (3OHB[a]P). Calibrations were performed with OHPAH mixed standards in methanol, except for 1OHNaph and 1OHAce that were quantified with a matrix-matched calibration curve because of some matrix effects, based on at least 6 calibration points. Elution of 1OHNaph and 1OHAce have the same retention times and same mobile phase composition (70:30 *v/v* of methanol/water) for the optimum excitation/emission wavelength pair, so these compounds were co-eluted and quantified together.

The minimum detectable amount of analyte LOD and LOQ, with a signal-to-noise ratio of 3:1 and 10:1, ranged, respectively, 0.02 (3OHB[a]P) to 6.97 (1OHNaph+1OHAce) and 0.07 (3OHB[a]P) to 23.2 (1OHNaph+1OHAce). The concentrations of OHPAHs were normalized with the urinary creatinine levels (mol/mol), measured by the Jaffe colorimetric method (Kanagasabapathy & Kumari, 2000). Creatinine is excreted in the urine at a constant rate and has been used to minimize the variability caused by daily water intake and regular practice of physical exercise. For more accurate comparisons, only normalized OHPAH concentrations were utilized, presented in μmol/mol creatinine.

Blanks and standards were prepared and scanned daily, and all determinations were performed in triplicate.

3.5. Statistical analysis

Statistical analysis was performed using Microsoft Office Excel (v. 16.0 Microsoft Corporation, EUA), and SPSS (version 28.0, Armonk, NY, USA) statistical software. When the concentration of a PAH or OHPAH was below its LOD, the concentration value was replaced by LOD/$\sqrt{2}$ (Hornung & Reed, 1990). When applicable, the chromatographic areas of the compounds detected above the LOD were subtracted from the respective areas of the analyzed samples. The total concentrations of PAHs in the air samples were calculated as the sum of the median value for each PAH in the acquired stages (stage 1 to stage 14) *per* location. The total concentrations of urinary OHPAHs were calculated as the sum of the value for each metabolite before (t=0), immediately after (t=1), and in the next morning (t=2) after the active participation in a controlled.

4. Results and discussion

4.1. Firefighters' characterization

Table 2 summarizes the firefighters' characterization that participated in this study. All participants were male, with ages ranging between 28 and 50 years old. Body mass index (BMI) is a measure of body fat in relation to heigh and weight of an individual. BMI values ranged between 22.1 and 35.6 kg/m^2, with 60% of firefighters falling in the healthy weight range (18.5 kg/m^2 < BMI < 24.9 kg/m^2), 33% with BMI values within the overweight range (25.0 kg/m^2 < BMI < 29.9 kg/m^2), and 7%, representing one participant, whose BMI value corresponds to the obese range (BMI > 30.0 kg/m^2) (CDC, 2024). Firefighters with BMI values above the healthy range are at risk, given the link between obesity and several health problems (WHO, 2024). Firefighters included in this study work full-time, with years of service ranging between 3 years and 31 years and a median of 14 years working as a firefighter in FS1 and 12.8 years in FS2.

Smoking habits were also analyzed and 53% of the sampled firefighters are smokers. Smoking is related to various health diseases, such as cardiovascular and pulmonary diseases, as well as cancer (Varghese & Muntode Gharde, 2023). Firefighters reported not being involved in any firefighting activities within the week before exposure to the controlled fires and sample collection. During sample collection, firefighters from FS1 spent an average of 3.5 hours firefighting, with this time varying from 2.5 hours to 6.0 hours, and

all firefighters from FS2 spent 7.0 hours firefighting. PAHs are common pollutants, so firefighters filled in a questionnaire concerning possible exposures prior to the study, in which it was not reported any exposures outside occupational exposure beyond smoking. Furthermore, possible exposures related to food consumption were not considered, assuming firefighters had similar dietary habits.

Table 2. Characteristics of firefighters that participated in the study.

	FS1	FS2
n	7	8
Gender (%)	Male (100)	Male (100)
Age (median; min – max; years)	39 (31 – 45)	34 (28 – 50)
Body mass index (median; min – max; kg/m^2)	24.8 (23.1 – 35.6)	24.1 (22.1 – 29.4)
Employment duration (median; min – max; years)	14.0 (7.3 – 26.1)	12.8 (3.0 – 31.0)
Smokers (%)	57.1	50
Non-smokers (%)	42.9	50
Time dedicated to firefighting within the week before sample collection (hours)	0	0
Time dedicated to firefighting during sample collection (median; min – max; hours)	3.5 (2.5 – 6.0)	7.0 (7.0 – 7.0)

n – number of samples

4.2. Exposure to PM-bound PAHs

Acquired levels of PM-bound PAHs in the different microenvironments inside the fire stations, including PPE's storage room, garage and common area are summarized in Figure 7. All participating firefighters were enrolled in a controlled forest fire.

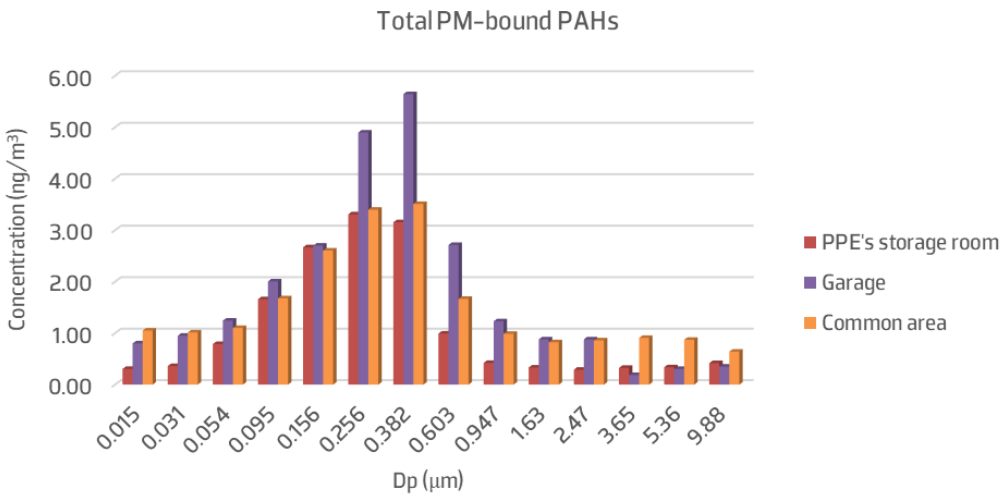


Figure 7. Concentrations (median and range; ng/m³) of PM-bound total PAHs for each collected PM fraction (Dp, µm) in the sampled area of the fire station.

Levels of PM-bound total PAHs were determined through the sum of concentrations obtained for each PM fraction and grouped according to the Dp into ultrafine (0.015 < PM < 0.095 µm), fine (0.156 < PM < 2.47 µm), and coarse (3.65 < PM < 9.88 µm) PM. Higher levels of PAHs were found in the garage, with concentrations of total PM-bound PAHs of 24.781 ng/m³, followed by the common area and the PPE's storage room, corresponding, respectively, to 21.112 ng/m³ and 15.345 ng/m³. As expected, the garage had higher concentrations of PM-bound PAHs, which can be attributed to sources such as fire vehicles exhaust as well as the tools used during firefighting that can retain the fire emissions. These are sustained in the air by the entrance and departure of fire and medical vehicles in the garage of fire stations as well as the movement of firefighters through the fire station while performing routine tasks (*e.g.*, warm up the meals), contributing to some variation on the particles' concentration throughout the day. The similar values found in total PM-bound PAHs inside garages and PPE's storage room is probably due to the absence of physical separation (*e.g.*, a closed door) between these two microenvironments, which allow particles to dissipate to the adjacent divisions. Although the existence of a physical separation from the common area to other locations (for instance a wooden door to the outside) prevents the entrance of outdoor pollution, the fire stations are situated in a highly traffic area with its main entrance door permanently open.

The profile distribution of the PM-bound PAHs, according to the Dp, in each microenvironment is represented in Figure 8.

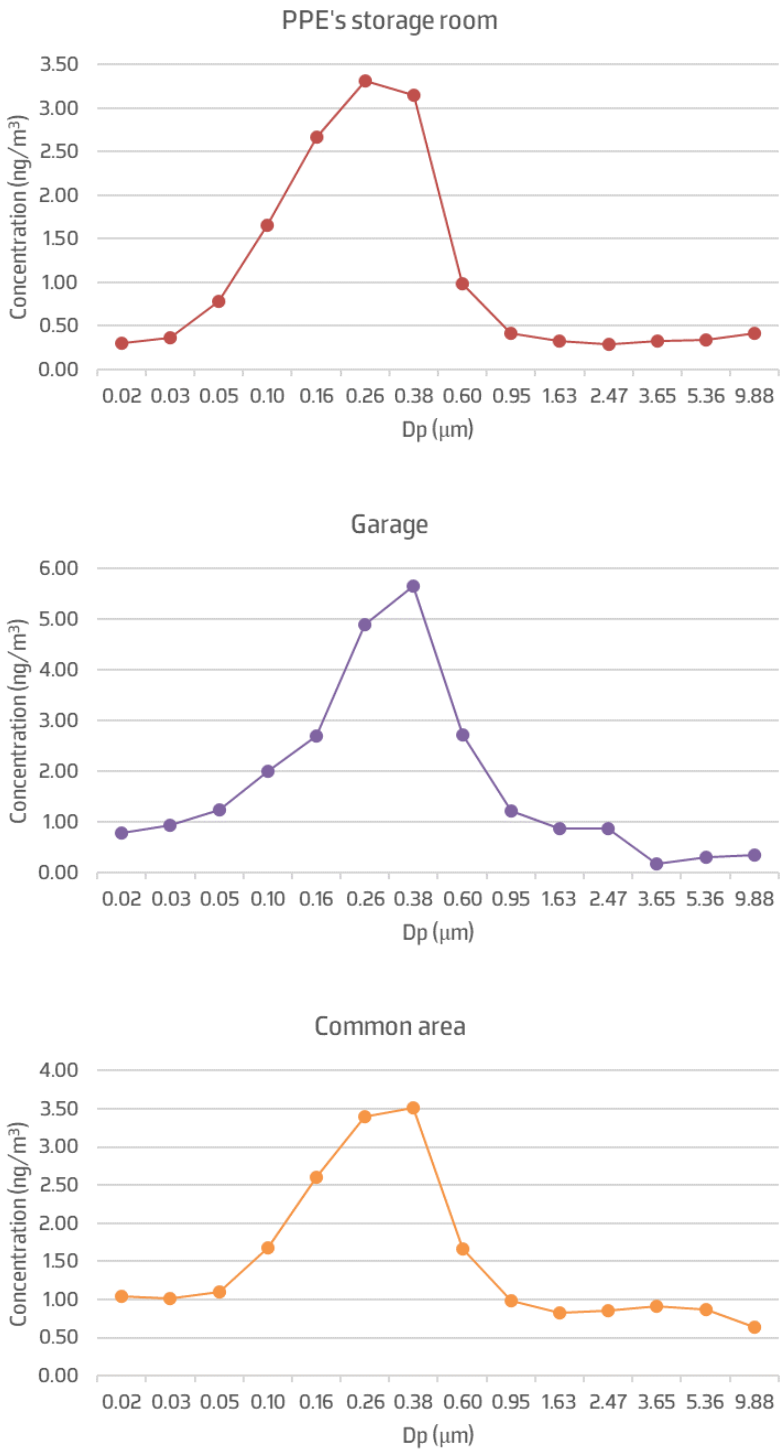


Figure 8. Profile distribution of total PM-bound PAH concentrations according to the specific stage cut-off diameter (Dp) in each area sampled at the fire station.

The concentration of PAHs in ultrafine PM ranged from 0.306 to 2.006 ng/m³, in fine PM varied between 0.290 and 5.642 ng/m³, while in coarse PM ranged from 0.185 to 0.907 ng/m³, representing a mean of 21.1%, 71.8%, and 7.1% of the total levels, respectively.

Fine PM comprised 72.7% (11.163 ng/m^3) in the PPE storage room, 76.4% (18.842 ng/m^3) in the garage, and 65.6% (13.851 ng/m^3) in the common area. This was followed by ultrafine PM, which accounted for 20.3% (3.112 ng/m^3), 20.2% (4.999 ng/m^3), and 22.9% (4.842 ng/m^3) in the respective locations. Coarse PM made up 7.0% (1.079 ng/m^3) in the PPE storage room, 3.4% (0.840 ng/m^3) in the garage, and 11.5% (2.420 ng/m^3) in the common area. These results are in line with other studies that also reported more elevated levels of ultrafine and fine PM in fire station microenvironments, in comparison to coarse PM (Baxter et al., 2014; Teixeira, Sousa, et al., 2023). Ultrafine and fine particles pose increased risks to human respiratory health due to their ability to penetrate airways and its tissues and may reach the lung alveoli (HEI, 2013). Due to the small size, after inhalation, ultrafine particles may pass through the alveolar-capillary membrane and enter bloodstream, thereby contaminating cells through the body, damaging structures, contributing to induction of oxidative stress. Having access to the bloodstream, ultrafine particles contribute to systemic inflammation, primarily in the lungs (Li et al., 2003; Schraufnagel, 2020; Schraufnagel et al., 2019). Coarse particles are predominantly filtered by human mucous membranes in the upper airways (Schraufnagel et al., 2019). Coarse particles can also affect human health, potentially decreasing the lung function and increasing the airways irritation, which can promote the development of lung and heart diseases (USEPA, 2024a). Coarse particles are often found in dust, being anticipated that during the hot season where forest fires occur, the levels of coarse, fine, and ultrafine PM inside the fire station are expected to be higher. This increase would be due to the transport of dust by vehicles and firefighting tools, as well as on firefighters' PPEs.

In all the sampled locations, Acy and B[g,h,i]P were not detected. Representing 100% detection, Naph was detected in all samples, followed by Ace and Phe, with a detection of 97.9%, and Pyr with 95.8% of detection. Possible and probable carcinogenic PAHs (Groups 2A and 2B) were detected in 54.2% to 100% of the samples, and B[a]P, considered carcinogenic to humans (Group 1), was detected in 68.8% of the samples. The median levels of PAHs determined in the breathing air zones of the different microenvironments inside the fire station are summarized in Table 3. To compare the levels of each PAH, the median value *per* PAH detected in all stages of each microenvironment was calculated.

Table 3. Total concentrations (median and range; ng/m³) and detection rate (Det, %) of PM-bound PAHs for each area inside the fire station.

PAHs	Det (%)	PPE's storage room	Garage	Common area
Naph	100	6.84×10^{-2} (3.41×10^{-2}–0.166)	4.77×10^{-2} (2.78×10^{-2}–9.85×10^{-2})	4.91×10^{-2} (2.73×10^{-2}–8.12×10^{-2})
Ace	97.9	0.187 (6.89×10^{-2}–0.631)	0.408 (2.77×10^{-2}–0.728)	0.713 (0.495-1.05)
Flu	70.8	8.29×10^{-3} (8.29×10^{-3}–2.81×10^{-2})	2.08×10^{-2} (8.22×10^{-3}–4.9×10^{-2})	3.24×10^{-2} (8.31×10^{-3}–6.67×10^{-2})
Phe	97.9	1.97×10^{-2} (5.08×10^{-3}–8.38×10^{-2})	3.64×10^{-2} (8.23×10^{-3}–0.170)	2.32×10^{-2} (8.37×10^{-3}–9.21×10^{-2})
Ant	60.4	3.64×10^{-3} (1.97×10^{-3}–0.111)	8.81×10^{-3} (1.95×10^{-3}–0.224)	6.93×10^{-3} (1.98×10^{-3}– 0.219)
Fln	83.3	7.66×10^{-3} (3.22×10^{-3}–0.175)	1.89×10^{-2} (3.19×10^{-3}–0.156)	1.09×10^{-2} (3.22×10^{-3}–7.88×10^{-2})
Pyr	95.8	6.71×10^{-3} (2.37×10^{-3}–9.77×10^{-2})	2.98×10^{-2} (6.59×10^{-3}–0.257)	1.35×10^{-2} (2.38×10^{-3}–7.62×10^{-2})
B[a]A	66.7	5.72×10^{-3} (1.61×10^{-3} – 0.120)	9.28×10^{-3} (1.59×10^{-3}–0.262)	3.01×10^{-3} (1.61×10^{-3}–9.91×10^{-2})
Chry	70.8	2.62×10^{-3} (2.19×10^{-3}–4.05×10^{-2})	2.61×10^{-2} (2.17×10^{-3}–0.540)	5.68×10^{-3} (2.20×10^{-3}–0.211)
B[b+j]F	70.1	3.32×10^{-2} (4.94×10^{-3}–0.755)	5.17×10^{-2} (4.90×10^{-3}–0.940)	1.84×10^{-2} (4.95×10^{-3}–0.476)
B[k]F	56.3	4.09×10^{-3} (1.67×10^{-3}–0.160)	8.84×10^{-3} (1.65×10^{-3}–0.153)	3.02×10^{-3} (1.67×10^{-3}–0.100)
B[a]P	68.8	1.85×10^{-2} (2.04×10^{-3}–0.477)	4.13×10^{-2} (2.02×10^{-3}–0.672)	1.31×10^{-2} (2.04×10^{-3}–0.349)
D[a,l]Pyr	62.5	9.75×10^{-3} (4.22×10^{-3}–0.185)	2.59×10^{-2} (4.18×10^{-3}–0.579)	6.66×10^{-3} (4.23×10^{-3}–0.186)
D[a,h]A	54.2	4.92×10^{-3} (4.92×10^{-3}–0.374)	1.94×10^{-2} (4.88×10^{-3}–0.562)	9.39×10^{-3} (4.94×10^{-3}–0.409)
InP	89.6	6.39×10^{-2} (2.84×10^{-3}–0.309)	5.41×10^{-2} (1.20×10^{-2}–0.794)	2.06×10^{-2} (2.84×10^{-3}–0.484)

Naph – naphthalene, Ace – acenaphthene, Flu – fluorene, Phe – phenanthrene, Ant – anthracene, Fln – fluoranthene, Pyr – pyrene, B[a]A – benz[a]anthracene, Chry – chrysene, B[b+j]F – benzo[b+j]fluoranthene, B[k]F – benzo[k]fluoranthene, B[a]P – benzo[a]pyrene, D[a,l]Pyr – Dibenzo(a,l)pyrene, D[a,h]A – dibenz[a,h]anthracene, Ind – indeno[1,2,3– cd]pyrene.

The total median concentrations of PAHs were increased in the common area (0.929 ng/m³), followed by the garage (0.808 ng/m³), and PPE's storage room (0.444 ng/m³) (Table 3). In all sampled microenvironments, Ace is the most concentrated PAH, with a

median value of 0.713 ng/m^3 in the common area, 0.408 ng/m^3 in the garage and 0.187 ng/m^3 in the PPE's storage room. Since Ace is found in emissions from vehicle exhausts, coal, cigarettes and tobacco, it was expected more elevated levels in the garage, but given the layout of fire stations, PAHs can dissipate and contaminate other microenvironments within the fire stations. Other factors that can modify the results are the location where firefighters practice smoking and the type of cooking utilized (USEPA, 2008). Levels of Phe and Naph varied between 5.08 ×10^{-3} ng/m^3 to 0.170 ng/m^3 and from 2.73 × 10^{-2} ng/m^3 to 0.166 ng/m^3 (Table 3). Sources of Phe and Naph can be cigarette and tobacco smoke, or exposure to burning wood, corn, coal or oil, as well as contaminated foods (USEPA, 2024d, 2024b). In each microenvironment, the less predominant compounds were Chry in the PPE's storage room, Ant in the garage, and B[a]A in the common area (2.19 × 10^{-3}–4.05 × 10^{-2} ng/m^3, 1.95 × 10^{-3}–0.224 ng/m^3, and 1.61 × 10^{-3}–9.91 × 10^{-2} ng/m^3, respectively). Overall, the acquired levels of each PM-bound PAH were lower than the ones reported in previous studies on indoor air quality of fire stations in the north of Portugal (Oliveira, Slezakova, Alves, et al., 2017; Oliveira, Slezakova, Fernandes, et al., 2017; Teixeira et al., 2024.

LMW PAHs (2-3 rings; Naph, Ace, Flu, Phe and Ant) were more prevalent in all microenvironments, corresponding to 64.7% of total PAHs in PPE's storage room and garage and 88.8% in the common area. PAHs with 4 rings (Fln, Pyr, B[a]A and Chry) accounted for 5.1%, 10.4%, and 3.6% of total PAHs, respectively. HMW compounds (5-6 rings; B[b+j]F, B[k], B[a]P, D[a,l]Pyr, D[a,h]A and Ind) were more predominant in the PPE's storage room (30.2%), as well as in the garage (24.9%), when compared to the common area (7.7%). These results are consistent with previous studies that also described higher concentrations of LMH PAHs in comparison to HMW PAHs (Oliveira, Slezakova, Alves, et al., 2017; Oliveira, Slezakova, Fernandes, et al., 2017; Teixeira et al., 2024). The concentrations of total carcinogenic PAHs found in the microenvironments were increased in the garage (0.293 ng/m^3), followed by the PPE's storage room (0.215 ng/m^3) and the common area (0.136 ng/m^3). Figure 9 presents the contribution of carcinogenic PAHs to the total levels of PM-bound PAHs in fire stations.

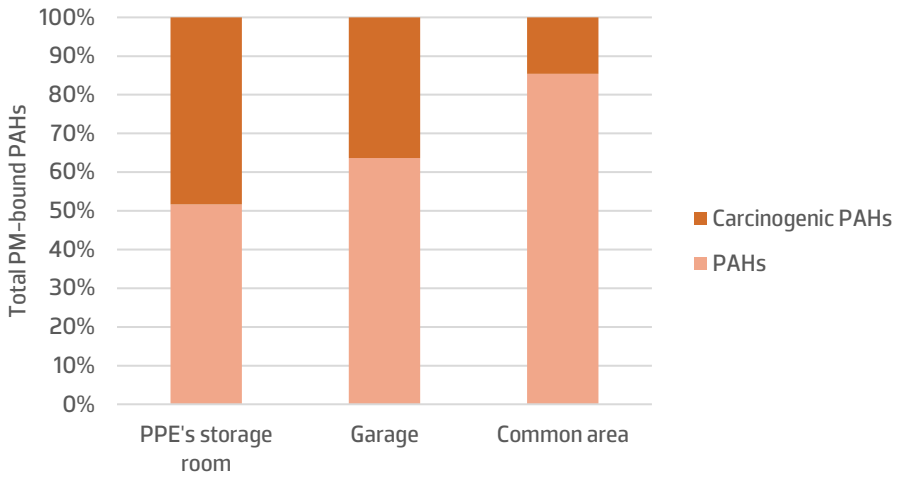


Figure 9. Daily total PM-bound PAHs and carcinogenic PAHs in each sampling site of the fire station.

As expected, the PPE's storage room has a higher percentage of carcinogenic PAHs in comparison to the garage and the common area. In the PPE's storage room 48.3% of PAHs were carcinogenic, ranging from 1.61×10^{-3} ng/m³ to 0.755 ng/m³, followed by the garage with 36.3% of carcinogenic PAHs (1.59×10^{-3} ng/m³ to 0.940 ng/m³), and the common area with 14.6% (1.61×10^{-3} ng/m³ to 0.484 ng/m³). The fact that PPEs are not cleaned often and are exposed to hazardous emissions and materials from different sources, the air in the PPE's storage room can become contaminated as particles dissipate.

4.3. Urinary concentrations of monohydroxyl-PAHs

Urinary levels of OHPAHs were normalized with creatinine values of each firefighter, allowing to minimize variability among participants. Average creatinine concentrations ranged from 0.75 g/L to 2.02 g/L and were within the guidelines defined by WHO for healthy people (0,3 g/L < creatinine < 3.0 g/L) (WHO, 1996).

The urinary concentrations of total OHPAHs determined in firefighters are presented in Figure 10.

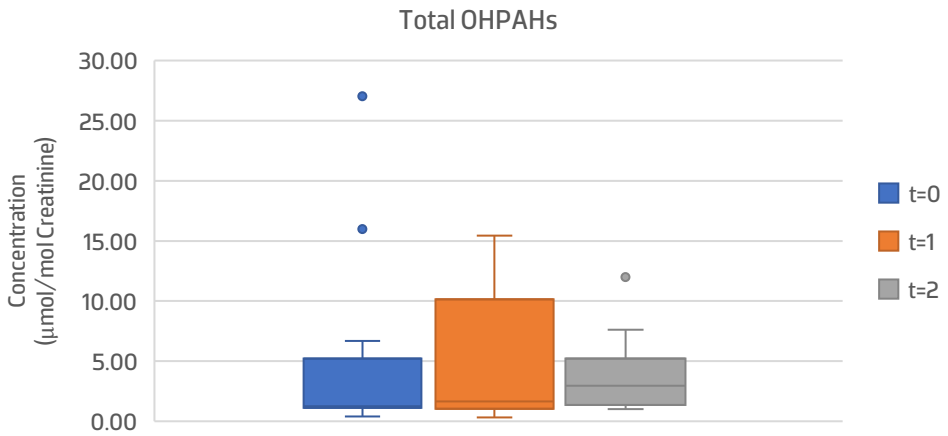


Figure 10. Total concentrations (median, 25%-75% and range; µmol/mol creatinine) of monohydroxyl-PAHs in urine samples of firefighters before (t=0), immediately after (t=1), in the next morning (t=2), after participation in a controlled forest fire.

Total median concentrations of OHPAHs were increased after firefighting (1.6596 µmol/mol creatinine in t=1 and 2.9565 µmol/mol creatinine in t=2) comparatively with pre-exposure levels (1.2468 µmol/mol creatinine). This work demonstrates the contribution of firefighting activities to increased urinary levels of OHPAHs as a consequence of exposure to PAHs released during forest fires and regardless of the use of PPE. Other studies have also found higher concentrations of urinary OHPAHs in firefighters after exposure to wildland fires in comparison to pre-exposure levels (Adetona et al., 2017; Oliveira et al., 2016; Paiva et al., 2024). Urinary metabolite 3OHB[a]P, the biomarker of exposure to BaP, a PAH classified as carcinogenic to humans (Group 1) by IARC, was not detected in the analyzed samples, as it is primarily eliminated through feces (EPA, 2017; IARC, 2023). These findings are coherent with the findings reported by previous studies (Barros et al., 2024; Paiva et al., 2024; Yamano et al., 2014).

Tobacco smoke is classified by IARC as carcinogenic to humans (Group 1) and represents an additional health risk for the occupation of firefighters (IARC, 2023). The concentrations of total OHPAHs in smokers and non-smokers firefighters are presented in Figure 11.

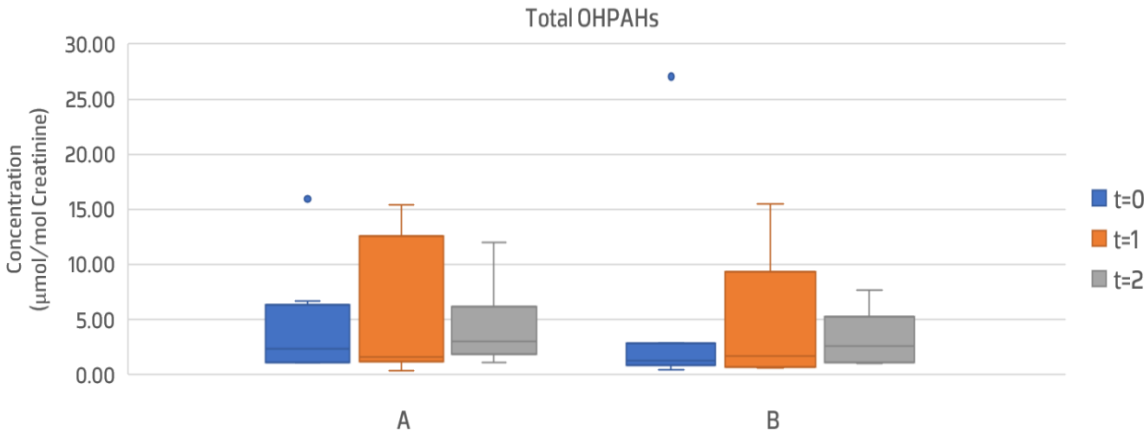


Figure 11. Total concentrations (median, 25%-75% and range; µmol/mol creatinine) monohydroxyl-PAHs in the urine samples of smokers (A) and non-smokers (B) firefighters before (t=0), immediately after (t=1), in the next morning (t=2), after participation in a controlled forest fire.

Smokers have a baseline level of OHPAHs more elevated than non-smokers (2.3461 µmol/mol creatinine *versus* 1.2468 µmol/mol creatinine for t=0; Figure 11). Indeed, smoking emissions are known for the contribution to human exposure to PAHs and many other hazardous substances (Lawal, 2017). In non-smokers firefighters, it was possible to observe a gradual increase in concentrations of OHPAHs after exposure to fire emissions (1.2468 µmol/mol creatinine at t=0 *versus* 1.6939 µmol/mol creatinine at t=1 *versus* 2.5627 µmol/mol creatinine at t=2; Figure 11). Regarding smokers, the impact of firefighting is also observed after some hours after the end of the fire (2.3461 µmol/mol creatinine at t=0 *versus* 1.6253 µmol/mol creatinine at t=1 *versus* 3.0364 µmol/mol creatinine at t=2; Figure 11). These findings agree with previous studies that also found higher concentrations of OHPAHs in smokers and in firefighters after participation in firefighting activities. Oliveira *et al.* (2020) compared 3 groups of firefighters and reported more elevated levels of OHPAHs in firefighters with smoking habits and exposed to fire emissions in comparison to exposed non-smokers, further demonstrating the effect of smoking in OHPAHs levels. Furthermore, it reported that concentrations of OHPAHs were least abundant in the non-smoking and non-exposed firefighters' group allowing to understand the impact of the fire emissions on the OHPAHs levels. Paiva *et al.* (2024) found a similar relation in the evaluated groups of firefighters, as exposed smokers had more elevated levels of OHPAHs in comparison to exposed non-smokers, followed by non-exposed and non-exposed non-smokers.

Regarding outlier points, at t=0, two firefighters had elevated concentrations of OHPAHs, each corresponding to different groups. The concentrations were 15 μmol/mol creatinine for a smoker and 27 μmol/mol creatinine for a non-smoker. While the elevated OHPAH levels in the smoker can be attributed to smoking habits, the other outlier may have elevated levels due to reasons such as activities outside firefighting or dietary habits that lead to increased PAH exposures.

The percentage distributions of OHPAHs in urine samples of smokers and non-smokers firefighters before and after firefighting are represented in Figure 12.

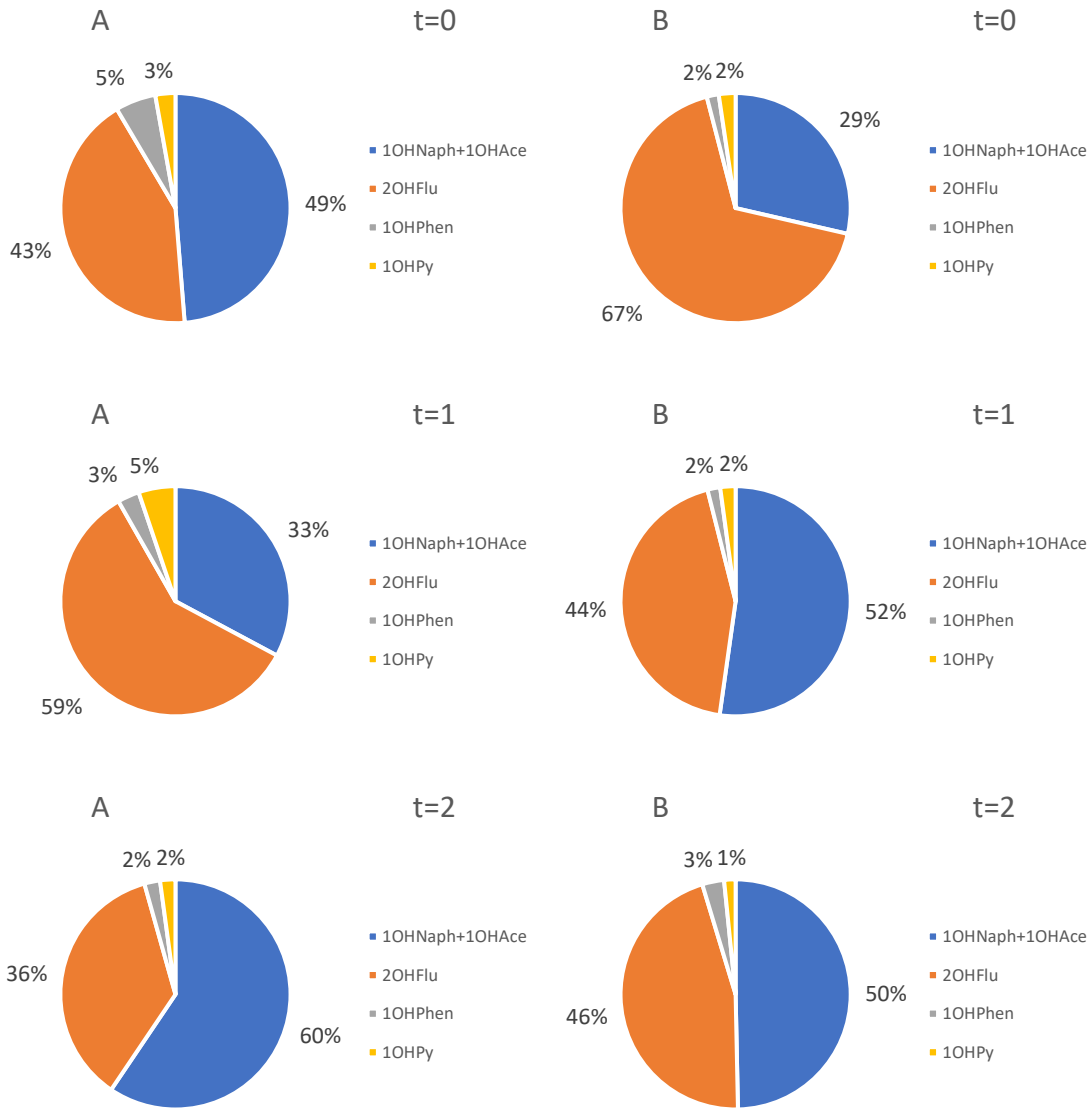


Figure 12. Distributions of concentrations of individual monohydroxyl-PAHs (1OHNaph + 1OHAce, 2OHFl, 1OHPHen and 1OHPy) in the urine samples of smokers (A) and non-smokers (B) firefighters before (t=0), immediately after (t=1), in the next morning (t=2), after participation in a controlled forest fire.

1OHNaph+1OHAce and 2OHFlu were the most abundant metabolites, accounting to 33-60% and 36-59% of the total OHPAHs in smokers and 29-52% and 44-67% in non-smokers. These OHPAHs are commonly found in higher concentrations given that their parent PAHs (Naph, Ace, and Flu) as LMW PAHs are easily metabolized and eliminated through the urine.

Regarding 1OHNaph+1OHAce levels, it was found a higher increase of concentrations in non-smokers than in smokers firefighters after firefighting (0.3764 μmol/mol creatinine in t=0 *versus* 0.7736 μmol/mol creatinine in t=1 and 0.7736 μmol/mol creatinine in in t=2 and 0.8909 μmol/mol creatinine in t=0 *versus* 0.4770 μmol/mol creatinine in t=1 and 1.8083 μmol/mol creatinine in in t=2, respectively).

The increment of urinary 2OHFlu were not so evident in both groups. However, a tendency of slightly increased levels after exposure to the fire was observed in smokers (0.7821 μmol/mol creatinine in t=0, 0.8567 μmol/mol creatinine in t=1 and 1.1010 μmol/mol creatinine in t=2). These findings can be attributed to the cumulative contribution due to exposures to fire and tobacco smoke. A previous study reported a higher elevation in the concentrations of 2OHFlu in smoking firefighters when compared to a controlled group of non-exposed and non-smoking firefighters (Oliveira et al., 2020).

Urinary 1OHPhen and 1OHPyr were the least prevalent monohydroxyl-PAHs in all firefighters, corresponding to 2-5% of the total OHPAHs measured concentrations in smokers and 1-3% in non-smokers (Figure 12). Small increments in the levels of 1OHPyr were observed in smokers firefighters (0.0515 μmol/mol creatinine at t=0 versus 0.0753 μmol/mol creatinine ate t=1 versus 0.0654 μmol/mol creatinine at t=2).

A relation between 1OHPhe levels and occupational exposure might be present in non-smokers since concentrations gradually increased after the firefighting exercise (0.0222 μmol/mol creatinine at t=0 *versus* 0.0265 μmol/mol creatinine at t=1 *versus* 0.0478 μmol/mol creatinine at t=2 for non-smokers and 0.1040 μmol/mol creatinine at t=0 *versus* 0.0445 μmol/mol creatinine at t=1 *versus* 0.0671 μmol/mol creatinine at t=2 for smokers.

Other studies also reported 1OHNaph+1OHAce and 2OHFlu as the most abundant OHPAHs and 1OHPyr and 1OHPhe as the least predominant metabolites in urine samples (Barros et al., 2024; Oliveira et al., 2016; Oliveira, Slezakova, Alves, et al., 2017).

5. Conclusions

This study characterized firefighters' exposure to PAHs by monitoring the PM-bound PAHs in microenvironments inside the fire station and the changes on the urinary OHPAHs during a controlled forest fire.

Overall, levels of PM-bound PAHs were more elevated in the garage (24.781 ng/m^3), followed by the common area (21.112 ng/m^3) and the PPE's storage room (15.345 ng/m^3). LMW PAHs (2-3 rings; Naph, Ace, Flu, Phe and Ant) were more predominant in all microenvironments (64.7% of the total PAHs in the PPE's storage room and in the garage; 88.8% of the total PAHs in the common area) than PAHs with 4 rings (Fln, Pyr, B[a]A and Chry: 5.1%, 10.4% and 3.6% of the total PAHs, respectively) and HMW PAHs (5-6 rings; B[b+j]F, B[k], B[a]P, D[a,l]Pyr, D[a,h]A and Ind: 30.2%, 24.9% and 7.7% of the total PAHs, respectively). Carcinogenic PAHs were more present in the PPE's storage room (48.3% of the total PAHs), followed by the garage (36.3% of the total PAHs) and the common area (14.6% of the total PAHs). Sources of PAHs are primarily related to vehicles emissions and fire emissions that cling to PPEs, vehicles and firefighting tools. Further studies should be conducted on occupational exposure, especially during summer, as levels of PM-bound PAHs might be more elevated, given the higher occurrence of forest fires.

Regarding the urinary concentrations of OHPAHs, it was found that 1OHNaph+1OHAce (33%-60% of the total OHPAHs in smokers and 29%-52% of the total OHPAHs in non-smokers) and 2OHFlu (36%-59% of the total OHPAHs in smokers and 44%-67% of the total OHPAHs in non-smokers) were more prevalent in all urine samples. This work suggests that emissions from fires cause a general elevation in urinary OHPAHs in firefighters recently enrolled in firefighting activities. This is more noticeable in non-smokers firefighters, whose levels of OHPAHs increased from 1.24680 μmol/mol creatinine before expose, to 1.6939 μmol/mol creatinine after exposure and 2.5627 μmol/mol creatinine in the morning after the controlled forest fire. However, additional studies including a higher number of firefighters are needed to better explore these preliminary results. Also, a more realistic approach to the occupational exposure of firefighters during fire combat would be using pre- and post-exposure urine samples during real fires, instead of controlled fires, and combining with inhalation exposure to PAHs during fire combat.

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