Science of the Total Environment 926 (2024) 171801

Contents lists available at ScienceDirect

Science of the Total Environment

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

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Biomonitoring of polycyclic aromatic hydrocarbons exposure and short-time health effects in wildland firefighters during real-life fire events

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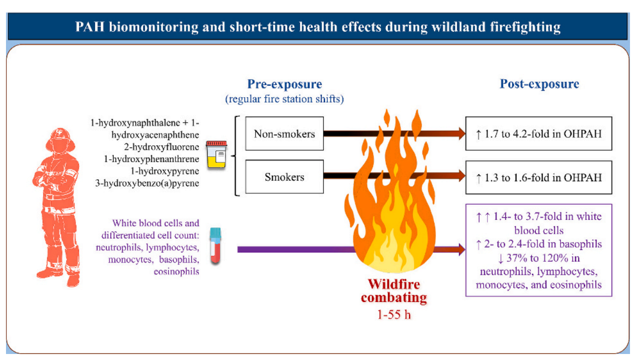
HIGHLIGHTS

- PAH metabolites (OHPAH) were analysed pre- and post- real-life wildfire combating.
- Exposure to wildfire emissions significantly elevated the levels of all OHPAH.
- The greatest discriminant of exposure to wildfire emissions was 1OHNaph + 1OHAce.
- 2OHFlu and 1OHPy correlated positively with the daily number of smoked cigarettes.
- OHPAH were associated with total white blood cells and differential count.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Lingyan Zhu

Keywords:
Occupational exposure
Human biomonitoring
Wildfire emissions
PAH

ABSTRACT

Human biomonitoring data retrieved from real-life wildland firefighting in Europe and, also, worldwide are scarce. Thus, in this study, 176 Portuguese firefighters were biomonitored pre- and post- unsimulated wildfire combating (average:12–13 h; maximum: 55 h) to evaluate the impact on the levels of urinary polycyclic aromatic hydrocarbons hydroxylated metabolites (OHPAH; quantified by high-performance liquid chromatography with fluorescence detection) and the associated short-term health effects (symptoms, and total and differentiated white blood cells). Correlations between these variables and data retrieved from the self-reported questionnaires were also investigated. Firefighters were organized into four groups according to their exposure to wildfire

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https://doi.org/10.1016/j.scitotenv.2024.171801
Received 9 November 2023; Received in revised form 27 February 2024; Accepted 16 March 2024
Available online 19 March 2024

Tobacco consumption
White blood cells count

emissions and their smoking habits: non-smoking non-exposed (NSNExp), non-smoking exposed (NSExp), smoking non-exposed (SNExp), and smoking and exposed (SExp). The most abundant metabolites were 1-hydroxynaphthalene and 1-hydroxyacenaphthene (1OHNaph + 1OHAce) (98–99 %), followed by 2-hydroxyfluorene (2OHFlu) (0.2–1.1 %), 1-hydroxyphenanthrene (1OHPhen) (0.2–0.4 %), and 1-hydroxypyrene (1OHPy) (0.1–0.2 %); urinary 3-hydroxybenzo(*a*)pyrene was not detected. The exposure to wildfire emissions significantly elevated the median concentrations of each individual and total OHPAH compounds in all groups, but this effect was more pronounced in non-smoking (1.7–4.2 times; $p \leq 0.006$) than in smoking firefighters (1.3–1.6 times; $p \leq 0.03$). The greatest discriminant of exposure to wildfire emissions was 1OHNaph + 1OHAce (increase of 4.2 times), while for tobacco smoke it was 2OHFlu (increase of 10 times). Post-exposure, white blood cells count significantly increased ranging from 1.4 (smokers, $p = 0.025$) to 3.7-fold (non-smokers, $p < 0.001$), which was accompanied by stronger significant correlations ($0.480 < r < 0.882$; $p < 0.04$) between individual and total OHPAH and total white blood cells (and lymphocytes > monocytes > neutrophils in non-smokers), evidencing the impact of PAH released from wildfire on immune cells. This study identifies Portuguese firefighters with high levels of biomarkers of exposure to PAH and points out the importance of adopting biomonitoring schemes, that include multiple biomarkers of exposure and biomarkers of effect, and implementing mitigations strategies.

1. Introduction

Firefighters face a high risk of experiencing health consequences due to their regular exposure to air pollutants released from fires (Adetona et al., 2017). In 2022, the International Agency for Research on Cancer (IARC) updated the classification of occupational exposure as a firefighter from "possibly carcinogenic to humans" (Group 2B) to "carcinogenic to humans" (Group 1), supported by its association with the development of mesothelioma and bladder cancer (Demers et al., 2022; IARC, 2023). Polycyclic aromatic hydrocarbons (PAH) are ubiquitous organic compounds that originate from the combustion of organic materials, making them one of the most important pollutants released from fires. Exposure to PAH causes carcinogenic, teratogenic, and genotoxic effects (Patel et al., 2020), and, thus, they are included in the lists of priority substances of the US Environmental Protection Agency (US EPA, 2014), the Agency for Toxic Substances and Disease Registry (ATSDR, 2022), and the Human Biomonitoring for Europe initiative (HBM4EU, 2021a). Although wildland firefighters are typically equipped with personal protective equipment (PPE) during fire combating activities, the equipment may not offer complete protection against pollutants. PAH can still find their way into the undergarments and the air inside the PPE, subsequently penetrating the respiratory system and skin of firefighters (Barros et al., 2023; Engelsman et al., 2023; Sousa et al., 2022). This highlights the importance of applying a biomonitoring approach to capture firefighters′ total body burden, *i.e.*, regardless of the exposure route (Choi et al., 2023; Oliveira et al., 2020b). PAH are excreted in urine mostly as hydroxylated metabolites (OHPAH) (Choi et al., 2023). The most characterized OHPAH is 1-hydroxypyrene (1OHPy), the main metabolite of pyrene (Banks et al., 2021a), however, previous studies (Oliveira et al., 2020b, 2017a, 2016) have been demonstrating the importance of incorporating other biomarkers such as 3-hydroxybenzo(*a*)pyrene (3OHB(*a*)P), the main metabolite of benzo(*a*)pyrene (classified as carcinogenic to humans; IARC, 2023). Due to the unpredictable and challenging nature of wildland fire events, most of the studies have characterized urinary OHPAH during simulated events, *i.e.* controlled fires (Banks et al., 2021a), prescribed burns (Adetona et al., 2019; Adetona et al., 2017) and firefighting training exercises (Fent et al., 2020; Fent et al., 2019; Mayer et al., 2023; Řiháčková et al., 2023; Rossbach et al., 2020; Wingfors et al., 2018); scarce are those, particularly from Europe, reporting data from real wildland fires scenarios (Oliveira et al., 2020b, 2017a, 2017b, 2016). Hence, the aim of the present study was to assess the impact of fire combating on the levels of OHPAH (five besides 1OHPy) and the associated short-term health effects in wildland firefighters during real-life fire events, providing data for this population, as requested by the Human Biomonitoring for Europe initiative (HBM4EU, 2020, 2021a), and simultaneously contributing with valuable information to set OHPAH guideline values (for several compounds) for occupational exposure to PAH. This was achieved through the determination of the urinary PAH biomarkers 1-hydroxynaphthalene (1OHNaph), 1-hydroxyacenaphthene (1OHAce), 2-hydroxyfluorene (2OHFlu), 1-hydroxyphenanthrene (1OHPhen), 1OHPy and 3OHB(*a*)P in (non-smoking and smoking) wildland firefighters. Additionally, paired urine samples (pre- and post-exposure of the same firefighter) were obtained allowing for better characterization of the internal dose of PAHs absorbed during wildfire combat activities. Further, unlike most studies which often exclude smokers or group non-smokers and smokers together, in this study these were separated to assess both individual and cumulative impacts of wildfire combating and tobacco consumption on the OHPAH concentrations, as well as the short-term health effects (including symptoms and white blood cells count – total and differentiated –). Correlations between these variables and data retrieved from the self-reported questionnaires were also investigated. This is the first study that reports such a comprehensive biomonitoring scheme in wildland firefighters and includes the largest number of subjects that has been published in the existing literature.

2. Materials and methods

2.1. Study location and characterization of the participants

Portugal is frequently affected by wildfires every year, especially in the north and central regions of the country, due to its large areas of forests and Mediterranean climate. Rainy seasons followed by hot and dry summers are ideal for the proliferation of forest vegetation (Oliveira et al., 2020a; Meneses et al., 2018). A total of 176 male firefighters working at the fire stations of the Northern region (Bragança) of Portugal were enrolled in this study. All firefighters agreed to voluntarily participate and signed an informed consent form, previously approved by the Ethic Committee of the University of Porto, Portugal, in accordance with the Declaration of Helsinki (approved protocol – Report Nr. 92/CEUP/2020). They filled in a structured questionnaire to provide personal, medical, and professional information, identify their exposure to relevant sources of PAH (*e.g.*, cigarette smoking and dietary habits), and short-term effects (*e.g.*, headaches, and eye and respiratory tract irritation) felt during and within 8 h after firefighting activities. The participants were organized into four groups according to their smoking habits and recent (<3 days) participation in wildfires: non-smoking and non-exposed to fire emissions (NSNExp) subjects, non-smoking and exposed (NSExp), smoking and non-exposed (SNExp), and smoking and exposed (SExp) firefighters (Table 1). Individuals ($n = 4$) who reported recent participation in urban fires were excluded, due to the different exposures and type of PPE used (self-contained breathing apparatus (SCBA)), which promote a different protection than the PPE worn by wildland firefighters (helmet, balaclava, gloves, eye protection glasses, firefighter uniform and boots).

Table 1
Characterization of firefighters from the north of Portugal. NSNExp: non-smoking and non-exposed to fire emissions; NSExp: non-smoking and exposed to fire emissions; SNExp: smoking and non-exposed to fire emissions; SExp: Smoking and exposed to fire emissions; n.a.: Not applicable.

Characteristic	NSNExp	NSExp	SNExp	SExp
n	96	20	70	25
Gender				
Male (%)	100	100	100	100
Age (mean; min – max; years)	40 (20–65)	40 (21–56)	33 (20–60)	32 (20–55)
BMI (mean; min – max; kg/m^2)	27.7 (18.5–36.8)	27.5 (24.2–35.9)	27.0 (18.9–41.3)	26.8 (19.4–33.7)
normal weight (18.5–25 kg/m^2; %)	23	28	37	26
overweight (25–30 kg/m^2; %)	52	55	38	61
obesity (>30 kg/m^2; %)	25	17	25	13
Number of years as firefighter (mean; min – max; years)	17 (2–43)	18 (3–38)	13 (0.3–43)	13 (0.3–34)
<10 years (%)	28	33	49	42
10–20 years (%)	29	28	24	37
≥20 years (%)	43	39	27	21
Number of hours per day spent at the fire station				
<8 h (%)	14	28	15	8
≥8 h (%)	86	72	85	92
Number of cigarettes smoked per day (mean; min–max)	n.a.	n.a.	16 (1–50)	18 (5–35)
Time dedicated to firefighting activities before sample collection (mean; min – max; h)	n.a.	13 (1.0–55)	n.a.	12 (1.0–48)
<5 h (%)	n.a.	53	n.a.	40
5–10 h (%)	n.a.	0	n.a.	12
≥10 h (%)	n.a.	47	n.a.	48

2.2. Urine sampling

A total of 211 spot urine samples were collected between July 2021 and July 2022. Non-exposed firefighters, *i.e.*, firefighters who did not participate in firefighting activities within 3 days prior to sample collection, provided a pre-exposure sample. Exposed firefighters, *i.e.*, those who were involved in the combat of a wildfire, collected a post-exposure sample after returning to their fire stations. Sterilized 100 mL polycarbonate containers were used to collect the urine samples, which were then stored at a temperature of −20 °C until analysis.

2.3. Analysis of OHPAH concentrations

Briefly, urine samples were buffered with acetate buffer at pH 5.0 and subsequently incubated with the enzyme β-glucuronidase/arylsulfatase and antioxidant TBHQ for 120 min at 37 °C after purging with nitrogen (analytical blank samples were prepared in the same way as samples but replacing the equivalent volume of urine by acetate buffer). Following this, OHPAH extraction was performed through Solid-Phase Extraction, and separation and quantification through High-Performance Liquid Chromatography with a fluorescence detector as previously described (Oliveira et al., 2016). Individual calibration curves were plotted for each OHPAH, except for 1OHNaph and 1OHAce that were quantified together. The coefficients of determination of the calibration curves varied from 0.987 to 0.999. The OHPAH limits of detection (LOD) and quantification (LOQ) ranged from 0.0773 μg/L (3OHB(*a*)P) to 7.34 (1OHNaph + 1OHAce) μg/L, and between 0.258 μg/L to 24.5 μg/L, respectively. Analytical quality control was performed by the analysis of spiked samples [0.016 μg/L urine (2OHFlu) to 1 μg/L urine (1OHNaph + 1OHAce)] during 6 consecutive days and evaluated through the relative standard deviation (RSD). For intra-day precision, the RSD values varied between 1.3 % (2OHFlu) to 6.4 % (1OHPhen), and for inter-day precision between 1.3 % (1OHNaph + 1OHAce) and 8.1 % (1OHPy). Additionally, the accuracy of the methodology was assessed by recovery assays through a pooled urine sample, and the recovery rates ranged from 70.0 % to 117 %.

The Jaffe colorimetric method was applied to quantify the creatinine concentration in each urine sample (Kanagasabapathy and Kumari, 2000). Creatinine is excreted at a constant rate through the kidneys, allowing to overcome dilution variations due to differences in fluid intake, physical exercise, and body temperature among study subjects (Oliveira et al., 2020b). Hence, to better compare individual levels of urinary OHPAH between firefighters, data were normalized with urinary creatinine concentrations and are presented as μmol/mol creatinine.

All samples were analysed in triplicate.

2.4. Blood analysis

Blood collection was executed following World Health Organization guidelines (WHO, 2010), *i.e.*, firefighters were in a quiet, clean, and well-lit zone and were asked for their full name and confirmed consent for the procedure. Transportation and sample analysis were conducted on the same day of collection, 3 to 4 h after venipuncture. Blood analysis was carried out in a haematology analyser PentraES60 (Horiba Medical Diagnostics, Montpellier, France), which integrates a blend of the following technologies: cytochemistry, impedance, and flow cytometry. This instrument used double hydrodynamic sequential and the multi-distribution sampling systems, respectively, for internal cellular structure analysis and blood sample homogenization; previous calibration was performed according to the manufacturer's instructions. The micro-sampler injected 30 μL and 53 μL for complete blood count and differential count, respectively. Both the count and percentage of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were analysed.

2.5. Statistical analysis

The statistical analysis was conducted using the software SPSS (IBM Statistics 29). Since data did not follow a normal distribution according to the Shapiro-Wilk´s test ($p < 0.05$), concentrations were compared through the Mann-Whitney U non-parametric test for unpaired samples and through the Wilcoxon Signed-Rank non-parametric test for paired samples. An exception was observed for blood lymphocytes in which a normal distribution was obtained ($p > 0.05$), thus an independent samples *t*-test was used. To test for confounders of PAHs sources, statistical differences were explored in urinary OHPAHs by dietary choices *i.e.*, type of most consumed meals on the last three days prior urine collection (roast/grilled/barbecue, stewed, or mix), consumption ("yes" *versus* "no", and frequency: "no" *versus* "weekly" *versus* "daily") of tea, coffee, smoked/processed food, fruit, and vegetables using data retrieved from the questionnaire. Possible correlations between individual OHPAH, total OHPAH (∑OHPAH), and variables extracted from the questionnaire that can influence PAH exposure, as well as blood parameters were estimated through Spearman correlation coefficients (*r*). The statistical significance was defined as $p \leq 0.05$. Principal components analysis (PCA) was performed using XLSTAT extension (v. 2023.3.1, XLSTAT) of Excel (v. 16.0.4266, Microsoft Corporation, USA).

3. Results and discussion

3.1. Population characterization

The characteristics of each group of firefighters are presented in

Table 1. Firefighters included in this study were all male and aged 20 to 65 years. No significant differences regarding body mass index (BMI), number of cigarettes, or recent exposure were observed among the groups, with the exception of the number of years as firefighter between the NSNExp and SNExp groups. Most of the participants (72–92 %) spent 8 or more daily hours in the fire station, and the overall time spent in wildland firefighting activities prior to sample collection ranged from 1 to 55 h, with an average duration of 13 h (NSExp) and 12 h (SExp).

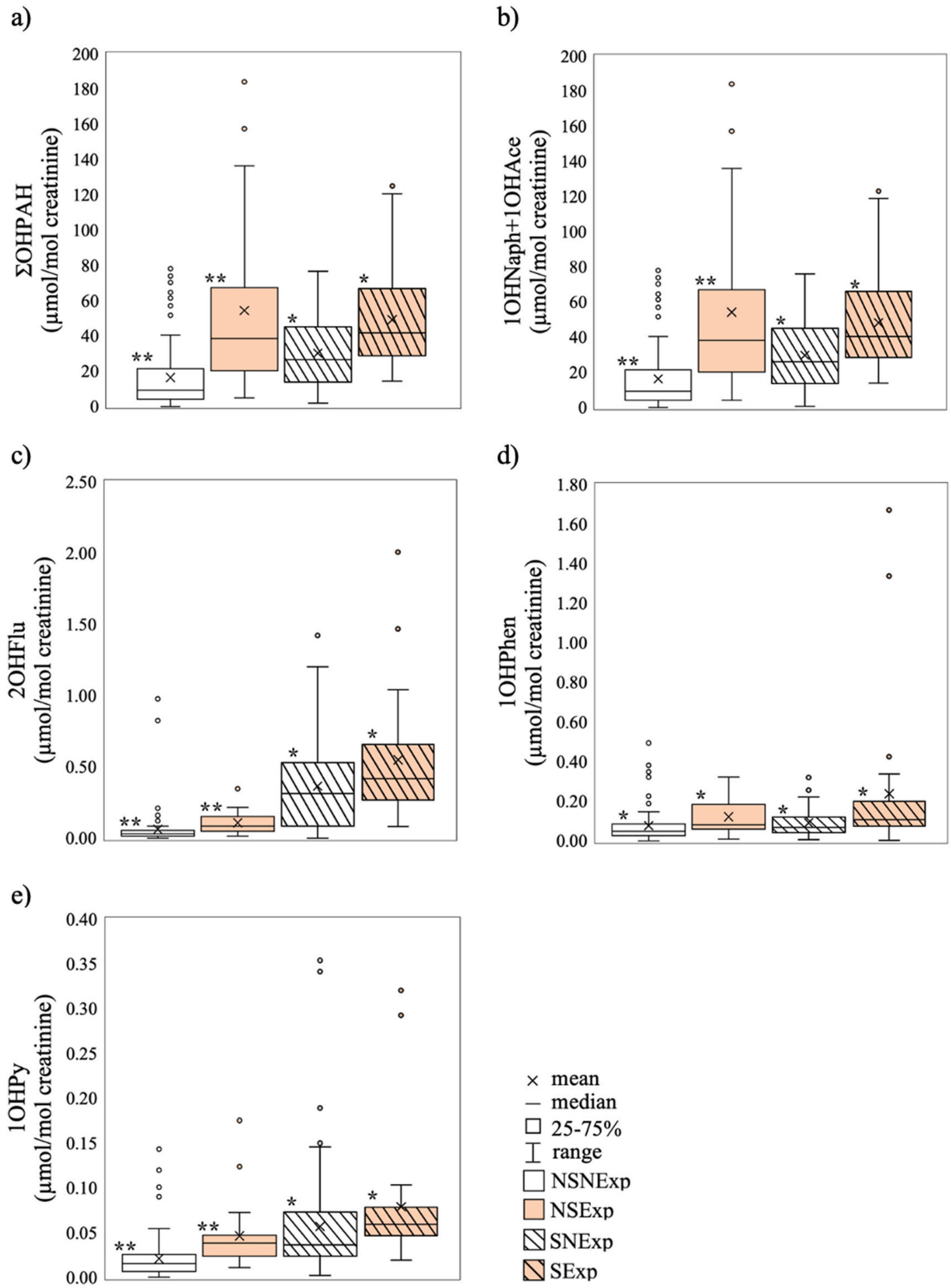

Fig. 1. Concentrations of urinary PAH metabolites (median, P25-P75 percentiles, and range; μmol/mol creatinine) in non-smoking and non-exposed to fire emissions (NSNExp), non-smoking exposed (NSExp), smoking non-exposed (SNExp), and smoking exposed (SExp) firefighters: a) ∑OHPAH, b) 1OHNaph + 1OHAce, c) 2OHFlu, d) 1OHPhen, and e) 1OHPy. ∑OHPAH: total of OHPAH; 1OHNaph + 1OHAce: 1-hydroxynaphthalene and 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene. *Statistically significant differences at $p \leq 0.05$; **Statistically significant differences at $p \leq 0.001$.

3.2. Urinary OHPAH concentrations

Overall, detection rates of OHPAH were all higher or equal to 90 %–98 % except for 3OHB(*a*)P (biomarker of exposure to carcinogen PAH) that was not detected in the collected samples. This biomarker has a complex metabolism and is primarily eliminated through faeces rather than in urine (Barbeau et al., 2014). Previous works also reported 3OHB(*a*)P as non-detected among firefighters (Oliveira et al., 2020b; Wingfors et al., 2018) or at a very low detection frequency (about 1 %) (Řiháčková et al., 2023).

Fig. 1 shows the creatinine corrected urinary concentrations of total and individual OHPAH among the four characterized groups of firefighters. The median concentrations of ∑OHPAH were 2.9 to 4.5 times higher ($p < 0.001$) in SNExp (26.7 μmol/mol creatinine), NSExp (38.5 μmol/mol creatinine) and SExp (41.7 μmol/mol creatinine) than in the NSNExp group (9.29 μmol/mol creatinine) (Fig. 1 a)). Therefore, firefighters' exposure to wildfire emissions and/or smoking effectively impacts the urinary levels of ∑OHPAH. In addition, within smokers, the concentrations were statistically different between non-exposed and exposed firefighters ($p = 0.002$), with the exposure to wildfires resulting in a 56 % increase in the levels of ∑OHPAH. Conversely, no statistical difference was found among the exposed groups (SExp 8 % higher than NSExp), suggesting a greater impact of wildfire emissions than tobacco smoke on the urinary levels. Within the defined groups, no significant differences were obtained in OHPAH concentrations according to consumption of coffee, fruit, vegetables, and smoked meat/products nor the most eaten type of meal in the last three days.

The biomarkers with the highest concentrations were 1OHNaph + 1OHAce, which represented 98–99 % of ∑OHPAH in the four groups. Contributions of the other metabolites ranged from 0.2 to 1.1 % for 2OHFlu, 0.2–0.4 % for 1OHPhen, and 0.1–0.2 % for 1OHPy. The decreasing order of contribution for ∑OHPAH was 1OHNaph + 1OHAce > 1OHPhen > 2OHFlu > 1OHPy for NSNExp firefighters and 1OHNaph + 1OHAce > 1OHPhen = 2OHFlu > 1OHPy for NSExp firefighters, while an inversion of 2OHFlu and 1OHPhen occurred for smoking firefighters, suggesting that tobacco smoke may have a stronger influence over 2OHFlu than 1OHPhen. 1OHPy was also reported as the characterized biomarker with the lowest or second lowest concentration in previous works with wildfires (Oliveira et al., 2016), prescribed burn shifts (Adetona et al., 2017) and firefighting training exercises (Fent et al., 2019; Rossbach et al., 2020). The different rates of excretion may contribute to explain the lowest rises observed in the urinary levels of 1OHPhen and 1OHPy. Li et al. (2015) reported greater half-lives for the excretion of 1OHPhen (13.8 h) and 1OHPy (23.5 h) than for 1OHNaph (6.6 h) and 2OHFlu (8.4 h) after woodsmoke exposure.

The ascending order of the median concentrations of 1OHNaph + 1OHAce was NSNExp (9.14 μmol/mol creatinine) < SNExp (26.2 μmol/mol creatinine) < NSExp (38.3 μmol/mol creatinine) ≈ SExp (40.6 μmol/mol creatinine) (Fig. 1 b)). Analogously to ∑OHPAH, and with similar rises in median concentration (ranging from 2.9 to 4.4 -fold), the 1OHNaph + 1OHAce median concentrations were statistically different among all groups ($p < 0.003$), except between the exposed groups, and the NSExp and SNExp groups (Fig. 1 b)). This implies that exposure to wildfire emissions significantly elevated the levels of 1OHNaph + 1OHAce among firefighters, and exposure to tobacco smoke significantly augmented the levels of 1OHNaph + 1OHAce among the non-exposed firefighters. Naphthalene and acenaphthene are the most volatile PAH that were biomonitored in this study and, consequently, could have penetrated the layers of the PPE and clothing more easily than other PAH (Mayer et al., 2023). Additionally, these compounds can easily off-gas from contaminated PPE, tools, and vehicles providing additional sources, thus explaining the observed increased concentrations in firefighters (Banks et al., 2021b; Sparer et al., 2017). Although 1OHNaph + 1OHAce were also earlier detected as the most abundant metabolites in the urine of firefighters (Oliveira et al., 2020b, 2017a, 2017b, 2016), the concentrations determined in this study are 2.5–66 times higher than those previously reported for non-exposed firefighters (0.138–3.59 μmol/mol creatinine), 7.2–54 times higher than in firefighters exposed to wildfire emissions (0.71–5.61 μmol/mol creatinine), and 5.0–6.0 times higher than for prescribed burns (geometric mean: 6.92 μmol/mol creatinine) (Table S1, Supplementary Material). An explanation for the higher attained concentrations in exposed firefighters may be the duration of recent exposure to fire emissions. Firefighters in the previous studies (Table S1) were exposed to fire emissions during firefighting training exercises [that lasted 10 min (Fent et al., 2019), 6 min (Mayer et al., 2023), and 2 h (Rossbach et al., 2020)], prescribed burns shifts (on average for 7.6 h (Adetona et al., 2017)), and wildfire combat (for 3 h (Oliveira et al., 2020b)). Meanwhile, NSExp and SExp firefighters in the present study reported an average duration of 13 h (median: 4 h; maximum of 55 h) and 12 h (median: 5 h; maximum of 48 h) of exposure before sample collection (Table 1). In the other study concerning wildfire exposure, 10 % of firefighters were exposed for >10 h (Oliveira et al., 2016), while in the present work, 47 % (NSExp) and 48 % (SExp) were exposed for >10 h. Moreover, PAH mixture distribution varies with the type and moisture of fuel, temperature, and wind, and so, differences in the exposure of PAH are likely to occur across different geographic locations (Miranda et al., 2012). In the present study, firefighters were exposed to wildfires where bushes, oak, pine, broom, and straw were burned, as reported in the questionnaires. Moreover, PPE can have different protection efficacies among firefighters from different countries, due to the materials, age, and laundry/decontamination processes.

After 1OHNaph + 1OHAce, 2OHFlu had the biggest increases post-exposure to wildfire emissions, and significant differences were found between all groups ($p \leq 0.03$) (Fig. 1 c)). The median of 2OHFlu was 2.9 times higher in NSExp than in the NSNExp, while for combined exposure (smoking and wildfire emissions), the SExp group displayed a 1.3- fold increment than the SNExp group (Fig. 1 c)). Among firefighters non-exposed to fire emissions, the median concentration was 10 times higher in smokers than non-smokers (0.314 *versus* 0.0303 μmol/mol creatinine for SNExp and NSNExp), making 2OHFlu the most discriminant biomarker for tobacco consumption. This agrees with the results reported by Helen et al. (2012) among adult subjects from the USA and Poland where hydroxyfluorenes (including 2OHFlu) increased the most when comparing smokers with non-smokers (Helen et al., 2012; HSE, 2020). Additionally, the concentration of the SExp group was 4.8 times higher than the concentration of the NSExp group, showing that 2OHFlu is a strong discriminant of tobacco consumption even among subjects exposed to wildfires. When comparing the SExp group with the NSNExp group, 2OHFlu exhibited a 14-fold increase, establishing it as the most significantly impacted biomarker by the combined effects of both sources, *i.e.*, wildfire emissions and tobacco consumption. 2OHFlu median concentrations are in agreement with those previously reported for Portuguese firefighters, which varied between 0.0139 and 1.53 μmol/mol creatinine (Table S1). Simultaneously, NSNExp and NSExp firefighters had median concentrations 42–92 % lower than those observed for firefighters from Germany, Check Republic, and the USA, before (0.12–0.20 μmol/mol creatinine) and after training exercises (0.15–1.08 μmol/mol creatinine), respectively (Table S1).

Regarding the urinary levels of 1OHPhen, the median concentrations of SNExp, NSExp and SExp firefighters showed significant ($p \leq 0.006$) increases of 1.4, 1.7 and 2.2-fold when compared to the NSNExp group (0.0484 μmol/mol creatinine), respectively (Fig. 1(d)). Significant differences were also found between the SNExp and SExp groups (0.0691 *versus* 0.107; $p = 0.01$) (Fig. 1 d)). The 1OHPhen levels were 33 % higher in SExp than in NSExp firefighters (0.107 *versus* 0.0810; $p > 0.05$). Urinary 1OHPhen seems to be the least affected biomarker by the tobacco consumption and/or wildfire smoke exposure. 1OHPhen median concentration are similar as those reported for Portuguese firefighters (0.008–0.204 μmol/mol creatinine) (Table S1). The NSNExp and NSExp firefighters displayed median concentrations 19–89 % below the range described for German and USA firefighters (0.06–0.75 μmol/mol creatinine), but were, respectively, 4.8 times larger (0.0484 *versus* 0.01 μmol/mol creatinine) and withing the range (0.0810 *versus* 0.05–0.10 μmol/mol creatinine) compared to firefighters from the Czech Republic (Table S1).

As for 1OHPy, the most characterized PAH biomarker in the literature, median concentrations (μmol/mol creatinine) were 2.3 times increased for SNExp (0.0367; $p < 0.001$), 2.4 times for NSExp (0.0383; $p < 0.001$), and 3.8 times for SExp (0.0597; $p < 0.001$) comparatively to the NSNExp group (0.0157) (Fig. 1 e)). Thus, the influence of tobacco consumption and emissions from wildfires on the urinary levels of 1OHPy is demonstrated, however the individual contribution of these variables is similar. Although not so evident as for 2OHFlu, the levels of 1OHPy in SExp firefighters were 56 % higher than in NSExp ($p = 0.001$). The median concentrations of 1OHPy were in line with the results previously described among Portuguese firefighters (0.009–0.462 μmol/mol creatinine) but generally lower than the values reported for firefighters from other countries (Table S1). For those referred non-smoking (German, Swedish, Czech, American, and Australian; Table S1) firefighters, the concentrations detected after performing regular tasks at fire stations (median: 0.031–0.14; geometric mean: 0.05–0.16; mean: 0.06–0.72 μmol/mol creatinine) and after participation in training exercises, controlled fires or fire missions (median: 0.0518–1.1; geometric mean: 0.10–0.12; mean: 0.10–1.3 μmol/mol creatinine) were about 1.2 to 29 times higher than the levels determined in this work. Furthermore, a recent study (including smoking and non-smoking firefighters) reported 1.4–3.2 times higher median concentrations of 1OHPy before and after their participation in fire combating missions (0.05 and 0.06 μmol/mol creatinine, respectively), such as residential building, vehicle fire, blazes, vegetation fires, and underground facilities fire than the concentrations determined in the present study, except for the SExp group which had a similar concentration (Taeger et al., 2023). Mean concentrations for exposed firefighters in the present study were 11–187 % higher than firefighters from Canada (mostly non-smokers) who participated in urban and industrial fires (Cherry et al., 2019).

OHPAH concentrations were augmented after fire combating, despite the usage of PPE. Although adequate use and maintenance of PPE provide significant protection for the user against PAH exposure through inhalation and dermal adsorption, data showed that exposure still occurred, in agreement with authors who found PAH on skin wipes [after firefighting training exercises (Wingfors et al., 2018), controlled compartment fires (Banks et al., 2021a), emergency fire suppressions (Keir et al., 2020), and wildfires (Cherry et al., 2023)], PPE and clothes worn by firefighters underneath the PPE [after simulated compartment particleboard fires (Engelsman et al., 2023), emergency fire suppressions (Keir et al., 2020), and controlled fires in a fully-furnished structure (Mayer et al., 2019)], and air inside the PPE [after firefighting training exercises (Mayer et al., 2023; Wingfors et al., 2018).]. Noteworthy, a recent biomonitoring study characterized the impact of several operations on the 1OHPy urinary levels of urban German firefighters (Taeger et al., 2023). Despite the use of SCBA, which is not used by wildland firefighters, 1OHPy concentrations doubled after firefighting missions. Imperfections of PPE, including the flash hood, may allow fine and ultrafine particles, and semi-volatile compounds released from fires to infiltrate through them, especially naphthalene and acenaphthene, the most volatile PAH included in this study (Wingfors et al., 2018). The long median time of wildland firefighting, as this is frequently the case in real-life missions, may also have contributed to the high exposure. Further, firefighters may remove part of their PPE when they feel out of danger like in the aftermath of fire events, increasing the risk of exposure to PAH and other pollutants (Sousa et al., 2022; Wingfors et al., 2018).

In Portugal, there is currently no exposure guideline regulating the urinary levels of OHPAH in both occupational and non-occupational scenarios, however, levels for Portuguese adults are available on the online dashboard of the HBM4EU initiative (HBM4EU, 2021b), a collaborative project across European countries to standardize human biomonitoring practices, providing valuable data on human exposure to chemicals. The concentrations of 1OHNaph + 1OHAce for Portuguese firefighters were 12–87 times above the concentration of 1OHNaph for Portuguese men (median: 0.734 μmol/mol creatinine) (HBM4EU, 2021b) and European adults (geometric mean: 0.477 μmol/mol creatinine) (Govarts et al., 2023). 2OHFlu concentrations for the non-smoking groups were 34–90 % lower than Portuguese men (median: 0.304 μmol/mol creatinine) and European adults (geometric mean: 0.128 μmol/mol creatinine), while the smoking groups were 3–224 % higher than these populations. Compared to the 1OHPhen levels in Portuguese men (median: 0.0648 μmol/mol creatinine) and European adults (geometric mean: 0.054 μmol/mol creatinine), the concentrations in the present study were 6.6–114 % greater, except for the NSNExp group, for which concentrations were 17–25 % lower. In contrast, 1OHPy concentrations in Portuguese firefighters were 21 % to 85 % lower than in Portuguese men (median: 0.107 μmol/mol creatinine) and European adults (median: 0.046 μmol/mol creatinine), except for the SExp group, that had a concentration 40 % higher than European adults. Median concentrations of 1OHPy were below the limit of biological exposure (0.93 μmol/mol creatinine, originally 2.5 μg/L) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 2019). Moreover, the highest concentration observed in the participants of this study was 0.35 μmol/mol creatinine, 63 % lower than the available limit. Median and maximum concentrations of 1OHPy were also lower than the post-shift biological monitoring guidance value (4.0 μmol/mol creatinine) established by The British Health & Safety Executive (HSE, 2020) and the limit for occupational exposure to PAH with no observed genotoxic effects (1.0 μmol/mol creatinine) suggested by Jongeneelen (2014). The data obtained also emphasize the importance of implementing biomonitoring strategies that encompass multiple metabolites, rather than exclusively relying on 1-OHPy, as is the common practice in many studies (Barros et al., 2023). This broader approach is necessary to capture the more substantial changes.

3.3. Individual pre- to post-fire urinary concentrations

To help understand the impact of wildfire emissions on the urinary levels of PAH biomarkers, the levels of ∑OHPAH for firefighters who simultaneously collected urine before and after firefighting were compared (70 paired samples; Fig. 2 a)). This approach allows the evaluation of personal exposure since each firefighter acts as his own control promoting a lower variation in the individual characteristics/habits from pre- to post-fire. Generally, urinary ∑OHPAH levels were higher after wildfire combat and the concentrations before and after wildfire exposure were significantly different for each OHPAH for non-smokers ($p \leq 0.02$) and smokers ($p \leq 0.05$), except for 1OHPhen (Fig. S1, Supplementary Material). The levels of ∑OHPAH after exposure increased 3.3–580 times (median: 6.6-fold; $p = 0.001$) in 86 % of non-smoking firefighters and 1.4–9.1 times (median: 1.6-fold; $p = 0.001$) in 76 % of smoking participants (Fig. 2)). No common factor was found between the firefighters (2 non-smokers and 5 smokers) who had lower concentrations (0.1 to 0.6 times) after exposure, since they belonged to different corporations, and fought different wildfires for different durations. For smokers, it is possible that they smoked less cigarettes while on fire combating duty, thus contributing to the higher pre-exposure levels. The values corresponding to the relative differences between post- and pre-exposure ∑OHPAH levels for non-smoking and smoking subjects are significantly different ($p = 0.001$) (Fig. 2 b)). In addition, non-smoking firefighters displayed higher increases in urinary ∑OHPAH levels after firefighting than smoking subjects (median relative differences and inter quartile range (IQR) of 935 %, 271–1096 % *versus* 64 %, 38–208 %, respectively) (Fig. 2 b)). The observed relative differences are also statistically different between non-smokers and smokers ($p \leq 0.001$; Fig. S2) for 1OHNaph + 1OHAce (median and IQR of 948 %, 276–1101 % for non-smokers; 64 %, 37–211 % for smokers) and 1OHPy (median and IQR of 229 %, 48–486 % for non-smokers; 66

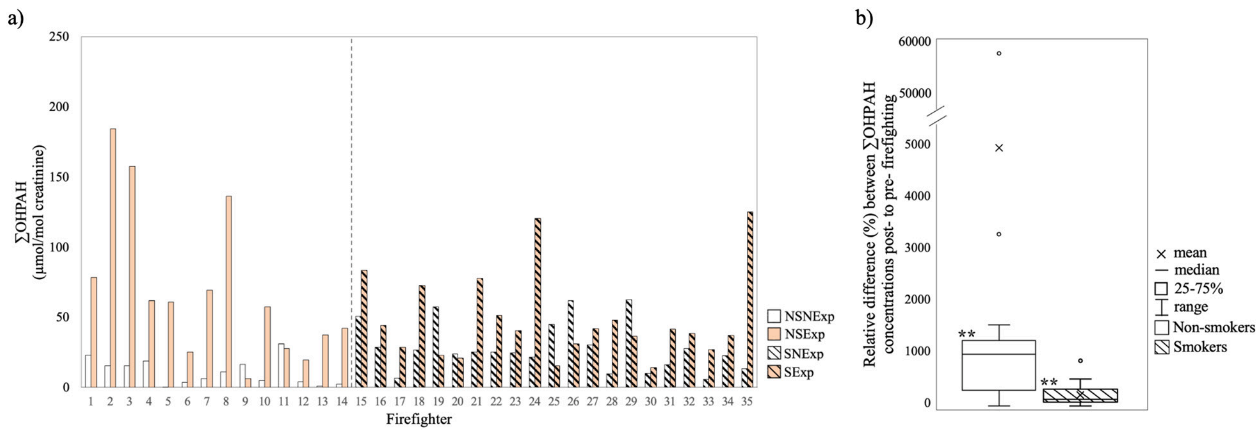

Fig. 2. a) Paired total median concentrations of urinary PAH metabolites (μmol/mol creatinine) for the same individual before and after wildland firefighting. b) Relative differences (median, P25-P75 percentiles, and range) between ∑OHPAH concentrations post- to pre- firefighting among non-smoking and smoking firefighters. NSNExp: Non-smoking Non-exposed; NSExp: Non-smoking Exposed; SExp: Smoking Exposed; SNExp: Smoking Non-exposed. **Statistically significant differences at $p \leq 0.001$.

%, −14 to 109 % for smokers). Taeger et al. (2023) also suggested that non-smoking firefighters are more affected by firefighting activities than smoking firefighters, although the latter reached higher median concentrations. Meanwhile, no statistical differences were observed between smokers and non-smokers regarding the relative differences post- and pre-exposure of 2OHFlu [median and IQR of 127 %, 67–415 % for non-smokers; 34 %, −7 to 121 % for smokers] or 1OHPhen (median and IQR of 118 %, 13–232 % for non-smokers; 67 %, −26 to 242 % for smokers) (Fig. S2). Considering that most OHPAH levels were more affected by wildfire emissions than tobacco consumption, the impact of wildfire emissions was further evaluated without discrimination by smoking status (*i.e.* non-smoking and smoking firefighters were included in the same group although significant differences were observed between them). The concentrations of the individual metabolites were statistically higher after exposure to wildfire emissions ($p \leq 0.01$). The highest increase in median concentrations post wildfire exposure was also observed for 1OHNaph + 1OHAce (2.2-fold) and the lowest was for 1OHPhen (1.5-fold), and the ∑OHPAH median concentration had a significant increase of 2.2-fold ($p < 0.001$). The variability observed in the urinary OHPAH after firefighting can be attributed to the duration of exposure and the position/task performed by the participant during the fire, smoke composition (affected by the type and moisture content of the burnt vegetation, fire temperature and wind direction), and the metabolism of each firefighter (Miranda et al., 2012). PAHs exposure has been associated with increased risk of cardiovascular disease development through increased blood pressure, inflammation, and atherosclerosis (Qigang et al., 2023). Smoking can contribute to airway and systemic inflammation in firefighters (Orysiak et al., 2022). Moreover, Klingbeil et al. (2014) highlighted that the cumulative exposure to PAHs from tobacco consumption and other sources such as air pollution can have synergetic effects *via* epigenetic changes. Therefore, through common mechanisms, PAHs exposure from firefighting and tobacco consumption can synergistically impact the cardio-respiratory system.

3.4. Correlations of urinary OHPAHs with occupational and personal factors

Correlations were estimated between individual and total OHPAH to study their relations, and between variables related to firefighters' occupation and personal characteristics to understand their influence on PAH exposure.

Fig. 3 shows the Spearman correlation coefficients used to estimate the associations within OHPAH concentrations. The correlations between urinary ∑OHPAH and 1OHNaph + 1OHAce were positive and very strong ($0.999 < r < 1.000$; $p < 0.001$). Considering that 1OHNaph + 1OHAce significantly contribute to the total concentrations (at least 98 % of ∑OHPAH in all groups), their strong correlation was expected. In the NSNExp group (Fig. 3 a)), 2OHFlu, 1OHPhen, and 1OHPy were positively and significantly correlated with each other ($0.284 < r < 0.462$; $p \leq 0.005$), which indicates that their parent compounds could have a common source. The very low correlation coefficients between these biomarkers and 1OHNaph + 1OHAce ($-0.007 < r < 0.050$; $p > 0.05$) suggest that naphthalene and acenaphthene may have originated from different sources compared to fluorene, phenanthrene and pyrene. Information retrieved from the personal questionnaires revealed that 72–92 % of firefighters spent >8 h per day at fire stations (Table 1). Oliveira et al. (2017c) have reported naphthalene and acenaphthene as the main compounds in the $PM_{2.5}$ of the breathing air zone of firefighters at Portuguese fire stations, although they predominantly exist in the gaseous phase. In Polish fire stations, Rogula-Kozłowska et al. (2020) detected naphthalene and acenaphthene only in the gaseous phase, being the most abundant PAH. Moreover, the highest concentrations were observed at the changing rooms and truck bay areas, which were generally higher compared to ambient air, in line with findings of other studies (Keir et al., 2020; Sparer et al., 2017). Additionally, as previously mentioned, several authors have found considerable levels of PAH in firefighters' PPE and underneath clothes after different types of fire events suppression [emergency fire suppressions (Keir et al., 2020), simulated compartment particleboard fires (Engelsman et al., 2023), and controlled fires in a fully-furnished structure (Mayer et al., 2019)]. While combating a fire or during firefighting training exercises, PAH accumulate in the PPE, promoting cross-contamination. In turn, the equipment can then contaminate the inside of the firefighters' vehicles and some areas inside fire stations with PAH through off-gassing and deposition on surfaces, leading to inhalation and dermal exposure of these compounds during regular work-shifts (Banks et al., 2021b; Rogula-Kozłowska et al., 2020; Sparer et al., 2017). Further, vehicular exhaust emissions are also an important source of PAH (Sparer et al., 2017) and, therefore, contribute to increased levels of these compounds in truck bay areas, especially if these lack ventilation. Consequently, levels of PAH tend to be higher in the indoor air of fire stations compared to ambient air and are dependent on several factors such as method and frequency of laundering of equipment post-exposure, frequency and type of fire exposure, number of vehicles and ventilation of the fire station (Keir et al., 2020; Rogula-Kozłowska et al., 2020; Sparer et al., 2017).

After exposure to wildfires, non-smokers showed negative correlations between urinary 1OHNaph + 1OHAce and 2OHFlu ($r = -0.259$; $p > 0.05$), 1OHPhen ($r = -0.006$; $p > 0.05$) and with 1OHPy ($r = -0.182$; $p > 0.05$), strongly suggesting different sources of exposure (Fig. 3 b)).

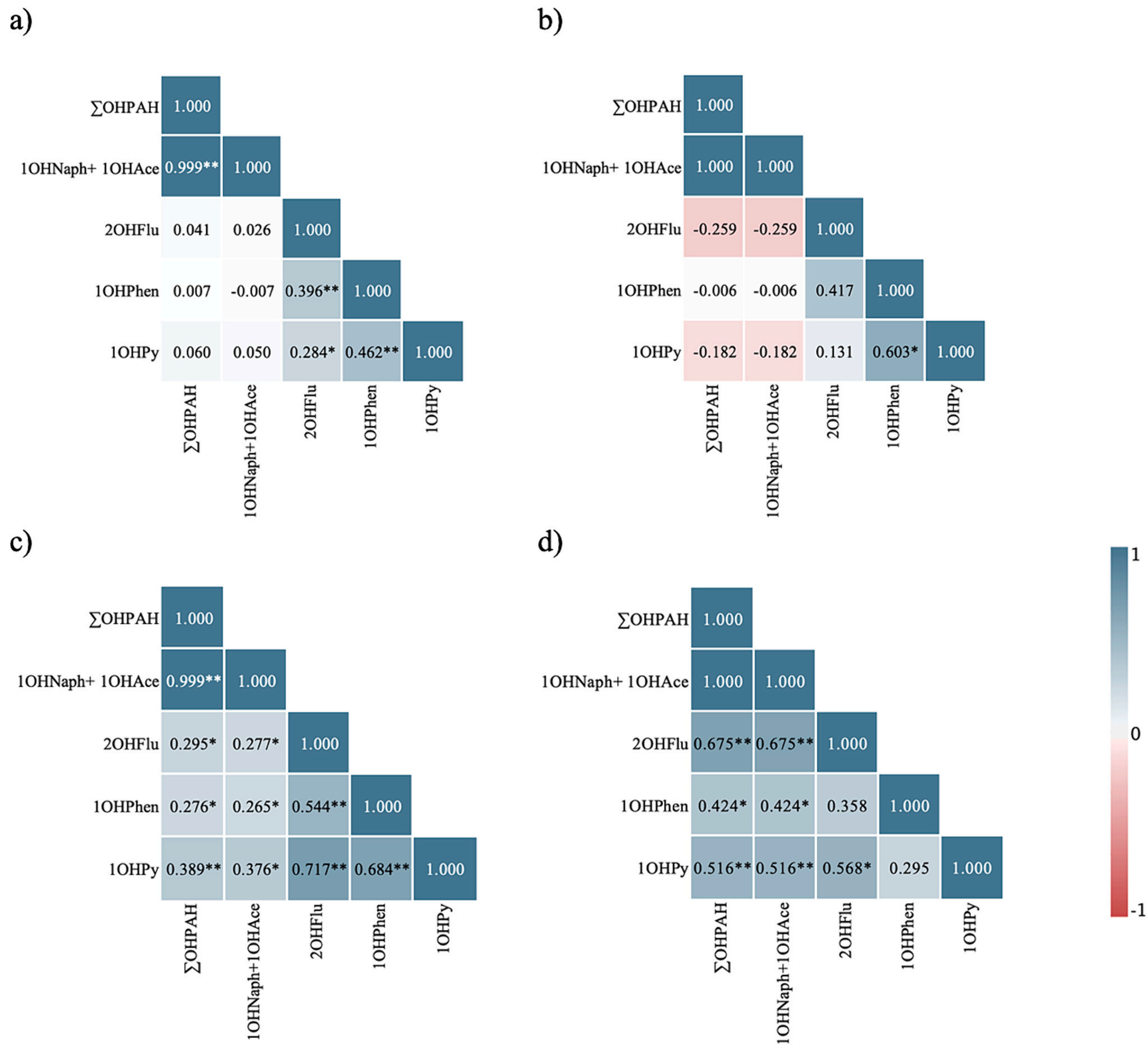

Fig. 3. Spearman's correlation matrix between the urinary PAH metabolites and ∑OHPAH in: a) non-smoking non-exposed firefighters, b) non-smoking exposed firefighters, c) smoking non-exposed firefighters, and d) smoking exposed firefighters. ∑OHPAH: sum of individual OHPAH; 1OHNaph + 1OHAce: 1-hydroxynaphthalene and 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene. *Statistically significant differences at $p \leq 0.05$; ** Statistically significant differences at $p \leq 0.001$.

Apart from the correlation between 1OHPhen and 1OHPy ($r = 0.603$; $p = 0.005$), the correlations determined in the same group (NSExp) were non-significant ($0.131 < r < 0.417$; $p > 0.05$). These differences could be attributed not only to distinct sources of exposure but also to variations in PAH metabolism, especially since 1OHPhen and 1OHPy may have similar half-lives, as reported by Rossbach et al. (2020).

On the other hand, all correlations between ∑OHPAH and each OHPAH in SNExp firefighters (Fig. 3 c)) were positive and significant ($0.276 < r < 0.717$; $p \leq 0.03$), evidencing that cigarette smoking was a shared source of PAH. Furthermore, smoking non-exposed firefighters consumed on average 16 cigarettes per day (Table 1). Consequently, these individuals are constantly exposed to tobacco-related PAH throughout the day comparatively with non-smokers and, therefore, PAH are continuously being metabolised and excreted. As a result, time of post-exposure is not as critical as it is for non-smoking exposed firefighters, which corroborates the previous findings of the individual pre- to post-fire urinary concentrations comparison.

Moderate to strong correlations were observed for SExp firefighters (Fig. 3 d)) between urinary 1OHNaph + 1OHAce and 2OHFlu ($r = 0.675$; $p < 0.001$), 1OHPhen ($r = 0.424$; $p = 0.04$), and 1OHPy ($r = 0.516$; $p = 0.008$). However, 1OHPhen was weakly correlated with 2OHFlu and 1OHPy ($0.295 < r < 0.358$; $p > 0.05$) in this group, which may indicate that these firefighters were exposed to additional sources of phenanthrene (*e.g.*, vehicle exhaust emissions, outdoor air pollution, cooking processes such as grilling, roasting). Rogula-Kozłowska et al. (2020) identified phenanthrene as one of the few PAH in firefighters changing rooms that did not originated from indoor sources.

A PCA was performed based on 1OHNaph + 1OHAce, 2OHFlu, and 1OHPy concentrations of the four groups (Fig. S3, Supplementary Material). Together, F1 and F2 account for 89.3 % of the original data, and therefore constitute a good approximation of it. 2OHFlu and 1OHPy are positively correlated (indicated by the small angle between their vectors) and not as well-correlated with 1OHNaph + 1OHAce (large angle between their vectors), which is in agreement with the Spearman's correlation matrix (Fig. 3). F1 effectively separated the NSNExp group (89 % of samples on the negative side of F1) from the remaining groups (73–96 % of samples on the positive side of F1), *i.e.* there was a clear separation of the group without exposure to tobacco smoke or wildfire emissions from the groups under at least one of these sources of exposure. This is in line with the statistical differences found between the NSNExp and the remaining groups for 1OHNaph + 1OHAce, 2OHFlu, and 1OHPy concentrations. While the clusters of the NSExp and SExp observations are predominantly on the positive side of F1 and F2, possibly influenced by the lack of statistical difference for 1OHNaph + 1OHAce between these two groups, the NSExp cluster is displaced towards lower values of F1 (0–1.5) and higher values of F2 (0.5–2) and, conversely, the SExp cluster tends towards higher values of F1 (1.5–3) and lower values of F2 (0–0.5). This mild separation is likely due to the statistical differences in the 2OHFlu and 1OHPy concentrations between these two groups. Projecting the observations of the SExp cluster onto the vertical axis, these firefighters are positioned higher than the bulk of the SNExp firefighters (who, despite somewhat dispersed, are mostly on the positive side of F1 and negative side of F2), thus separating these two groups, though not as evident as the one observed for NSNExp and NSExp. Accordingly, as previously mentioned, while both non-smokers and smokers are impacted by wildfire emissions, the impact is stronger among non-smokers.

Regarding the correlations with the occupational factors retrieved from the questionnaires, no significant positive correlations were observed between any OHPAH and the number of hours of firefighting activities. The strongest positive correlation was for the urinary levels of 2OHFlu among SExp firefighters ($r = 0.327$; $p > 0.05$). The half-lives of OHPAH are not yet well established in particular for inhalation and dermal contact, and when multiple routes of exposure occur. Available literature is mainly focused on 1OHPy, yet half-lives for other OHPAH have been proposed: i) 6–35 h for inhalation (1OHPy); ii) 2.3–23.5 h for ingestion (1OHNaph, 2OHFlu, 1OHPhe, 1OHPy), and iii) 13 h for dermal contact (1OHPy) (Gill and Britz-McKibbin, 2020; Li et al., 2012). These knowledge gaps pose a substantial challenge when it comes to determining the optimal sampling times, especially when multiple biomarkers are being assessed, and considering that different exposure routes play a part in firefighters' exposure while fire combating. Further, multiple chemical exposures can result in synergetic or antagonistic effects on the metabolism of PAH.

Regarding the years of service as a firefighter, moderate negative correlations were observed with the urinary levels of $\sum$OHPAH and 1OHNaph + 1OHAce in NSExp subjects ($r = -0.556$; $p = 0.02$), while in the SExp subjects these correlations were moderate and positive ($r = 0.411$; $p = 0.05$). The other correlations obtained were weak to moderate and lacked statistical significance. Řiháčková et al. (2023) found significant correlations between the career length of professional non-smoking firefighters and the concentrations of 1OHPhen ($r = 0.5$) and 1OHPy ($r = 0.3$). Despite their relatively quick excretion after exposure, PAHs are lipophilic compounds, and as such, can bioaccumulate in the human body, namely in fat tissues (Oliveira et al., 2020c; ATSDR, 1995). Thus, they can be mobilized when fat is burned, which may explain the low but significant *r*. One possible explanation for the observed negative correlation could be age-related slower metabolism due to a less active lifestyle and natural ageing (Bao et al., 2023). In this study, firefighters' median age was nearer middle-age class (median: 32–39; maximum of 65 years old), and almost one third of subjects had >20 years of service (Table 1). Therefore, further research is necessary to elucidate the relationship between the number of years spent working as a firefighter and the urinary levels of metabolites in exposed firefighters.

The correlations between BMI and the urinary levels of each and total OHPAH were negative and non-significant ($-0.295 < r < -0.018$; $p > 0.05$) for smoking firefighters, except for 1OHNaph + 1OHAce and $\sum$OHPAH ($0.034 < r < 0.035$; $p > 0.05$) in non-exposed subjects. For the non-smoking groups, the correlations were weak but mostly positive ($-0.386 < r < 0.243$; $p > 0.05$), and the only significant correlation was observed for 2OHFlu ($r = 0.227$; $p = 0.03$) among non-exposed firefighters. These findings were expected since no significant differences were observed in the BMI of participants (Table 1). However, it is expected that smoking habits can increase the individuals' metabolic rate and contribute to the loss of appetite, thus lowering the BMI of some individuals (Audrain-McGovern et al., 2011). Moreover, cigarette smoking can increase PAH exposure while simultaneously decreasing BMI, explaining the negative correlations. Řiháčková et al. (2023) found significant positive correlations between the BMI and 1OHPhen and 1OHPy ($r = 0.3$) in non-smoking professional firefighters and no correlation for new trainees.

Among SNExp firefighters, the daily number of smoked cigarettes exhibited the strongest correlation with urinary 2OHFlu ($r = 0.344$; $p = 0.004$), the metabolite that showed the largest increase when comparing smokers to non-smokers (Fig. 1 c)). Levels of 1OHPy also presented a significant correlation with the number of cigarettes consumed per day ($r = 0.307$; $p = 0.01$). However, the correlation coefficients determined for 2OHFlu and 1OHPy were lower among SExp firefighters, suggesting that the number of smoked cigarettes per day was less impactful among firefighters exposed to fire emissions. It is still worth noting that 1OHNaph + 1OHAce (and $\sum$OHPAH) had higher correlation coefficients among SExp than SNExp ($r = 0.067$; $p > 0.05$ *versus* $r = -0.102$; $p > 0.05$) and that the firefighters that reported the highest number of daily cigarettes (35 and 30) were the ones with the highest $\sum$OHPAH post-exposure.

3.5. Short-term health effects

Exposure to smoke causes short-term health effects, thus firefighters are likely to experience them during and/or after firefighting. The most reported symptoms by firefighters in the present study were eye and respiratory tract irritation while firefighting (27 %) and headaches within 8 h post-fire (13 %) (Fig. S4, Supplementary Material). While the percentage of firefighters having shortness of breath dropped to 2 % within 8 h, 11 % still reported irritated eyes and respiratory tract, indicating that this was a more prolonged symptom. Vomiting was not reported during or after firefighting. 1OHPy concentrations were significantly higher in firefighters who reported at least one symptom within 8 h after firefighting compared to those who experienced none (0.690 *versus* 0.454 μmol/mol creatinine; $p = 0.03$), and in those who had eye and respiratory tract irritation within 8 h after firefighting compared to those who did not (0.637 *versus* 0.456 μmol/mol creatinine; $p = 0.04$). Interestingly, 1OHNaph + 1OHAce levels (and $\sum$OHPAH) were statistically higher in firefighters who did not have eye and respiratory tract irritation within 8 h after wildfire combating compared to those who presented these symptoms (42.0 *versus* 30.5 μmol/mol creatinine; $p = 0.02$). More studies are needed to better characterize the influence of PAH exposure on short-time health effects and to understand the relationships with biomarkers levels and the duration of fire combat activities.

The levels of white blood cells count (total, and differential count, *i.e.*, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) (Fig. 4A) and percentage distribution of cell subtypes were also examined. After exposure (Fig. 4B), median total white blood cells count was significantly increased 1.4 (smokers, $p = 0.025$) to 3.7-fold (non-smokers, $p < 0.001$). This immune cell profile alteration was mostly basophilic (2 to 2.4-fold, $0.008 < p < 0.023$), which is characteristic of an allergenic reaction that followed a significant reduction in the number of neutrophils (−30 to −42 %, $0.001 < p \leq 0.05$), lymphocytes (−27 to −55 %, $0.001 < p \leq 0.02$), monocytes (−21 to −46 %, $0.001 < p \leq 0.03$), and eosinophils (−33 to −35 %, $0.017 < p \leq 0.03$) (Fig. 4B). A different pattern was observed in firefighters and fire service instructors after exposure to heat (Walker et al., 2015; Watt et al., 2016), structural fire training instructors (Watkins et al., 2021, 2019), and in firefighters after a forest fire exercise (with and without wood smoke exposure; Swiston et al., 2008); in these studies, a neutrophil/monocyte/

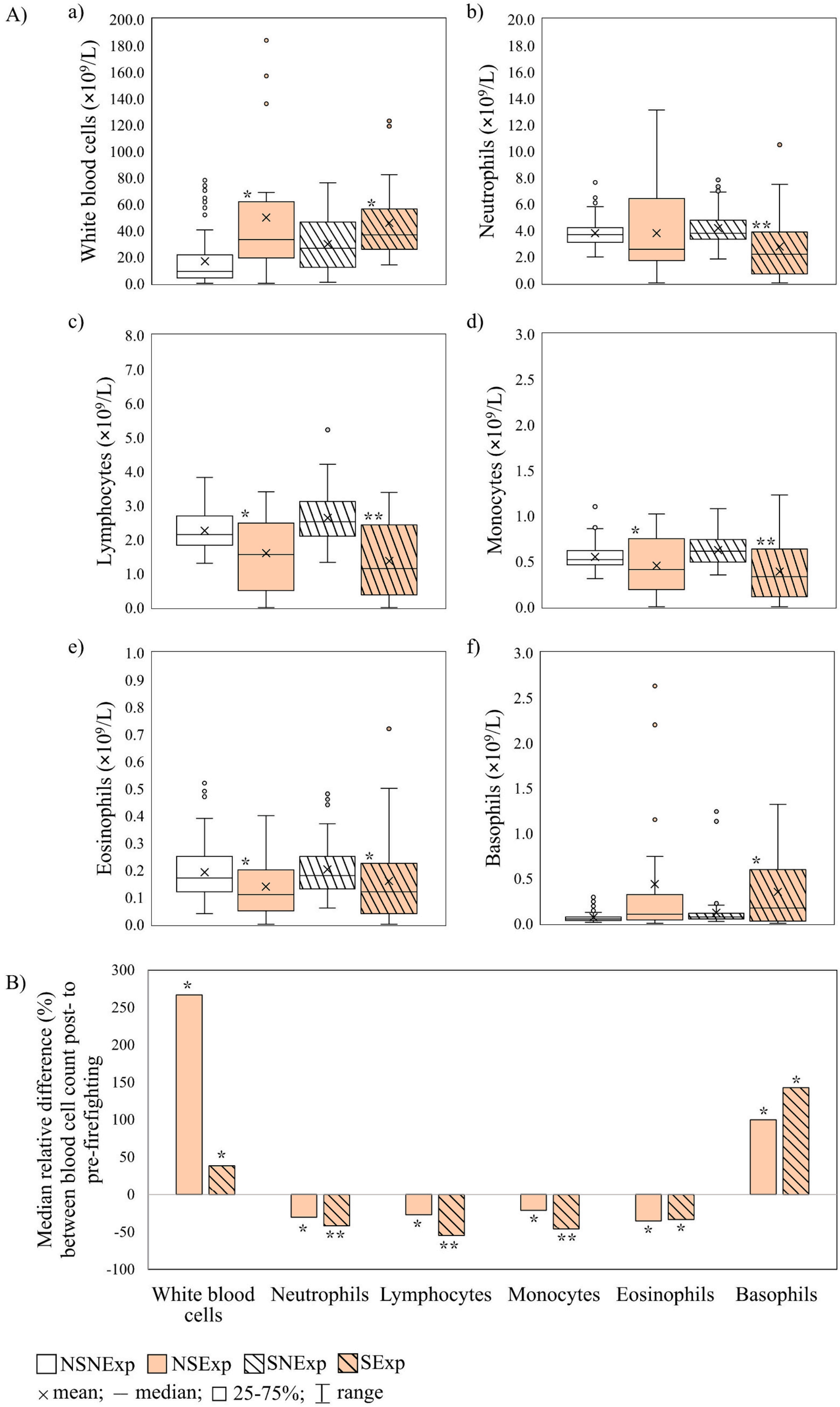

Fig. 4. A) Levels of a) total white blood cells and differential count, *i.e.*, b) neutrophils, c) lymphocytes, d) monocytes, e) eosinophils and f) basophils, among firefighters according to exposure and smoking status. B) Relative difference (median, %) between blood count of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils post- to pre-firefighting among non-smoking and smoking firefighters. NSNExp: Non-smoking Non-exposed; NSExp: Non-smoking Exposed; SExp: Smoking Exposed; SNExp: Smoking Non-exposed. *Statistically significant differences at $p \leq 0.05$; **Statistically significant differences at $p \leq 0.001$.

lymphocyte variation was more evident. Nonetheless, the observation of lower blood monocytes and lymphocytes (count and percentage), along with higher basophil production, could be related to post-exposure inflammation in the lungs, promoting the recruitment of immune cells from blood into the lungs for a localized response. This has been observed in firefighters after wildfire combat simulations (Ferguson et al., 2016). A high percentage of neutrophils, lymphocytes, and monocytes have also been identified in firefighters' sputum after combat activities (Barros et al., 2021). Moreover, Swiston et al. (2008) reported the impact of physical activity on total blood cells to be higher after firefighting exercising throughout the day (without smoke exposure) and that it did not significantly differ from the same training with exposure to smoke in wildland firefighting trainees. However, comparing controlled exposure during training to live wildfire events is not fully appropriate due to large differences in the number of work-hours and total exposure to fire emissions, which are more intense during live fire events than in training, thus supporting the different pattern in white blood cell count observed in post-exposed Portuguese firefighters. Differences in the type of PPE could have influenced the exposure and health impacts since the airway route of wildland firefighters might be less protected than structure firefighters who use SCBA. Furthermore, positive correlations between urinary OHPAH and blood haematological parameters (count) were observed for: i) NSNExp: white blood cells (1OHNaph + 1OHAce and $\sum$OHPAH: $0.999 < r < 1.0$; $p < 0.001$), neutrophils (1OHNaph + 1OHAce and $\sum$OHPAH: $0.281 < r < 0.286$; $p < 0.008$), monocytes (1OHNaph + 1OHAce, 1OHPy and $\sum$OHPAH: $0.221 < r < 0.230$; $p < 0.04$); ii) SNExp: white blood cells (1OHNaph + 1OHAce, 2OHFlu, 1OHPhe, 1OHPy, and $\sum$OHPAH: $0.314 < r < 1.0$; $p < 0.01$), lymphocytes (1OHPhe and 1OHPy: $0.248 < r < 0.275$; $p < 0.05$), monocytes (1OHPy: $r = 0.309$; $p = 0.04$), basophils (1OHPy: $r = 0.343$; $p = 0.006$); iii) NSExp: white blood cells (1OHNaph + 1OHAce and $\sum$OHPAH: $r = 0.840$, $p < 0.001$), neutrophils (1OHNaph + 1OHAce, $\sum$OHPAH: $r = 0.488$; $p = 0.03$), lymphocytes (percentage, 1OHPhe and 1OHPy: $0.480 < r < 0.675$; $p < 0.04$), monocytes (1OHNaph + 1OHAce and $\sum$OHPAH: $r = 0.534$; $p = 0.02$), and eosinophils (percentage, 1OHNaph + 1OHAce and $\sum$OHPAH: $r = -0.524$; $p = 0.02$); iv) SExp: white blood cell (1OHNaph + 1OHAce, 2OHFlu, 1OHPy, and $\sum$OHPAH: $0.518 < r < 0.882$; $p < 0.02$). Weak correlations between immune cells in blood and individual and total levels of OHPAH in non-exposed firefighters turned into moderate to strong correlations after wildland firefighting, evidencing the impact of PAH exposure on blood immune cells count. This association was more evident for 1OHNaph + 1OHAce > $\sum$OHPAH >1OHPy > 1OHPhe, with most associations related with an effect on total white blood cells > lymphocytes > monocytes > neutrophils. OHPAH-basophils associations were not observed (except in SNExp), whereas a negative association between OHPAH and eosinophils percentage was detected in NSExp, empathizing that wildfire PAH exposure may be linked to a migration of eosinophils from the blood to a localized allergenic response. An indirect induction of a pro-allergic immune process by PAH has been observed *in vitro* (Schober et al., 2007) and *in vivo* (Zakharenko et al., 2017), that could justify the lack of a direct correlation between OHPAH and basophils, and OHPAH with the reduced percentage of blood eosinophils. Moreover, current research has highlighted that aryl hydrocarbon receptor (PAH agonist) can have a dual-effect and mediate both the activation of type-2 immunity (humoral response, *i.e.*, B lymphocyte-mediated) and allergenic response (Yu et al., 2022), thus corroborating the hypothesis of a similar effect of these compounds in wildland firefighters. On the other hand, for 2OHFlu, correlations were only significant in smokers, supporting the previous findings that this metabolite is more discriminant of tobacco consumption than wildland firefighting exposure, justifying the observed limited correlations with haematologic parameters. The chronic effects of cigarette smoking on the blood immune cell population have been documented (Qiu et al., 2017). In that sense, even though a significant increased total white blood cell count was detected in SExp, the assessment of exposure effects in differential cell types was limited. To the best of the authors' knowledge, this study represents the first attempt to investigate the relationships between urinary PAH metabolites and haematological parameters in firefighters. Therefore, further data are required.

It is of interest to investigate correlations between the concentration of the studied biomarkers of exposure and biomarkers of effect in firefighters. Positive correlations have been found between 1OHPy and malondialdehyde, which is a biomarker of effect related to oxidative stress, in firefighters exposed to prescribed burns, and also DNA strand breaks in the blood of trainees after live-firefighting exercises (Adetona et al., 2019; Andersen et al., 2018). Oliveira et al. (2020b) found positive correlations between the urinary levels of OHPAH (1OHNaph + 1OHAce, 2OHFlu, 1OHPhe, and 1OHPy) and DNA oxidative damage and cardiac frequency.

Overall, further studies involving real-life scenarios, and including both non-smoking and smoking firefighters, that investigate the associations of OHPAH with biomarkers of effect are needed to better characterize the health risks of increased exposure to PAH that these professionals face. These should include (if possible due to the challenging operational study design) the monitoring of the parent compounds in the air during firefighting occurrences due to the high variability of smoke composition, and also those deposited in the skin of firefighters. This would aid in determining the specific contribution of each source to the total body burden, that was not undertaken in this study. Some knowledge gaps need also to be surpassed, namely, it is crucial to define the half-lives of biomarkers for different routes of exposure in order to develop the best sampling strategy, having into account the presence of other chemicals that can interact with them and, therefore, interfere with their half-lives. Lastly, considering the variability of biomarkers concentrations within the same individual with time, several samples should be collected after exposure to wildfire emissions; this option was not considered within the scope of this study. However, under real-life wildland firefighting activities, besides being physically and psychologically exhausted, firefighters may display different degrees of dehydration after several hours and days of fire combating, which also hinder the application of highly structured and complex study designs.

4. Conclusion

This study assessed the impact of PAH exposure of firefighters in real-life wildfires, *i.e.*, unsimulated fire events where firefighters are involved for varying periods of time, performing different tasks and being exposed to smoke with changing compositions due to meteorological conditions and burned materials (trees, vegetation, wastes, *etc.*). Exposure to wildfire emissions promoted significantly increased urinary levels of $\sum$OHPAH, 1OHNaph + 1OHAce, 2OHFlu, 1OHPhen, and 1OHPy in firefighters, regardless of their smoking habits. However, non-smoking firefighters were more affected than smoking firefighters by wildfire emissions. Out of the six considered metabolites, urinary 1OHNaph + 1OHAce were the biomarkers that exhibited the highest increase due to exposure to wildfire emissions, whereas 2OHFlu was the most affected by tobacco smoke and both sources combined. Apart from 3OHB(*a*)P that was not detected, 1OHPy (the established biomarker of exposure to PAH) was the least abundant OHPAH in all groups of participants. These findings highlight the importance of including different biomarkers of exposure when assessing occupational exposure to PAH. Furthermore, following exposure, there was a notable significant rise in the white blood cells count, with strengthened OHPAH associations with total white blood cells (and lymphocytes, monocytes, and neutrophils in non-smokers), showing that PAH released from wildfire impact the blood immune cells.

The attained data, and considering the scarcity of human biomonitoring assessment information about real-life wildland firefighting in Europe, but also worldwide, identify Portuguese firefighters with an increased level of exposure to 1OHNaph + 1OHAce (as compared to the

Portuguese and European general population, and to 2OHFlu, 1OHPhen, and 1OHPy depending on the group), while contributing to establish reference values, and strengthening the need to apply mitigation measures.

Funding

This work was financially supported by the project PCIF/SSO/0017/2018 (doi:10.54499/PCIF/SSO/0017/2018) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds.

Ethics approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project (PCIF/SSO/0017/2018): "A panel of (bio)markers for the surveillance of firefighter's health and safety".

CRediT authorship contribution statement

Ana Margarida Paiva: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Bela Barros:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Marta Oliveira:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Sara Alves:** Conceptualization, Data curation, Methodology, Resources. **Filipa Esteves:** Data curation, Formal analysis, Investigation, Methodology, Supervision. **Adília Fernandes:** Investigation, Methodology, Supervision, Writing – review & editing. **Josiana Vaz:** Investigation, Methodology, Supervision, Writing – review & editing. **Klára Slezáková:** Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **João Paulo Teixeira:** Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Solange Costa:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Simone Morais:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgment

This work received support by UIDB/50006/2020 (DOI:10.54499/UIDB/50006/2020), UIDP/50006/2020 (DOI:10.54499/UIDP/50006/2020), LA/P/0008/2020 (DOI:10.54499/LA/P/0008/2020) and through the project PCIF/SSO/0090/2019 (doi:10.54499/PCIF/SSO/0090/2019) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. The authors would also like to thank to FCT and European Union through Fundo Social Europeu (FSE) which supported the scientific contract CEEC- Individual 2017 Program Contract CEECIND/03666/2017 (DOI:10.54499/CEECIND/03666/2017/CP1427/CT0007) and the PhD grant 2020.07394.BD, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171801.

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