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Individual and cumulative impacts of fire emissions and tobacco consumption on wildland firefighters' total exposure to polycyclic aromatic hydrocarbons

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Highlights:

- Impact of fire emissions and smoking on firefighters' exposure to PAHs was assessed.
- Smoking increased 76-412% the levels of total urinary metabolites (OH-PAHs).
- Firefighting activities raised 158-551% the total OH-PAHs concentrations.
- 2-hydroxyfluorene was the most affected metabolite by firefighting activities.
- 1OHNaph+1OHAce presented the more pronounced increase due to tobacco consumption.

Abstract

There is limited information about wildland firefighters' exposure to polycyclic aromatic hydrocarbons (PAHs), being scarce studies that included the impact of tobacco consumption. Thus, this work evaluated the individual and cumulative impacts of firefighting activities and smoking on wildland firefighters' total exposure to PAHs. Six urinary PAH metabolites (1-hydroxynaphthalene (1OHNaph), 1-hydroxyacenaphthene (1OHAce), 2-hydroxyfluorene (2OHFlu), 1-hydroxyphenanthrene (1OHPhen), 1-hydroxypyrene (1OHPy), and 3-hydroxybenzo[a]pyrene (3OHB[a]P)) were quantified by high-performance liquid chromatography with fluorescence detection. Firefighters from three fire stations were characterized and organized in three groups: non-smoking and non-exposed to fire emissions (NSNExp), smoking non-exposed (SNExp), and smoking exposed (SExp) individuals. 1OHNaph+1OHAce were the most predominant OH-PAHs (66-91% \sum OH-PAHs), followed by 2OHFlu (2.8-28%), 1OHPhen (1.3-7%), and 1OHPy (1.4-6%). 3OHB[a]P, the carcinogenicity PAH biomarker, was not detected. Regular

consumption of tobacco increased 76-412% \sum OH-PAHs. Fire combat activities promoted significant increments of 158-551% \sum OH-PAHs. 2OHFlu was the most affected compound by firefighting activities (111-1068%), while 1OHNaph+1OHAce presented the more pronounced increments due to tobacco consumption (22-339%); 1OHPhen (76-176%) and 1OHPy (20-220%) were the least influenced ones. OH-PAH levels of SExp firefighters were significantly higher than in other groups, suggesting that these subjects may be more vulnerable to develop and/or aggravate diseases related with PAHs exposure.

Keywords: Biomonitoring; Firemen; Occupational exposure; Tobacco smoking; Urinary monohydroxyl metabolites (OH-PAHs).

1 Introduction

Human biomonitoring allows assessment of total personal exposure, including environmental and workplace hazardous chemicals, through the analysis of chemicals and/or their metabolites in human exhaled air, blood, urine, milk, saliva, nails, hair, faeces, and adipose tissue [1-2]. Among the many human matrices, urine is by far the most common one [3-5]. Thus the use of human biomonitoring is a precious tool as it estimates the total internal dose regardless of the source, route, and duration of the exposure.

Occupational exposure of firefighters, i.e. firefighters directly involved in firefighting activities, was classified as a possible carcinogen to humans by the International Agency for Research on Cancer (IARC group 2B; [6]) and the US National Institute for Occupational Safety and Health (NIOSH; [7]). Wildland firefighters are frequently

exposed to numerous pollutants (particulate matter, carbon monoxide, nitrogen dioxide, volatile organic compounds including polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers, polychlorinated dibenzo-p-dioxin, *etc.*) that are released during fires [5,8-13]. Exposure to wildfire smoke is being associated with respiratory morbidity with growing evidence supporting an association with all-cause mortality, being consistently related with cardiovascular effects, specific causes of mortality, birth outcomes, and mental health disorders [14].

Tobacco consumption is an additional risk factor for potential health effects, since prolonged exposure to tobacco smoke is, by itself, the major cause of lung cancer [15]. Tobacco smoke is a complex mixture of thousands of chemical substances including over 69 known carcinogens; therefore, tobacco smoking and its exposure are classified as known human carcinogens by most of the international agencies [15-16].

In terms of the main constituents isolated from the mainstream smoke of cigarettes and air emissions released during wildland and agricultural fires, PAHs are a relevant group of compounds that cause carcinogenic effects [15]. PAHs are ubiquitous pollutants that are included in the lists of priority pollutants of the European Union [17] and US Environmental Protection Agency [18] due to their mutagenic and carcinogenic properties [19-21]. They are formed during incomplete combustion of organic matter and originate from both natural and anthropogenic sources [22-23]. Human exposure to PAHs occurs by all routes of exposure, namely inhalation, ingestion, and skin contact [8,24-28]. Once absorbed into blood stream, PAHs are biotransformed and distributed within human body, being mostly eliminated through urine as glucuronide and/or sulfate conjugates [22,29-30]. Thus, urinary metabolites of PAHs (OH-PAHs) reflect a more accurate estimation of the total exposure. Urinary 1-hydroxypyrene (1OHPy), the major metabolite of pyrene, is the most frequently used biomarker to assess PAHs exposure [1,11,31-33].

3-hydroxybenzo[a]pyrene (3OHB[a]P) is one of the metabolites of benzo[a]pyrene, and it is considered as the PAH biomarker of carcinogenicity [32]. 1-hydroxynaphthalene (1OHNaph) is frequently used to assess the exposure to naphthalene [34], a PAH that was selected as an important indicator of indoor air pollution by the World Health Organization (WHO) [35]. Due to the ubiquity of PAHs, their various sources and routes of exposure, the use of more than one PAHs biomarker better characterizes the total exposure of a subject.

Despite the undeniable role that firefighters play in maintaining population safety, limited information related with their occupational exposure to PAHs is available in the literature [4-5,11,31,33-34,36-38], being scarce studies that included the impact of tobacco consumption [34,37]. Moreover, biomonitoring of wildland firefighter's exposure to PAHs from fire emissions has been mostly performed during training exercises [11,34,37-38] and typically based exclusively on 1OHPy analysis [31,33,37-38]. Exposure to PAHs has been associated with the development and/or aggravation of lung function reduction, specifically with exacerbations of asthma and chronic obstructive pulmonary disease, some cardiovascular pathologies and cancer [39-41]. Excess morbidity and mortality in firefighters have been related with those diseases and some selected site-specific cancers comparatively with general population [42-47].

Thus, the present study aims to characterize the impact of tobacco consumption and occupational exposure on wildland firefighters' total exposure to PAHs. Six urinary PAH metabolites (1OHNaph, 1-hydroxyacenaphthene (1OHAce), 2-hydroxyfluorene (2OHFlu), 1-hydroxyphenanthrene (1OHPhen), 1OHPy and 3OHB[a]P) were determined in three different groups of subjects: i) non-smoking non-exposed firefighters (NSNExp) who were not involved in firefighting activities within the previous week of urine sampling, ii) smoking firefighters who were not exposed to any kind of fire emissions

(SNExp), and iii) smoking exposed firefighters (SExp) who actively participated in wildland fire combat activities (knockdown and/or overhaul) within the 48 hours prior the urine collection. Comparison between the urinary levels of individual and total OH-PAHs among the three groups was conducted in order to evaluate the individual and cumulative impacts of the tobacco consumption and participation in firefighting activities on wildland firefighters' total exposure to PAHs.

2 Materials and Methods

2.1 Characterization of the study population

A total of 108 healthy firefighters serving at three different fire stations, namely Vinhais (VNH), Mirandela (MDL) and Bragança (BRG) participated in this study. Fire stations were situated in Trás-os-Montes and Alto Douro Region, the Northeast area of the country that is every year strongly affected by wildfires [48]. This geographical area is characterized by very hot and dry summers, and cold winters. All firefighters of the selected fire stations were requested to fill a structured questionnaire, which was adapted from a validated form [49]. The questionnaire was used to collect firefighters' personal information on age, weight, number of years of occupational exposure as firefighters, time dedicated to firefighting activities in the last 48 hours and the use of personal protective equipment during that period; exposure to environmental tobacco smoke, tobacco smoking habits (including the number of cigarettes smoked per day for smokers), and the most frequently consumed meals (boiled, roasted, and grilled) during the week before urine collection. Also, some clinical parameters, such as cardiac frequency, individual diastolic and systolic blood pressure (Geratherm Desktop, Geratherm Medical AG, Geschwenda, Germany), and the arterial oxygen saturation (Oxy-100 pulse oximeter, Gima, Italy) were measured in each participant at the end of the work shift. Body mass

index (BMI; kg/m²) was determined for each fireman. According to the information collected through the questionnaires, firefighters were organized into three different groups: NSNExp, SNexp and Sexp. The protocol was reviewed and approved by the Ethic Committee of University of Porto, Portugal (Project Ethics Committee No. 12/CEUP/2015) and all participants gave a written informed consent.

2.2 Urine sampling, extraction and chromatographic analysis

Collection campaigns were performed at the three fire stations during June to September 2015. All firefighters were asked to collect a spot urine sample at the end of a regular work shift between Tuesday and Thursday in order to avoid the impact of different personal weekend activities. Urine samples were collected in sterilized 50 mL polycarbonate containers and frozen at -20 °C until analysis.

Extraction and chromatographic analysis of urinary OH-PAHs were done according to Oliveira et al. [5] and Chetianukornkul et al. [50]. Calibration curves were prepared with OH-PAH mixed standards in methanol except for 1OHNaph and/or 1OHAc that were quantified with a matrix-matched calibration curve; they presented good correlation coefficients (6 calibration points; $R^2 \geq 0.9979$). The methodology was validated by recovery experiments with values ranging from 70.0 to 117.5%. Detection limits varied between 0.0008 µg/L urine for 2OHFlu to 0.195 µg/L urine for 1OHNaph+1OHAc with respective quantification limits ranging from 0.0028 to 0.650 µg/L urine. Intra and inter-precision assays were performed during six consecutive days with RSD values ranging from 1.3% (2OHFlu) to 6.4% (1OHPhen) and between 1.3% to 8.1% (1OHNaph+1OHAc, and 1OHPy), respectively [5]. Representative chromatograms are exhibited in Figure 1S (Supplementary Material)

Urinary creatinine levels ($\mu\text{mol/mol}$) were quantified according to the Jaff colorimetric method [51] and used to normalize the urinary concentrations of OH-PAHs.

All determinations were performed in triplicate.

2.3 Statistical treatment

Statistical analysis was performed using SPSS (IBM SPSS Statistics 20) and Statistica (v. 7, StatSoft Inc., USA) software. Median values of individual and $\Sigma\text{OH-PAHs}$ were compared through the nonparametric Mann–Whitney U test since normal distribution was not observed by Shapiro-Wilk's test. Statistical significance was defined as $p \leq 0.05$.

3. Results and Discussion

3.1 Characterization of the study population

The overall mean ages were 34 years for NSNExp (22-48 years) and SNExp (21-60 years) firefighters, while SExp subjects had a mean age of 31 years (21-53 years); no statistical difference ($p > 0.05$) was observed between the three groups.

BMI, the most commonly used index of weight-for-height, is a tool commonly used to classify underweight ($< 18.5 \text{ kg/m}^2$), normal weight ($18.5\text{-}25 \text{ kg/m}^2$), overweight ($25\text{-}30 \text{ kg/m}^2$), and obesity (degree I: $30\text{-}35 \text{ kg/m}^2$; degree II: $35\text{-}40 \text{ kg/m}^2$; degree III: $\geq 40 \text{ kg/m}^2$) in adults [52]. The overall BMI values ranged between 19 kg/m^2 (SNExp of VNH and SExp of MDL) to 36 kg/m^2 (NSNExp of BRG) (mean 25 kg/m^2) (Table 1). A total of 61% of firefighters had normal BMI values; 22% of NSNExp, 3% of SNExp, and 14% of SExp individuals presented BMI values higher than 25 kg/m^2 , suggesting an overweight. Significant differences of BMI levels were observed between NSNExp (28 kg/m^2) and SNExp (24 kg/m^2 ; $p = 0.05$) groups, and among NSNExp and SExp subjects (24 kg/m^2 ; $p = 0.013$). NSNExp firefighters serving at MDL and BRG fire corporations had mean

BMI levels indicative of overweight, with some individuals revealing values of degree I and II of obesity [52] (Table 1). Obesity is a nutritional pathology that may conduct to the development of health problems [52]. The determined BMI levels are in accordance with the national report, which indicated that 52.8% of the Portuguese adult population was overweight in 2014 [53].

Arterial hypertension is an asymptomatic disease characterized by continually elevated blood pressure, usually diastolic blood pressure ≥ 90 mmHg and/or systolic blood pressure ≥ 140 mmHg [54]. The values of diastolic and systolic blood pressure in firefighters ranged between 49-114 and 120-152 mmHg for NSNExp, 61-97 and 108-152 mmHg for SNExp and from 60-98 to 113-156 mmHg for SExp subjects, respectively. A total of 83% of NSNExp and SNExp, and 61% of SExp firefighters presented normal values of blood pressure; 17% (NSNExp and SNExp) to 39% (SExp) of the participants revealed to have levels higher than the recommended limits (Table 1). The cardiac frequency observed in firefighters was overall within the recommended range of 60-100 heart beats/min [55]. The arterial oxygen saturation values were also within the normal range of 95-100% [55].

3.2 Detection rates of OH-PAHs

The detection of urinary OH-PAHs confirms the exposure to PAHs, regardless of the source. The collection of spot urine samples in occupational biomonitoring studies is frequently used, however the variability in the volume of urine collected and its total water content needs to be taken into consideration. To overcome these limitations, the creatinine adjustment is the most frequently used method to normalize the concentrations of urinary metabolites [56], thus it was applied in this study. Creatinine can be also used as a criterion of acceptability of the collected spot urine samples if its concentrations range between 0.3 g/L to 3.0 g/L [57]. In this study, urinary creatinine concentrations

varied from 0.706 to 2.90 g/L for NSNExp individuals, between 0.91 to 2.77 g/L for SNExp firemen, and 0.700 to 2.95 for SExp subjects. No significant differences were found between the groups ($p>0.05$). 1OHPhen was quantified in all samples, 1OHNaph+1OHAce, 2OHFlu, and 1OHPy were determined in 97% of the urine samples. 3OHB[a]P was never detected and thus it was excluded from the further analysis. Some authors have observed that PAHs with low molecular weight (2 and 3 aromatic rings, such as naphthalene, acenaphthene, fluorene, and phenanthrene) are predominantly eliminated as macromolecules conjugates through the urine [4-5,23,58-59]. Information related with pharmacokinetics of OH-PAHs is very scarce, reason why the elimination kinetics of PAH metabolites for each route of exposure remain unclear. For 1OHPy, the most characterized OH-PAH, the half-life excretion rate varies between 6-35 h [60-61], from 4.4-12 h [58,62] and up to 13 h [62-64] after inhalation, ingestion, and dermal exposures, respectively. For the other OH-PAHs, Li et al. [59] reported a half-life excretion rate of 3.3-6.2 h for 1OHNaph, 2.3-4.0 h for 2OHFlu, and 4.3-6.1 h for 1OHPhen for ingestion in non-smoking volunteers with no occupational exposure to PAHs. With increasing molecular weight of PAHs, the complexity of their metabolism also increases so metabolites of high molecular weight PAHs are predominantly eliminated through the feces rather than in urine [59,65]. Fernando et al. [34] detected 1OHPy and 3-hydroxyfluoranthene in less than 50% of the total samples. Other authors have also reported very low detection rates for 3OHB[a]P in exposed (smoking and non-smoking) workers [59,66-67].

3.3 Impact of tobacco smoke

The concentrations of individual compounds in NSNExp and SNExp groups, organized per fire station, are presented in Figure 1. The levels of 1OHNaph+1OHAce (0.033-0.98

$\mu\text{mol/mol}$ creatinine; 0.138-4.19 $\mu\text{g/L}$ urine), 1OHPhen (0.002-0.077 $\mu\text{mol/mol}$ creatinine; 0.007-0.362 $\mu\text{g/L}$ urine), and 1OHPy (0.004-0.089 $\mu\text{mol/mol}$ creatinine; 0.022-0.369 $\mu\text{g/L}$ urine) (Figure 1) determined in this study for NSNExp individuals were well below the reference values defined by the German Human Biomonitoring Commission for the general non-smoking population: <30 $\mu\text{g/L}$ urine for 1OHNaph [68], 0.6 $\mu\text{g/L}$ urine for 1OHPhen and 0.5 $\mu\text{g/L}$ urine for 1OHPy [68-70]. The levels were in a similar range as those reported by Haines et al. [71] for the Canadian population during the third cycle (2012-2013) of a large ongoing study (2007-2019) conducted by the Canadian Health Measure Surveys, and also in agreement with the concentrations previously reported for other NSNExp Portuguese firefighters [4]. In European countries, the consumption of any kind of tobacco was restricted and/or forbidden in enclosed public places, on public transport and in workplaces, with only limited exceptions allowed for some particular economic sectors [72]. Thus firefighters considered in this study were not exposed to environmental tobacco smoke in the facilities of the respective fire stations. Non-smoking firefighters also reported in their questionnaire that they were not exposed to environmental tobacco smoke outside of their fire corporation in the week preceding the urine collections. The quantification of urinary cotinine, the principal metabolite of nicotine (biomarker of exposure to tobacco smoke) allows validating data (obtained by the questionnaires) concerning the smoking habits of firefighters; however, it was not determined in this study. Still, there is generally a high concordance level between the information reported in the questionnaires of smoking industrial workers and the urinary concentrations of cotinine [73], being the differences observed between NSNExp and SNExp firefighters attributed to tobacco consumption. Also, it should be taken in consideration that the studied individuals reported diets exclusive of charbroiled and

deep-fried foods within the five days before urine collection, so the contribution of food to the overall PAHs exposure can be considered as negligible.

Levels of total OH-PAHs (\sum OH-PAHs) in Portuguese firefighters are exhibited in Table 2. At the three fire stations, the median concentrations of individual (Figure 1) and total (Table 2) compounds in SNExp were overall higher than in NSNExp subjects. Urinary concentrations of 1OHNaph+1OHAce for SNExp firefighters were increased from 22% (VNH: 0.097 *versus* 1.18 $\mu\text{mol/mol}$ creatinine; $p>0.05$) to 339% (MDL: 0.67 *versus* 2.96 $\mu\text{mol/mol}$ creatinine; $p=0.024$) comparatively with NSNExp individuals. The urinary levels of the other PAH metabolites followed the same tendency: 29% (BRG: 0.017 *versus* 0.022 $\mu\text{mol/mol}$ creatinine; $p>0.05$) to 188% (VNH: 0.118 *versus* 0.340 $\mu\text{mol/mol}$ creatinine; $p=0.009$) for 2OHFlu, 76% (VNH: 0.029 *versus* 0.051 $\mu\text{mol/mol}$ creatinine; $p=0.002$) to 176% (MDL: 0.074 *versus* 0.204 $\mu\text{mol/mol}$ creatinine; $p>0.05$) for 1OHPhen, and 20% (VNH: 0.020 *versus* 0.024 $\mu\text{mol/mol}$ creatinine; $p>0.05$) to 167% (BRG: 0.009 *versus* 0.024 $\mu\text{mol/mol}$ creatinine; $p=0.005$) for 1OHPy (Figure 1). Feunekes et al. [37] also found concentrations of 1OHPy that were 213% higher in the urine of smokers compared with non-smoking firefighters (0.47 *versus* 0.15 $\mu\text{mol/mol}$ creatinine; $p=0.01$) during a set of controlled fires in a confined training area. Overall, tobacco consumption promoted a \sum OH-PAHs concentration increase of 76% (BRG) to 412% (VNH) (Table 2). In agreement, Fernando et al. [34] previously reported higher levels of urinary OH-PAHs for smoking firefighters during training exercises at Canadian burn houses. In addition, the majority of available studies (mostly non-occupational ones) also concluded that smokers possess higher levels of urinary OH-PAHs than non-smoking subjects [74-75], which may be attributed to the occurrence of genetic polymorphisms in the carcinogen metabolizing enzymes [15,74]. Benzo[a]pyrene, the only known human carcinogenic PAH [17] has been detected in cigarette smoke only in small quantities and therefore it is

difficult to detect its metabolites in the urine of smoking people [15,61]. Haines et al. [71] detected benzo[a]pyrene, chrysene, and fluoranthene metabolites in less than 2% of the urine samples collected in Canadian citizens within the Canadian Health Measures Survey during the third cycle of the study. No significant differences were observed between the distribution profiles of OH-PAHs in NSNExp and SNEExp subjects, with 1OHNaph+1OHAce accounting for 66-91% of Σ OH-PAHs, followed by 2OHFlu (4.9-21%), 1OHPhen (1.8-6.9%), and 1OHPy (1.5-6.3%) (Figure 2a, b). Still, Lu and Zhu [76] described a similar profile for 17 airborne PAHs in the tobacco smoke of 12 commercial brand cigarettes (2-3 aromatic rings accounted with 74% of Σ PAHs; 4-ring compounds represented 11% of Σ PAHs and PAHs with 5 and more aromatic rings contributed with 15% of Σ PAHs).

The relation between the urinary levels of individual and total OH-PAHs with the number of smoked cigarettes for each SNEExp subject was explored through the determination of Spearman correlation coefficients (r). The attained correlations were predominantly medium to high ($r \geq 0.878$ and $p \leq 0.021$ at BRG; $r \geq 0.603$ and $p \leq 0.205$ at VNH; $r = 0.878$ and $p \leq 0.021$ at MDL, except for 1OHNaph+1OHAce and 2OHFlu at VNH and MDL fire corporations). Hagedorn et al. [74] also observed significant correlations ($r = 0.34$ to 0.63 and $p < 0.001$) between the levels of urinary OH-PAHs with the daily cigarette consumption in smokers, revealing that smokers of cigarettes with higher tar yield excreted significantly higher amounts of urinary 1- and 2OHNaph, 2OHFlu, 1+9OHPhen, 2+ 3OHPhen, and 1OHPy than non-smokers.

3.4 Impact of fire emissions

Since no significant differences were observed between the number of cigarettes consumed by SNEExp and SExp firefighters ($p > 0.05$), these two groups were used to study

the influence of fire emissions on firefighter's Σ PAHs exposure. Figure 3 presents the median concentrations of each OH-PAH and the respective ranges for SNExp and SExp firefighters per station, while the respective distribution (%) is exhibited in Figure 2 (b, c). Firemen who were directly involved in wildland fire combats reported the use of self-protective equipment with flame-retardant properties, helmet with eye protection, flashhood and gloves, as required by the Portuguese legislation [77]. However, the urinary median concentrations of individual (Figure 3) and Σ OH-PAHs (Table 2) was overall higher in SExp than in SNExp firefighters. 1OHNaph+1OHAce were by far the most predominant OH-PAHs, accounting for 72-91% and 66-84% of Σ OH-PAHs for (smoking) non-exposed and exposed subjects, respectively (Figure 2b, c). Their concentrations increased between 48% (MDL: 2.96 *versus* 4.38 $\mu\text{mol/mol}$ creatinine) and 268% (VNH: 1.18 *versus* 4.35 $\mu\text{mol/mol}$ creatinine) for SExp firefighters compared with SNExp subjects ($p \leq 0.024$; Figure 3). 2OHFlu was the second most abundant PAH metabolite (2.8-21% of Σ OH-PAHs for SNExp and 13-28% for SExp firefighters), followed by 1OHPy (1.5-4.2% *versus* 1.4-3.1%) and 1OHPhen (1.3-3.4% *versus* 1.6-2.6%). Urinary 2OHFlu was the compound with the highest increment in SExp individuals, increasing from 111% (VNH: 0.340 *versus* 0.718 $\mu\text{mol/mol}$ creatinine; $p=0.031$) to 1068% (BRG: 0.022 *versus* 0.257 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) while 1OHPy (83% (BRG: 0.044 *versus* 0.024 $\mu\text{mol/mol}$ creatinine, $p=0.001$) to 220% (MDL: 0.462 *versus* 0.144 $\mu\text{mol/mol}$ creatinine, $p > 0.05$)) and 1OHPhen were the least affected (87% (BRG: 0.016 *versus* 0.030 $\mu\text{mol/mol}$ creatinine; $p=0.005$) to 112% (VNH: 0.051 *versus* 0.108 $\mu\text{mol/mol}$ creatinine; $p > 0.05$)) by firefighters's exposure to emissions released during firefighting activities (Figure 3). These findings are in accordance with the small but significant increase of 1OHPy concentrations determined in the urine of 13 individuals at a firefighting training school [38]. Edelman et al. [36] reported urinary 1-

2-, and 3OHPhen and 1OHPy increments ranging from 22-61% and 76-81% in firemen who actively participated in firefighting activities during and after the World Trade Center collapse. Laitinen et al. [11] also observed urinary 1OHPy concentrations that were 50-537% higher in European firefighting trainers at gas and fire house diving simulators. More recently, Fernando et al. [34] found significantly elevated concentrations (up to 40-fold increase when compared with the baseline, 0.6 $\mu\text{g/g}$ of creatinine) of total post exposure urinary 1- and 2OHNaph, 2-, 3-, and 9OHFlu, and 2-, 3-, and 4OHPhen in 25 firefighters during training exercises at Canadian burn houses; similar trend was also reported by Oliveira et al. [5] for non-smoking firefighters that were directly involved in fire combat (knockdown and overhaul). BRG was the fire station where the differences between SNExp and SExp individuals were all statistically significant for individual (Figure 3c) and total (Table 2) OH-PAHs ($p \leq 0.05$). Within the SExp firefighters serving at BRG fire corporation, three individuals presented much higher levels of 1OHNaph+1OHAce (49.6-52.2 $\mu\text{mol/mol}$ creatinine) in comparison with the other exposed colleagues; no specific source was identified to justify these higher levels. The median $\sum\text{OH-PAH}$ concentrations ranged between 0.74 (BRG) and 2.06 $\mu\text{mol/mol}$ creatinine (MDL) for SNExp and from 1.91 (BRG) to 5.71 $\mu\text{mol/mol}$ creatinine (MDL) for SExp (Table 2). The determined levels of $\sum\text{OH-PAHs}$ increased between 158 (BRG) and 551% (VNH) ($p \leq 0.02$) in exposed firemen, reflecting the impact of firefighting activities on the total PAHs exposure (Table 2).

The inter-relation between individual and total OH-PAHs was also estimated. Overall, moderate to strong correlations were observed among individual compounds and between individual OH-PAHs with $\sum\text{OH-PAHs}$ for SExp individuals working at VNH ($r \geq 0.737$; $p \leq 0.006$, except between 1OHPhen with 1OHNaph+1OHAce and with $\sum\text{OH-PAHs}$) and BRG ($r \geq 0.514$; $p \leq 0.050$, except between 2OHFlu and 1OHNaph+1OHAce and with

1OHPy) fire stations. These findings indicated the predominant impact of common sources of PAHs exposure. In addition, the correlation between the urinary levels of individual and Σ OH-PAHs with the number of hours that each firefighter dedicated to combat activities were also estimated. In general, moderate to strong correlations were observed for all firefighters from the characterized fire stations: $r \geq 0.760$ and $p \leq 0.028$ at VNH; $r \geq 0.878$ and $p \leq 0.021$ except for 2OHFlu and 1OHPhen at MDL; $r \geq 0.413$ and $p \leq 0.023$ except for 1OHNaph+1OHAce and 1OHPy at BRG. These results show the influence of participation in fire combat activities on Σ PAHs exposure of firemen. Despite the short period of exposure, the use of self-protective equipment revealed to be insufficient to protect firemen from the emissions released during fires and thus preventive action measures should be implemented to reduce inhalation and dermal exposure to PAHs during fire combat.

In a view of the scarce information regarding firefighters' occupational exposure, comparison with the reported results is extremely difficult. Furthermore, the reported data are frequently not normalized with creatinine concentrations [11,33,36]. Still, the urinary levels of 1OHPy in SExp firefighters (overall range: 0.025 $\mu\text{mol/mol}$ creatinine at VNH to 0.462 $\mu\text{mol/mol}$ creatinine at MDL) were lower than those reported for 43 Canadian firefighters involved in a fire combat (0.080-3.63 $\mu\text{mol/mol}$ creatinine; [31]). The concentrations in SExp firefighters determined in this study were overall higher than the levels observed for NSNExp firefighters serving at other Portuguese fire stations [5].

Limited information is available concerning the reference standards and guidelines for urinary levels of OH-PAHs. In 2010, the American Conference of Governmental Industrial Hygienists recommended a 1OHPy limit of 0.5 $\mu\text{mol/mol}$ creatinine for occupational PAHs exposure [78]. Only three exposed firefighters from MDL fire station exceeded that reference guideline, the urinary OH-PAH concentrations of those firemen

being much lower than the no-biological effect limit of 1.4 $\mu\text{mol/mol}$ creatinine proposed by Jongeneelen [29].

3.5 Cumulative effects of tobacco smoke and fire emissions

The majority of occupational health effects, including the development of cancers, are predominantly caused by exposure to a multiple causal factors rather than to a specific agent; as a consequence, people exposed to more than one risk factor are more prompt to present the symptoms and develop the illness. Smoking firefighters are simultaneously exposed to the potential health effects of tobacco consumption and the hazardous fire emissions. In comparison with NSNExp firefighters, the urinary concentrations of $\Sigma\text{OH-PAHs}$ in SExp individuals increased from 355% (BRG: 1.91 *versus* 0.42 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) to 3237% (VNH: 5.34 *versus* 0.16 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) (Table 2). The median urinary concentrations of individual compounds were augmented as high as 308% (BRG: 0.394 $\mu\text{mol/mol}$ creatinine in NSNExp subjects *versus* 1.61 $\mu\text{mol/mol}$ creatinine in SExp; $p \leq 0.001$) to 550% (MDL: 0.674 *versus* 4.38 $\mu\text{mol/mol}$ creatinine; $p = 0.002$) for 1OHNaph+1OHAce, from 508% (VNH: 0.118 *versus* 0.718 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) to 1412% (BRG: 0.017 *versus* 0.257 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) for 2OHFlu, and from 97% (MDL: 0.074 *versus* 0.146 $\mu\text{mol/mol}$ creatinine; $p = 0.009$) to 275% (BRG: 0.008 *versus* 0.030 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$) for 1OHPhen. Urinary 1OHPy presented a similar trend with increments ranging between 25% (VNH: 0.020 *versus* 0.025; $p > 0.05$) to 579% (MDL: 0.068 *versus* 0.462 $\mu\text{mol/mol}$ creatinine; $p \leq 0.001$). The increments in the urinary levels of OH-PAHs reflect the potential cumulative effects of smoking consumption and occupational activities to the total PAHs exposure. In general, urinary 2OHFlu and 1OHNaph+1OHAce were the most affected compounds by the impact of both factors. The Bartlett sphericity test showed that

these metabolites were strongly correlated with each other ($r=0.600$; $p<0.001$). Principal component analysis (PCA) was performed based on urinary concentrations of those two OH-PAHs for NSNExp, SNEp, and SExp firefighters (Figure 2S, Supplementary Material). The Kaiser-Meyer-Olkin values were ≥ 0.5 for both variables. The first function (F1) issued from PCA explained up to 80.01% of the total variance, presenting the highest loadings for both variables (square cosines values >0.800). It allowed the differentiation of the majority of SExp and NSNEXP firefighters from the others, highlighting the discriminating power of urinary 1OHNaph+1OHAce and 2OHFlu. The second function (F2) contributed with 19.99% of the original data. NSNEXP subjects are grouped together with mostly negative F1 and (or near zero) F2 scores. Still, a higher number of subjects would be precious to corroborate the findings achieved in this study.

Previously, Feunekes et al. [37] also found significant influences of both tobacco smoking and exposure to smoke released during fires on the urinary levels of 1OHPy in trainers and instructors from a firefighting school. More recently, Fernando et al. [34] observed higher levels of urinary OH-PAHs and methoxyphenols in smoking firefighters compared with non-smoking subjects working at four Canadian fire stations during training exercises at burn houses. The concentrations of urinary OH-PAHs in Portuguese SExp firefighters were mainly much lower than the levels reported for other relevant occupations concerning PAHs exposure, such as coke-oven workers, iron foundry and aluminum production workers, mining workers, as well as employees from graphite electrodes, refractory materials, and special carbon products industrial productions [67,73,79-80].

Little information is available concerning the impact of short- and long-term exposure to fire emissions in firefighters' health. It has been reported that cardiovascular disease is the leading cause of excess morbidity and mortality in firefighters [9,42-43]. Some

investigators have noticed a higher incidence of respiratory and pulmonary symptoms as well as airways hyper-responsiveness and decay in lung function of firefighters during fire combat activities [9,81-82]. In addition, tobacco smoke is associated with the development of lung cancer among other types of cancers and diseases, namely leukemia and cancers of the urinary and aerodigestive tracts as well as of stomach, pancreas, liver, breast, prostate, and colorectal tissues [15]. Some authors have also described an increased risk of developing respiratory, inflammatory and cardiovascular illnesses in smokers [84-85]. Thus SExp firefighters are more vulnerable to the development and/or aggravation of cardio-respiratory diseases; cumulative and synergistic effects may occur. Certain life styles may also potentiate the health risks associated with firefighters' exposure to PAHs. Indeed, PAHs are electrophilic substances that have been associated with cardiovascular disease and adverse reproductive outcomes [86]. Unmetabolized PAHs are known for their teratogenic, mutagenic, and carcinogenic properties and therefore constitute a serious threat to the health of humans [19-20]. Positive association between the urinary levels of 1OHNaph, 2OHNaph, 2OHPhen and total low molecular weight OH-PAHs with *diabetes mellitus* have also been reported in American adults [87]. Moreover, significant associations have been described between the levels of urinary 1OHPy with asymmetric dimethylarginine in the plasma and also with adipocyte-fatty acid binding protein present in the serum of Mexican women [27-28]. However, the major concern is the ability of PAH reactive metabolites (in the form of epoxides and dihydrodiols) to bind to some proteins and DNA molecules, thus leading to biochemical disruptions and cell damage that may originate mutations, tumors and cancers [88]. Regarding the incidence of cancer in firefighters, some studies reported an elevated risk of developing several types of cancers, including urothelial, skin, lung, kidney and

testicular cancers throughout their professional career; however, some findings remain contradictory [89-91].

4 Conclusions

This study assessed the total exposure to PAHs in three different groups of wildland firefighters (NSNExp, SNEExp, and SExp) serving at three Portuguese fire stations through the determination of six urinary metabolites. SExp individuals presented levels of Σ OH-PAHs that were 355-3237% higher than for NSNExp subjects, with occupational exposure to fire combat activities promoting significant increments of 158-551% in Σ OH-PAHs. Tobacco consumption increased 76-412% the levels of Σ OH-PAHs for SNEExp firefighters. The main findings of this research indicated that individual and cumulative PAHs exposure occurs by participation in firefighting activities and tobacco consumption. More studies concerning both occupational exposure and characterization of other causal factors in firefighters, as well as, toxicological and epidemiological studies are needed to better estimate the overall risks and the potential health effects of firefighter's exposure to PAHs.

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Figure Captions

Figure 1. Concentrations of urinary monohydroxyl-PAHs (median, 25-75 percentiles and range; $\mu\text{mol/mol}$ creatinine; 1OHNaph+1OHAce: 1-hydroxynaphthalene and 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) determined in non-smoking non-exposed (NSNExp) and in smoking non-exposed (SNExp) firefighters' from three different fire stations (Vinhais (VNH), Mirandela (MDL), and Bragança (BRG)).
 *Statistically significant differences at $p \leq 0.05$ between non-smoking non-exposed and smoking non-exposed firefighters (non-parametric Mann-Whitney U test). The value of $\text{LOD}/\sqrt{2}$ was used whenever the concentration of OH-PAHs was below the LOD [90].

Figure 1

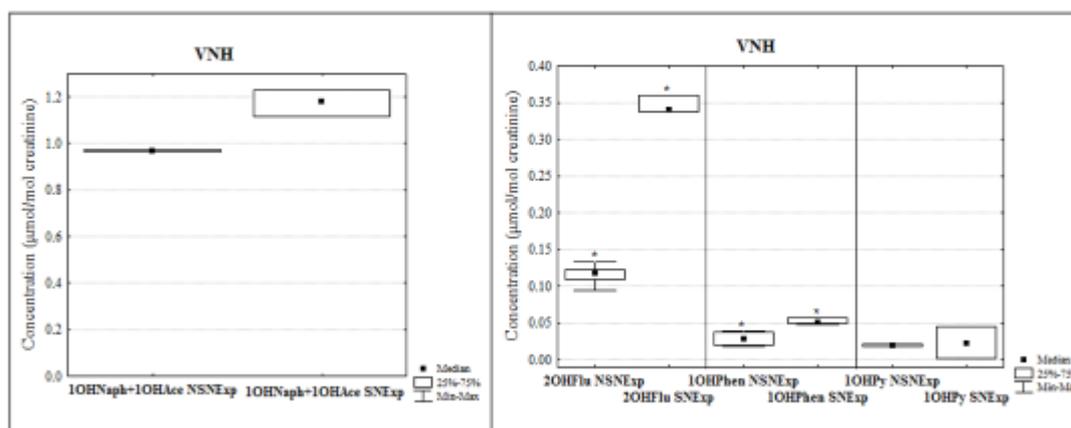


Figure 1-cont.

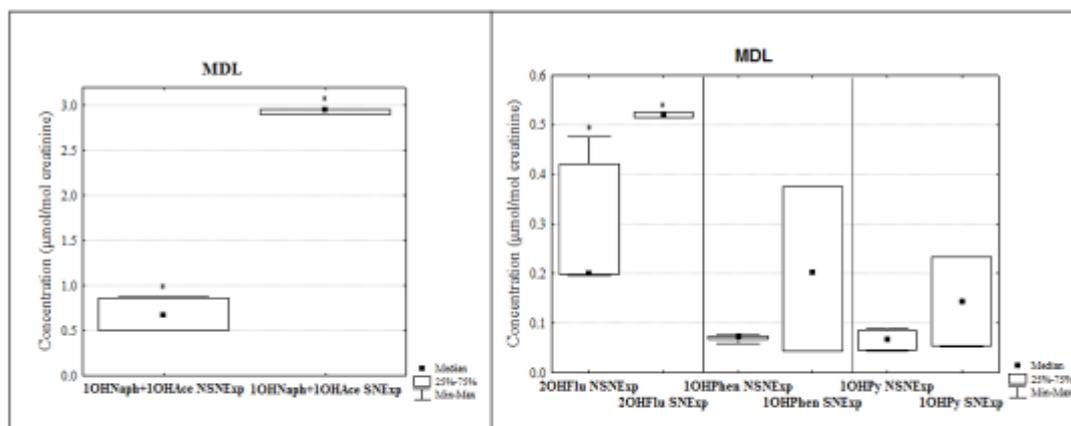


Figure 1-cont.

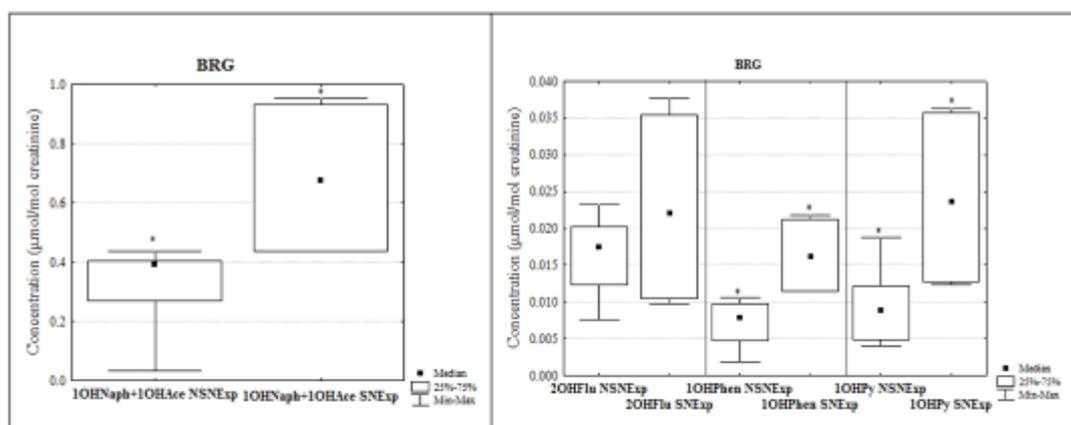
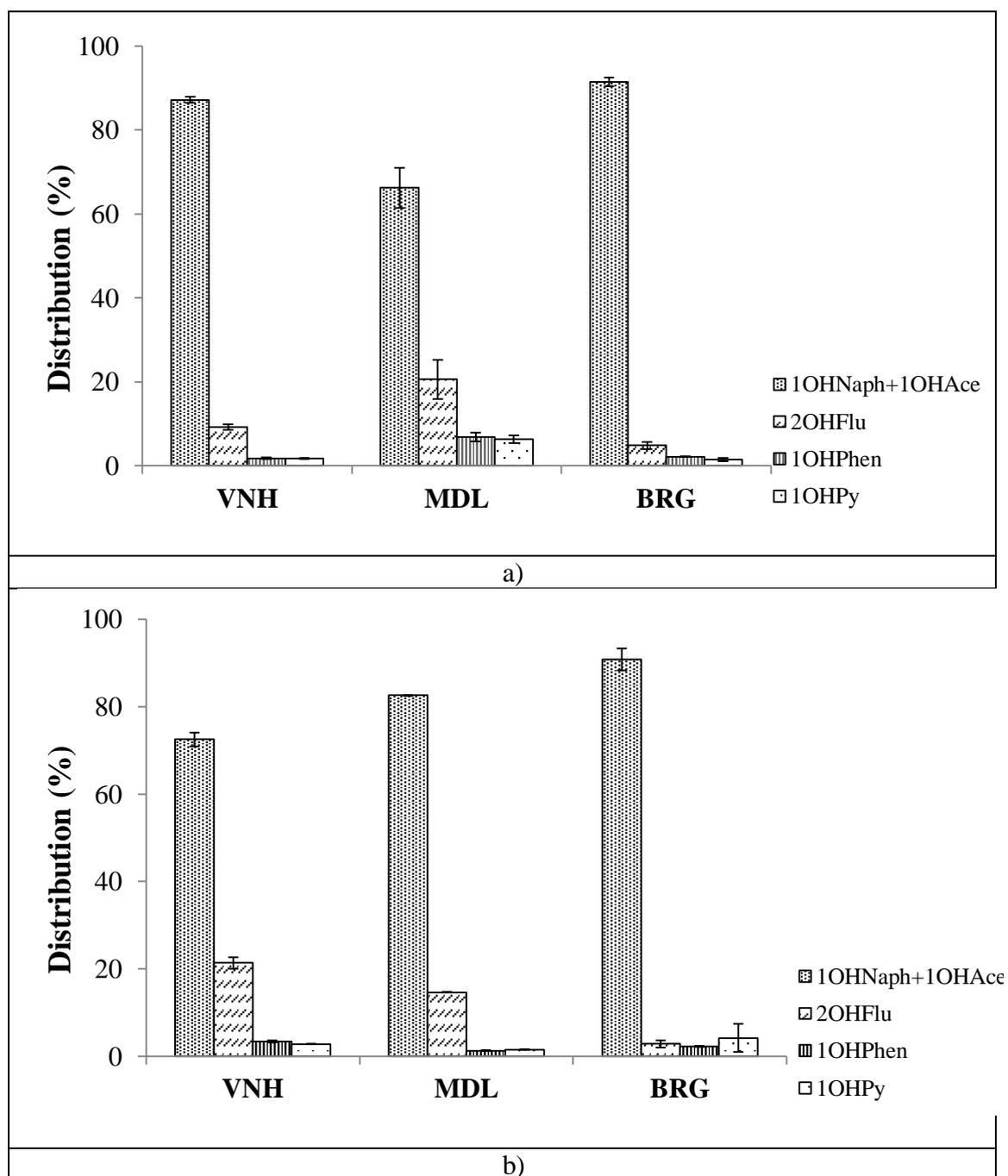


Figure2. Distribution of monohydroxyl-PAHs (1OHNaph+1OHAce: 1-hydroxynaphthalene and 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) in the urine of non-smoking non-exposed firefighters (a), smoking non-exposed (b) and smoking exposed (c) firefighters from different fire stations (Vinhais (VNH), Mirandela (MDL), and Bragança (BRG)).



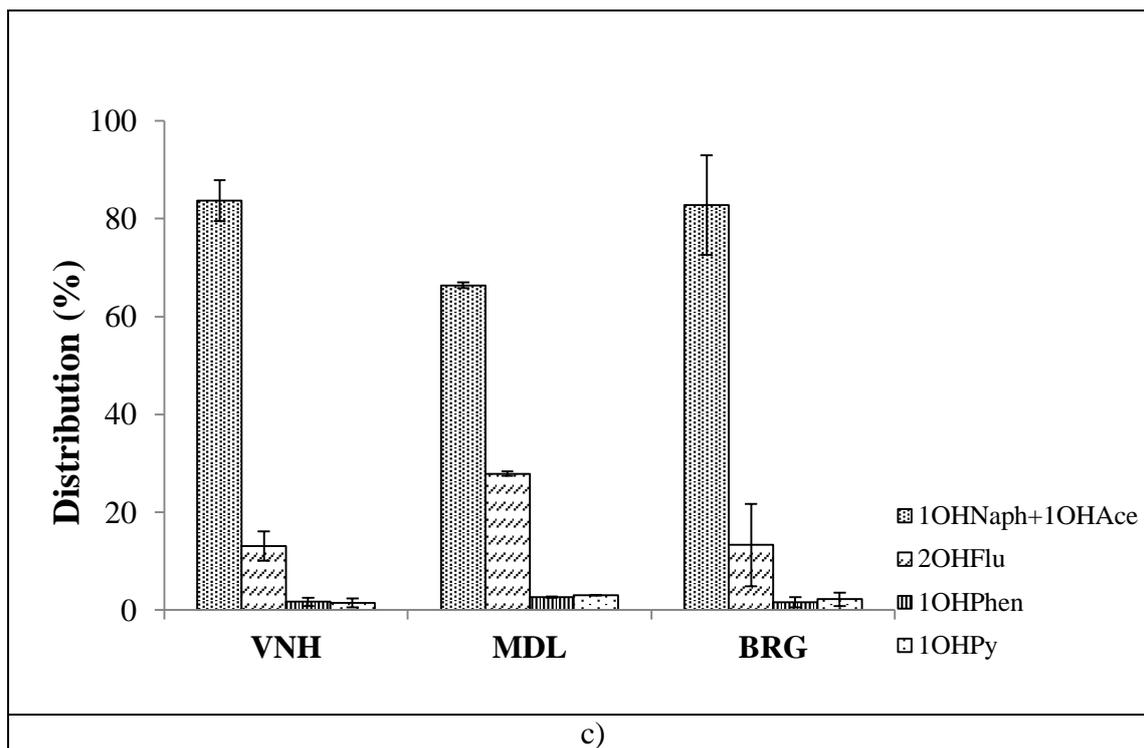


Figure 2.

Figure 3. Concentrations of urinary monohydroxyl-PAHs (median, 25-75 percentiles and range; $\mu\text{mol/mol}$ creatinine; 1OHNaph+1OHAce: 1-hydroxynaphthalene and 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) determined in smoking non-exposed (SNExp) and smoking exposed (SExp) firefighters' from three different fire stations (Vinhais (VNH), Mirandela (MDL), and Bragança (BRG)). *Statistically significant differences at $p \leq 0.05$ between smoking non-exposed and smoking exposed firefighters (non-parametric Mann-Whitney U test). The value of $\text{LOD}/\sqrt{2}$ was used whenever the concentration of OH-PAHs was below the LOD [90].

Figure 3

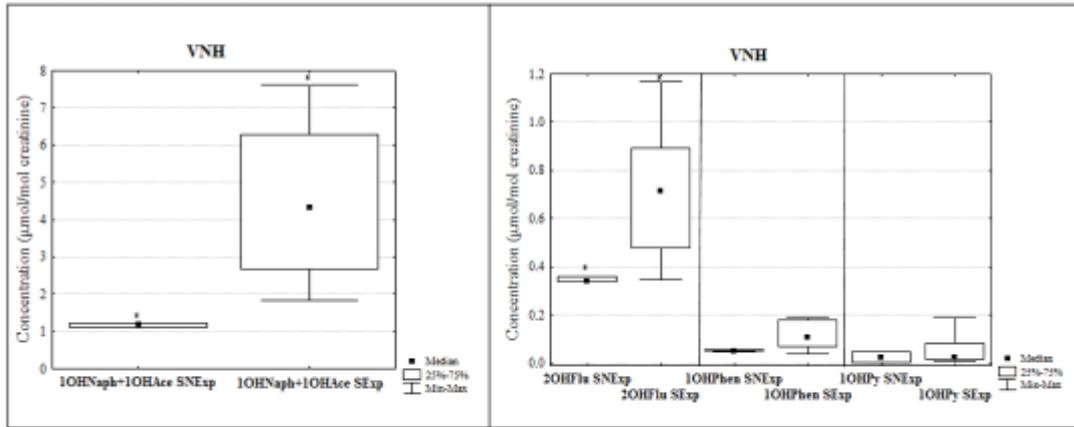


Figure 3-cont.

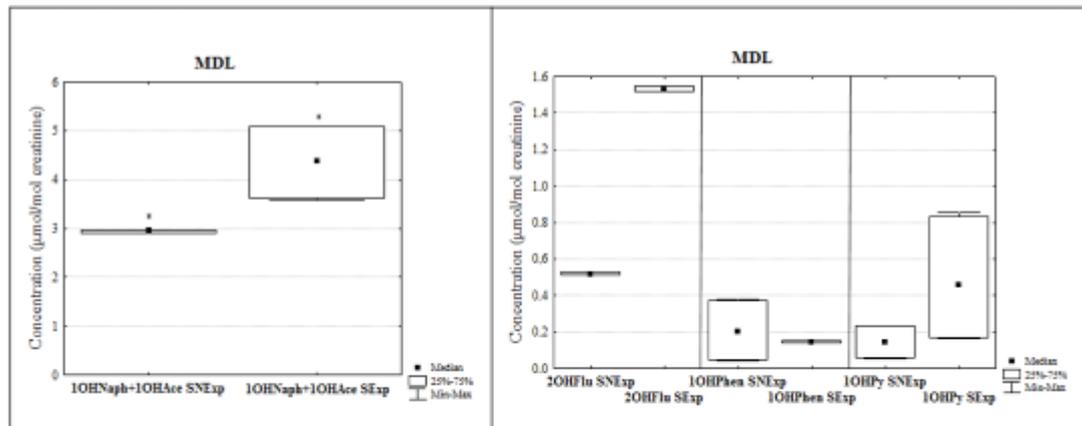


Figure 3-cont.

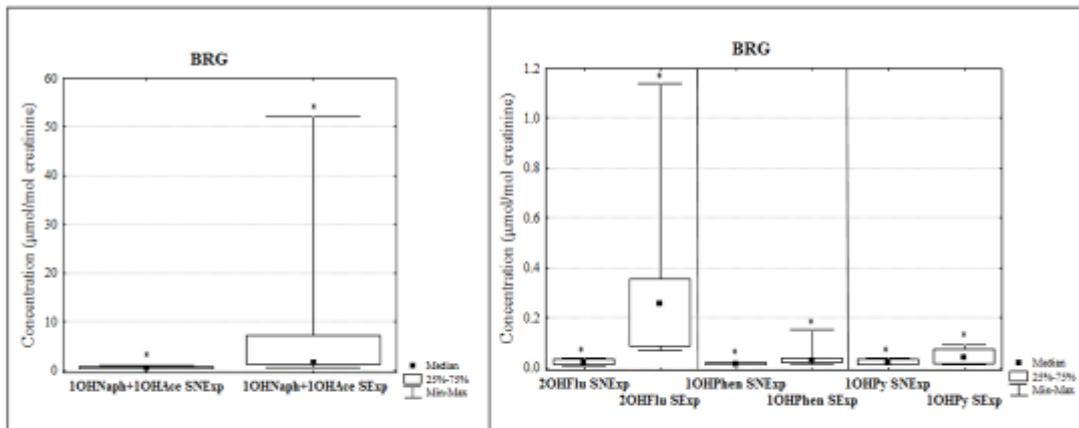


Table 1. Characterization of firefighters (n=108) from Vinhais (VNH), Mirandela (MDL) and Bragança (BRG) fire stations that participated in the study. NSNExp: non-smoking non-exposed; SNExp: smoking non-exposed; SExp: Smoking exposed individuals.

Characteristic	VNH			MDL			BRG		
	NSNExp	SNExp	SExp	NSNExp	SNExp	SExp	NSNExp	SNExp	SExp
Firefighters (n)	9	6	15	9	6	9	18	6	30
Age (mean \pm SD; min – max; years)	27 \pm 6.7 (23-35)	38 \pm 12 (30-47)	29 \pm 4.0 (25-35)	29 \pm 7.0 (22-36)	43 \pm 24 (26-60)	33 \pm 12 (25-48)	40 \pm 5.6 (34-48)	22 \pm 1.4 (21-23)	31 \pm 9.4 (21-53)
Body mass index (mean \pm SD; min – max; kg/m ²)	24 \pm 3.2 (21-26)	22 \pm 3.3 (19-24)	23 \pm 1.7 (21-24)	29 \pm 5.0 (26-35)	24 \pm 1.2 (23-25)	22 \pm 2.8 (19-25)	29 \pm 4.2 (25-36)	26 \pm 2.4 (24-28)	26 \pm 2.3 (22-29)
Diastolic blood pressure (mean \pm SD; min – max; mmHg)	81 \pm 4.5 (77-86)	65 \pm 5.7 (61-69)	87 \pm 7.6 (81-96)	80 \pm 33 (49-114)	73 \pm 1.4 (72-74)	84 \pm 18 (60-96)	86 \pm 11 (72-106)	92 \pm 7.1 (87-97)	83 \pm 9.9 (68-98)
Systolic blood pressure (mean \pm SD; min – max; mmHg)	136 \pm 2.5 (133-138)	110 \pm 3.5 (108-113)	138 \pm 6.3 (129-144)	128 \pm 9.7 (120-139)	127 \pm 0.71 (127-128)	132 \pm 23 (113-156)	137 \pm 11 (127-152)	138 \pm 19 (125-152)	132 \pm 11 (118-150)
Cardiac frequency (mean \pm SD; min – max; heart beats/min)	69 \pm 9.9 (58-76)	73 \pm 1.4 (72-74)	74 \pm 16 (54-87)	76 \pm 25 (56-105)	77 \pm 1.4 (76-78)	83 \pm 19 (54-112)	72 \pm 9.4 (62-84)	79 \pm 5.7 (75-83)	78 \pm 12 (64-101)
Arterial oxygen saturation (mean \pm SD; min – max; %)	98 \pm 1.2 (97-99)	99 ^a	98 \pm 0.7 (97-99)	99 ^a	97 \pm 0.7 (97-98)	98 \pm 0.6 (97-99)	97 \pm 1.2 (95-98)	97 \pm 0.7 (97-98)	97 \pm 1.4 (95-99)
Respiratory pathologies (n)	No	1 rhinitis; sinusitis allergic to pollens, dust, dust mites, varnishes	No	2 sinusitis	No	No	1 sinusitis	No	No
Number of cigarettes smoked per day (mean \pm SD; min – max)	n.a.	8 \pm 3 (6-10)	21 \pm 2.2 (20-25)	n.a.	15 \pm 7.1 (10-20)	16 \pm 7.6 (5-25)	n.a.	11 \pm 1.4 (10-12)	16 \pm 5.6 (4-20)
Number of years as firefighters									
≤ 10 years (%)	33	50	20	33	50	67	17	100	70
10 – 20 years (%)	67	50	80	67	0	0	33	0	20
≥ 20 years (%)	0	0	0	0	50	33	50	0	10
Time dedicated to firefighting activities (48 hours before sampling; mean \pm SD; min – max; h)	n.a.	n.a.	3.2 \pm 1.1 (2-4)	n.a.	n.a.	2.0 \pm 0.6 (1-4)	n.a.	n.a.	3.1 \pm 2.2(0.5-8)

na – Not applicable

^a – All firefighters from this group presented the same value of arterial oxygen saturation at the end of their work shift.

Table 2. Total urinary PAH metabolites (Σ OH-PAHs) concentrations (median and range; $\mu\text{mol/mol}$ creatinine) determined in non-smoking non-exposed (NSNExp), smoking non-exposed (SNExp), and smoking exposed (SExp) firefighters from the selected fire stations: Vinhais (VNH), Mirandela (MDL) and Bragança (BRG).

Fire station	Σ OH-PAHs ($\mu\text{mol/mol}$ creatinine)		
	NSNExp	SNExp	SExp
VNH	0.16 (0.12-1.12)	0.82* (0.05-1.67)	5.34* (2.20-8.59)
MDL	0.82 (0.56-1.24)	2.06* (0.59-3.59)	5.71* (5.44-5.94)
BRG	0.42 [#] (0.05-0.47)	0.74 ^{#*} (0.49-1.02)	1.91* (0.09-52.4)

[#]Statistically significant ($p < 0.05$) between non-smoking non-exposed and smoking non-exposed firefighters;

* Statistically significant ($p < 0.05$) between smoking non-exposed and smoking exposed firefighters.