Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose

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ABSTRACT

This work characterizes levels of eighteen polycyclic aromatic hydrocarbons (PAHs) in the breathing air zone of firefighters during their regular work shift at eight Portuguese fire stations, and the firefighters' total internal dose by six urinary monohydroxyl metabolites (OH-PAHs). Total PAHs (ΣPAHs) concentrations varied widely (46.4–428 ng/m^3), mainly due to site specificity (urban/rural) and characteristics (age and layout) of buildings. Airborne PAHs with 2–3 rings were the most abundant (63.9–95.7% ΣPAHs). Similarly, urinary 1-hydroxynaphthalene and 1-hydroxyacenaphthene were the predominant metabolites (66–96% ΣOH-PAHs). Naphthalene contributed the most to carcinogenic ΣPAHs (39.4–78.1%) in majority of firehouses; benzo[a]pyrene, the marker of carcinogenic PAHs, accounted with 1.5–10%. Statistically positive significant correlations ($r \geq 0.733$, $p \leq 0.025$) were observed between ΣPAHs and urinary ΣOH-PAHs for firefighters of four fire stations suggesting that, at these sites, indoor air was their major exposure source of PAHs. Firefighter's personal exposure to PAHs at Portuguese fire stations were well below the existent occupational exposure limits. Also, the quantified concentrations of post-shift urinary 1-hydroxypyrene in all firefighters were clearly lower than the benchmark level (0.5 µmol/mol) recommended by the American Conference of Governmental Industrial Hygienists

1. Introduction

Firefighting, along with construction, mining, and agriculture, ranks among the most dangerous professions, with its occupational exposure being regarded as possible carcinogen to humans by the International Agency for Research on Cancer (IARC) and the US National Institute for Occupational Safety and Health (NIOSH) [1,2]. Firefighting is among the most hazardous yet the least studied occupations in terms of exposures and their relationship to occupational diseases.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds that are released during the incomplete combustion or pyrolysis of organic material. PAHs are well known for their cytotoxic and mutagenic properties [3,4], with some

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of them being recognized as endocrine disrupting chemicals [5]; USEPA listed 16 priority PAHs [6]. PAHs possess an undeniable role in the induction of human carcinogenesis [7], especially if benzo[a]pyrene (known human carcinogenic) and benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, indeno[1,2,3-cd]pyrene, and naphthalene (possible human carcinogens; [4,8] are present. Dibenzo[a,l]pyrene and dibenz[a,h]anthracene have been also under scrutiny because they are regarded as probable carcinogens to humans due to their higher carcinogenic potency than benzo[a]pyrene [9–12]. Electrophilic compounds such as PAHs play a key role in environmental cancer and some recent evidences associate their role in cardiovascular disease [13]. Firefighters' occupational exposure has been associated with excess morbidity and mortality with cardiovascular disease being considered as the leading cause of death in approximately 45% of firefighters and a major cause of their morbidity [14–16]. As a consequence firefighters' occupational exposure to PAHs may promote the development or aggravation of cardiovascular illnesses. Fires are the major contributor to occupational exposure to PAHs [17–20]. Other relevant sources include motor-vehicle exhaust (especially diesel), industrial emissions, residential and commercial heating with wood, coal, or other biomass fuels, and tobacco smoke [21–23]. Despite some available information regarding firefighters' occupational exposure to PAHs during live fire combat activities in Australia [20] and USA [17–19,23–27], firefighters may also be exposed to PAHs when they are at fire stations. Recently some studies revealed that chemical contaminants from fires were tracked back to fire stations via fire vehicles and principally through firefighters' personal equipments such as boots, gloves, and turnout gear [18,20,28–31]. Only two studies were found regarding firefighters' occupational exposure to PAHs at fire stations [23,32], both performed in USA. No information exists concerning other countries, even though the available exposure data may not be directly applicable.

Biological monitoring is an important tool in the prevention of occupational diseases related to those exposed chemicals on a regular basis, particularly when multi-route exposure (inhalation, dermal, ingestion) or abnormal exposure takes place. 1-hydroxypyrene (1OHPy) is the most widely used biological indicator of internal dose of PAHs exposure [33,34]; 3-hydroxibenzo[a]pyrene (3OHBaP) is the main metabolite of the known human carcinogenic benzo[a]pyrene. Acenaphthene, fluorene, and phenanthrene are common PAHs in different matrices [10,35,36] and their major urinary metabolites are 1-hydroxylacenaphthene (1OHAce), 2-hydroxylfluorene (2OHFlu), and 1-hydroxyphenanthrene (1OHPhen), respectively. Regarding naphthalene there are more than thirty

Table 1
Concentrations of $PM_{2.5}$-bound PAHs (median[a]; min-max; ng/m^3) in the breathing air zone of firefighters at the fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

Compound	Fire station							
	MRD	TDC	SDM	MDL	TMC	VNH	BRG	FEC
Naphthalene	12.2 (8.51–15.1)	6.67 (5.61 – 6.99)	7.95 (6.04 – 10.3)	11.5 (10.9 – 13.5)	4.95 (4.27 – 8.74)	7.19 (5.95 – 8.53)	9.21 (4.23 – 13.0)	35.5 (33.4 – 38.6)
Acenaphthylene	110 (75.6 – 208)	24.0[a]	56.4 (27.8 – 86.9)	125[**] (24.0 – 198)	24.0[a]	43.9[***] (24.0 – 89.1)	27.2[***] (24.0 – 111)	199 (60.3 – 352)
Acenaphthene	88.5 (56.1 – 111)	2.48[**] (1.62 – 4.26)	18.7 (18.4 – 20.8)	97.3[**] (1.62 – 121)	10.3 (9.11 – 16.2)	1.62[a]	8.54[***] (1.62 – 18.2)	4.33[***] (1.62 – 8.02)
Fluorene	1.05 (0.540 – 1.49)	0.272[***] (0.272 – 0.588)	0.272[a]	1.86 (1.62 – 3.53)	0.446[**] (0.272 – 0.528)	0.272[a]	0.272[***] (0.272 – 0.450)	6.73 (3.13 – 9.94)
Phenanthrene	6.07 (3.74 – 7.27)	4.08 (3.71 – 5.97)	2.88 (2.76 – 3.00)	9.45 (6.83 – 9.88)	3.99 (3.39 – 4.42)	4.32 (3.42 – 5.01)	3.63 (2.97 – 4.84)	28.5 (21.0 – 35.9)
Anthracene	0.223[a]	0.223[***] (0.223 – 0.364)	0.223[a]	0.223[***] (0.223 – 1.59)	0.223[a]	0.223[***] (0.223 – 0.657)	0.223[a]	0.636 (0.601 – 0.675)
Fluoranthene	0.351[***] (0.351 – 0.603)	0.351[***] (0.351 – 0.592)	0.351[a]	1.50 (0.968 – 2.16)	0.351[***] (0.351 – 0.589)	0.596[**] (0.351 – 1.02)	0.351[***] (0.351 – 0.631)	1.88[***] (0.351 – 3.88)
Pyrene	1.24[**] (0.292 – 1.34)	0.292[a]	0.292[a]	2.28 (1.76 – 2.77)	0.473[**] (0.292 – 0.755)	0.582[**] (0.292 – 1.09)	0.292[a]	3.41 (2.01 – 4.92)
Benz[a]anthracene	0.205[a]	0.205[a]	0.205[a]	0.205[***] (0.205 – 0.866)	0.205[a]	0.205[***] (0.205 – 0.452)	0.205[***] (0.205 – 0.324)	0.446[***] (0.205 – 0.824)
Chrysene	0.145[***] (0.145 – 0.358)	0.145[a]	0.145[a]	0.145[***] (0.145 – 2.01)	0.365[**] (0.145 – 0.475)	0.196[***] (0.145 – 0.743)	0.145[a]	2.41 (2.01 – 3.08)
Benzo[b + j]fluoranthene	0.844[a]	0.844[a]	0.844[a]	0.844[***] (0.844 – 3.55)	0.844[a]	2.31[**] (0.844 – 3.51)	0.844[a]	24.5 (15.5 – 33.9)
Benzo[k]fluoranthene	0.238[**] (0.134 – 0.642)	0.134[a]	0.134[a]	0.134[***] (0.134 – 0.412)	0.134[a]	0.399[**] (0.134 – 0.594)	0.134[a]	3.84 (2.50 – 5.15)
Benzo[a]pyrene	0.277[***] (0.277 – 1.02)	0.277[***] (0.277 – 0.398)	0.277[a]	0.277[***] (0.277 – 1.24)	0.277[a]	1.22[**] (0.277 – 2.45)	0.277[a]	15.1 (9.76 – 20.5)
Dibenzo[a,l]pyrene	0.671[a]	0.671[a]	0.671[a]	0.671[a]	0.671[a]	0.671[a]	0.671[a]	0.671[a]
Dibenz[a,h]anthracene	3.97[**] (0.499 – 13.9)	0.499[***] (0.499 – 1.66)	0.499[a]	0.767[**] (0.499 – 3.63)	0.499[a]	5.85[**] (0.499 – 9.96)	0.499[a]	51.1 (38.1 – 65.5)
Benzo[ghi]perylene	3.09[**] (0.355 – 4.48)	5.08 (2.66 – 6.78)	1.66 (1.44 – 1.88)	3.80 (2.86 – 11.8)	3.17 (1.98 – 4.00)	4.83 (3.06 – 8.53)	2.29 (1.55 – 4.18)	32.9 (27.9 – 40.8)
Indeno[1,2,3-cd]pyrene	0.185[***] (0.185 – 1.18)	0.185[a]	0.185[a]	0.185[a]	0.185[a]	0.185[a]	0.185[a]	17.6 (13.9 – 23.3)
ΣPAHs	229 (200 – 296)	46.4 (44.0 – 49.4)	91.7 (64.5 – 124)	256 (77.6 – 352)	51.1 (48.8 – 57.5)	74.6 (44.4 – 125)	55.0 (49.8 – 137)	428 (250 – 631)
$\Sigma PAHs_{carc}$	20.8 (15.8 – 26.0)	9.82 (8.57 – 11.1)	10.9 (9.00 – 13.3)	16.8 (13.9 – 23.9)	8.24 (7.45 – 11.7)	19.6 (9.43 – 23.6)	12.2 (7.19 – 16.1)	150 (122 – 186)

Note: Detection frequency of each compound was 100% unless otherwise indicate.

[*] 80% ≤ detection frequency <100%.

[**] 60% ≤ detection frequency <80%.

[***] 15% ≤ detection. frequency <60%.

[a] When the concentration was below the LOD, the value of the respective LOD/$\sqrt{2}$ was used [50].

Monohydroxyl-PAH concentrations (median[#]; min-max; µmol/mol creatinine) measured in firefighters urine samples from different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

Compound[*]	Fire station							
	MRD	TDC	SDM	MDL	TMC	VNH	BRG	FEC
1OHNaph + 1OHAce	1.39	0.138[#]	1.48	0.887	0.707	1.63[#]	0.650	3.59
	0.823–1.60	0.138 – 1.50	0.731 – 2.25	0.722 – 2.31	0.231 – 5.32	0.138 – 8.06	0.458 – 1.90	3.26 – 4.14
2OHFlu	0.147	5.23×10^{-2}	1.39×10^{-2}	0.182	4.40×10^{-2}	3.58×10^{-2} [#]	9.35×10^{-2}	5.86×10^{-2}
	4.40×10^{-2} – 0.150	2.42×10^{-2} – 0.152	9.58×10^{-3} – 2.01×10^{-2}	6.95×10^{-2} – 0.212	3.40×10^{-2} – 8.75×10^{-2}	5.66×10^{-4} – 0.134	4.71×10^{-2} – 0.170	5.32×10^{-2} – 6.61×10^{-2}
1OHPhen	4.10×10^{-2}	4.17×10^{-2}	1.21×10^{-2}	7.44×10^{-2}	1.41×10^{-2}	3.93×10^{-2}	8.38×10^{-2}	3.10×10^{-2}
	6.71×10^{-3} –0.211	1.65×10^{-2} – 7.62×10^{-2}	1.04×10^{-2} – 1.28×10^{-2}	4.22×10^{-2} – 0.103	9.67×10^{-3} – 2.86×10^{-2}	3.05×10^{-2} – 4.54×10^{-2}	7.07×10^{-2} – 0.120	1.87×10^{-2} – 4.33×10^{-2}
1OHPy	3.59×10^{-2}	2.67×10^{-2}	1.35×10^{-2} [#]	0.146	2.10×10^{-2}	1.73×10^{-2}	5.18×10^{-2}	2.70×10^{-2}
	9.23×10^{-3} – 0.131	1.12×10^{-2} – 4.10×10^{-2}	1.06×10^{-3} – 2.79×10^{-2}	1.99×10^{-2} – 0.166	6.07×10^{-3} – 2.68×10^{-2}	7.67×10^{-3} – 4.36×10^{-2}	3.09×10^{-2} – 9.91×10^{-2}	2.35×10^{-2} – 3.13×10^{-2}
ΣOH-PAHs	1.61	0.259	1.52	1.29	0.786	1.79	0.879	3.71
	0.889 – 1.88	0.133 – 1.56	0.780 – 2.28	0.979 – 2.62	0.281 – 5.42	0.198 – 8.13	0.737 – 2.20	3.37 – 4.27

[*] 1OHNaph + 1OHAce: 1-hydroxynaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPHen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene.

[#] When the urine concentration was below the LOD, the value of the respective $\text{LOD}/\sqrt{2}$ was used [50].

identified metabolites, being 1-hydroxynaphthalene (1OHNaph) and 2-hydroxynaphthalene the main biomarkers of occupational exposure to naphthalene [37]. Only four studies were found in the literature concerning firefighters' biological monitoring to PAHs. Among them, two characterized exclusively 1OHPy levels [27,38], while the other two assessed the urinary levels of 1OHPy and 1OHNaph [31], and the concentrations of 1OHPy and three hydroxyphenanthrene metabolites [39], respectively. In order to evaluate firefighters' workplace conditions on a continuous basis, emphasis should be placed on environmental monitoring, complemented by biological monitoring. No study was found about firefighters' occupational exposure to PAHs that simultaneously assessed environmental exposure levels at fire stations and the urinary levels of one or more PAH metabolites. Furthermore, urinary 1OHAce, 2OHFlu, and 3OHB[a]P were never measured before in firefighters.

Thus, the present study aims to assess the levels of 18 PAHs (16 USEPA priority PAHs, dibenzo[a,l]pyrene, and benzo[j]fluoranthene recommended by EU [40]) in the breathing air zone of firefighters' during their work shift at Portuguese fire stations, and the firefighters' total internal dose by six biomarkers of exposure (1OHNaph, 1OHAce, 2OHFlu, 1OHPhen, 1OHPy, and 3OHB[a]P) in urine samples. The contribution of personal airborne individual (naphthalene, acenaphthene, naphthalene + acenaphthene, fluorene, phenanthrene, pyrene) and ΣPAHs exposure to respective individual (1OHNaph + 1OHAce, 2OHFlu, 1OHPhen, and 1OHPy) and ΣOH-PAHs excretion in urine was explored for the first time in firefighters.

2. Materials and Methods

2.1. Characterization of the sampling sites

Portugal is one of the five Southern European countries that are the most affected by forest fires every year [41], being the North and Centre regions the most disturbed areas [42]. In 2014 the district of Bragança, in the sub-region of Alto Trás-os-Montes, (north of Portugal), was the third Portuguese district with a higher incidence of burnt area [42]. This region is characterized by typically hot and very dry summers with absolute maximum temperatures exceeding the 30 °C; winters are usually long and cold with absolute minimum temperatures reaching values below 0 °C. Sampling was performed at eight different fire stations: Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC) (Fig. 1). Two pairs of fire stations, namely MDL and TDC, as well as MRD and SDM were 22–23 km away from each other. The fire stations FEC, MDL, and MRD were located in the urban center of the city, near commercial shops and busy roads, while the firehouses TMC and TDC were situated in villages. FEC, MDL, and MRD were constructed before 1984 while the other fire stations were constructed and/or restored after 2007. At FEC, MDL and MRD, the truck bay where firefighters' and emergency vehicles were parked on had direct access to the operational control center and social room where firefighters spend most of their time (Fig. 1S (a), Supplementary Material). The door that separated these two microenvironments was frequently opened. At fire stations TDC, SDM, TMC, VNH, and BRG, the control center and social room were indirectly (through corridors and with some divisions; Fig. 1S (b), Supplementary Material) connected with the truck bay.

Information on outdoor meteorological conditions, namely temperature, relative humidity, wind speed, precipitation, and solar radiation during the sampling campaigns were retrieved from the local meteorological station (Table 1S, Supplementary material). The concentrations of 24-h ambient particulate matter with aero-

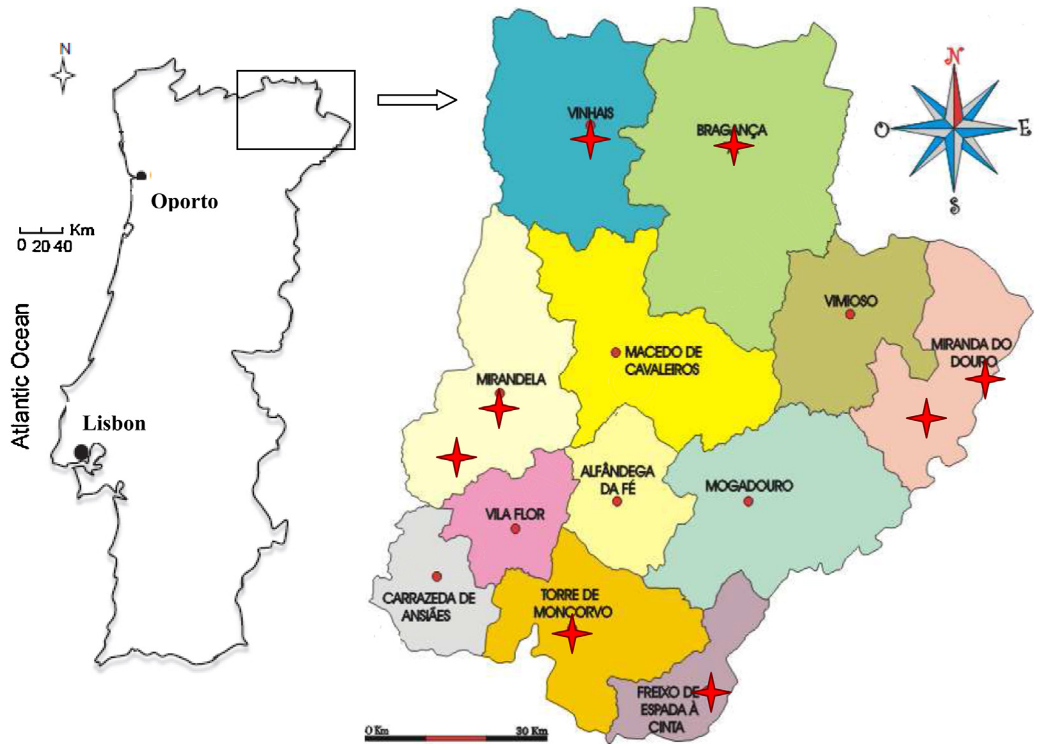

Fig. 1. Geographical location of the monitored fire stations in the district of Bragança.

dynamic diameter of 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$), nitrogen monoxide (NO), dioxide (NO_2) and oxides (NOx), sulphur dioxide (SO_2), and ozone (O_3) were also monitored from the meteorological station during the sampling campaign (Table 1S, Supplementary material).

2.2. *Personal air and urine sampling*

The study population consisted of eight units of professional firefighters serving at six municipal cities (Fig. 1). A structured questionnaire adapted from validated questionnaire [43] was filled by each firefighter. The questionnaire collected information on gender, age, number of years as firefighter (Table 2S, Supplementary material), and factors reported to be associated with PAH exposures, namely smoking habits, and the most consumed meals (boiled, roasted, and grilled) during the five days before urine collection. Subjects were excluded from the study if they participated in firefighting activities within five days prior the sampling campaigns and if they were exposed to tobacco smoke. Based on the information collected through the individual questionnaires, PAHs intake through food ingestion was considered not significant. All participants read and signed informed consent forms approved by the Ethic Committee of University of Porto.

Personal exposures were monitored during a period of fifty-four days (June and July 2014) in firefighters that were not directly involved in firefighting activities. At each fire station, air sampling campaigns of 18 selected PAHs in $PM_{2.5}$ were performed on a single day (6 individuals at SDM and FEC; 9 at MRD, MDL and TMC; 12 at TDC, VNH and BRG) and over a continuous 4-h period of a regular work shift inside the fire station; duplicate samples per individual were collected. During the sampling campaign firefighters were encouraged to move freely at the fire station as in a normal working day. Air sampling was done by personal constant flow samplers (Gilian, models GilAir3 and ProValue3; Sensidyne, USA) that were placed at the waist of each firefighter in a way that could not disturb the regular activities; an air flow rate of 2 L/min was used. The inlets were positioned at the breading zone of firefighters. Air samples were collected on polytetrafluoroethylene membrane filters with polymethylpentene support ring (2 μm porosity, Ø47 mm, SKC Ltd., United Kingdom). $PM_{2.5}$ masses were determined gravimetrically according to [44]. After the sampling, filters were stored in a freezer (−20 °C) before chemical analysis.

A spot urine sample was collected at the end of the firefighter work shift in sterilized 50 mL polycarbonate containers. Samples were frozen at −20 °C until analysis. Sampling campaigns were performed in triplicate.

During sample collection, a researcher was present to keep a record of potential source activities and ventilation system status (door and window positions). Potential sources of PAHs in each fire station were also collected. It was observed that during sampling campaigns, there were always vehicles (arriving, parking, or leaving) on the truck bay; smoking is not allowed at fire stations.

2.3. *PAHs and OH-PAHs chromatographic analysis*

Extraction and quantification of PAHs from $PM_{2.5}$ filters, and OH-PAHs from urine samples were performed by previously validated analytical procedures [45–47]. PAHs and OH-PAHs extracts were analysed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with photodiode array (PAD) and fluorescence (FLD) detectors on line. Separation of the compounds was performed in a C18 column (CC 150/4 Nucleosil 100–5C18 PAH, 150 × 4.0 mm; 5 μm particle size; Macherey–Nagel, Duren, Germany) maintained at room temperature (20 ± 1 °C). Fluorescence wavelength programming was used to perform better sensitivity and minimal interference. Each compound was detected at its optimum excitation/emission wavelength pair: 260/315 nm (naphthalene, acenaphthene and fluorene), 260/366 nm (phenanthrene), 260/430 nm (anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and dibenzo[a,l]pyrene), and 290/505 nm

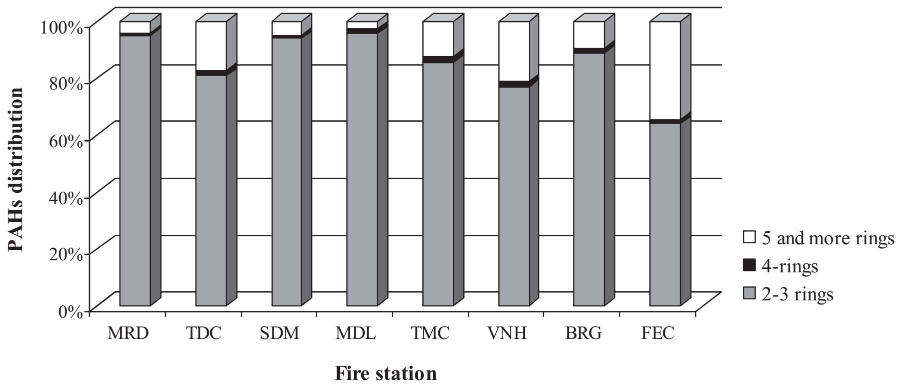

Fig. 2. PAHs distribution according to the number of aromatic rings at different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

(indeno[1,2,3–cd]pyrene) for PAHs, and 232/337 nm (1OHNapt and 1OHAce), 265/335 nm (2OHFlu), 263/363 nm (1OHPhen), 242/388 nm (1OHPy), and 308/432 nm (3OHB[a]P) for OH-PAHs. The PAH acenaphthylene, which shows limited fluorescence, was analysed at 254 nm in PAD.

Calibrations with PAHs and OH-PAHs mixed standards, using at least 6 calibration points, were performed in acetonitrile and methanol, respectively. Calibration curves were linearly fitted with correlation coefficients always higher than 0.9979. RSD values ranged from 1.8% (dibenzo[a,l]pyrene) to 9.1% (naphthalene) for PAHs extraction from filters. Regarding urinary OH-PAHs, RSD values varied between 1.3% (1OHNaph + 1OHAce and 2OHFlu) to 8.1% (1OHNaph + 1OHAce, 2OHFlu, and 1OHPy). Limits of detection (LODs) and quantification (LOQs) were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, respectively [48]. For PAHs, LODs between 1.0 pg/m^3 (for anthracene, benzo[k]fluoranthene, chrysene, benz[a]anthracene, phenanthrene and indeno[1,2,3-cd]pyrene) and 148 pg/m^3 (for acenaphthylene) were obtained, with corresponding LOQs in the range 3.4–492 pg/m^3. Regarding OH-PAHs, LODs ranged between 0.0008 μg/L urine (for 2OHFlu) to 0.195 μg/L urine (for 1OHNaph + 1OHAce), while the respective LOQs varied from 0.0028 μg/L urine to 0.650 μg/L urine.

All OH-PAH concentrations were normalized by urinary creatinine (mol/mol). Urinary creatinine measurements were performed according to the Jaffe colorimetric method [49].

Analytical blanks and standards were analysed daily and regularly. Each analysis was performed at least in triplicate.

2.4. *Statistical analysis*

Statistical analysis was performed using SPSS (IBM SPSS Statistics 20). PAHs and OH-PAHs median values were compared through the nonparametric Mann–Whitney *U* test, since normal distribution was not observed by Kolmogorov-Smirnov with Lilliefors correction and Shapiro–Wilk's tests. When the concentration was below the LOD, the value of the respective LOD/$\sqrt{2}$ was used [50]. Statistical significance was defined as $p \leq 0.05$. Spearman correlation coefficients were calculated to examine the relation between airborne individual and total PAH concentrations with firefighters' urinary individual and total OH-PAHs for each fire station.

3. Results and Discussion

3.1. *Personal exposure monitoring*

The concentrations of individual and total PAHs (ΣPAHs) in the breathing air zone of firefighters working at each fire station are presented in Table 1. Among the 18 PAHs considered, acenaphthylene was the most abundant compound (except in the environmental exposures of the rural sites of TMC and TDC where it was not detected) with concentrations representing 46.4% (at FEC) to 61.5% (at SDM) of ΣPAHs. Acenaphthylene is a component of crude oil, coal tar, tobacco smoke and a product of combustion which may be produced and released to the environment during fires [20,30,51]; emissions from wood combustion contain more acenaphthylene than other PAHs [52]. For workers at MRD, SDM, MDL, TMC, and BRG firehouses, the other most abundant PAHs were, by descending order, acenaphthene, naphthalene, and phenanthrene with contributions ranging from 15.5 (BRG) to 38.6% (MRD), 4.48 (MDL) to 16.8% (BRG), and from 2.65 (MRD) to 7.82% (TMC), respectively. Together with acenaphthylene, these four PM$_{2.5}$-bound PAHs represented 84.6–95.0% of ΣPAHs for those fire stations. In the breathing air zone of firefighters from FEC and VNH, dibenz[a,h]anthracene was respectively the second and third predominant PAH, accounting with 11.9% and 7.84% of ΣPAHs. Dibenz[a,h]anthracene concentrations in workers from the remaining six fire stations represented only 0.30–1.73% of ΣPAHs. The prevalence of this compound indicates a higher influence from light-duty gasoline vehicle emissions [53] in the surrounding areas of FEC and VNH fire stations. Globally PM$_{2.5}$-bound PAHs with 2–3 aromatic rings (naphthalene, acenaphthylene, and acenaphthene) represented 63.9% (FEC) to 95.7% (MDL) of ΣPAHs; 4 aromatic ring compounds (anthracene, fluoranthene, and pyrene) accounted with 0.79% (MRD) to 2.05% (TMC) and 5 or more aromatic ring PAHs corresponded to 2.74% (MDL) to 34.7% (FEC) of ΣPAHs (Fig. 2). It is worth to mention that lighter PAHs usually predominate in the gas phase [10,36] suggesting that the determined personal exposures may be underestimated. Overall airborne PAH profiles obtained at FEC and VNH exhibited the highest contributions of the heavier molecular weight compounds (the most hazardous), while those at MRD, SDM and MDL presented the lowest. As a general rule, PAHs toxicity increases as the number of rings increases (with the exception of naphthalene, 2-rings compound that is classified as possible human carcinogen; [8]).

Global mean ΣPAHs varied widely. The highest total median content of PAHs was found, by descending order, in the breathing air zone of firefighters from $\text{FEC} \gg \text{MDL} > \text{MRD} \gg \text{SDM} > \text{VNH} > \text{BRG} > \text{TMC} \approx \text{TDC}$, with levels at FEC (428 ng/m^3) 2 (256 ng/m^3, MDL) to 9 (46.4 ng/m^3, TDC) times significantly higher ($p \leq 0.036$) than at the other fire houses. Indoor PAH levels, and personal exposure, depend on activities conducted, occupancy rates, physical characteristics of buildings (permeability, particle size-specific difference), site specificity, ventilation habits, season and meteorology. The observed differences may be mainly attributed to site specificity (urban/rural) and characteristics of buildings. The

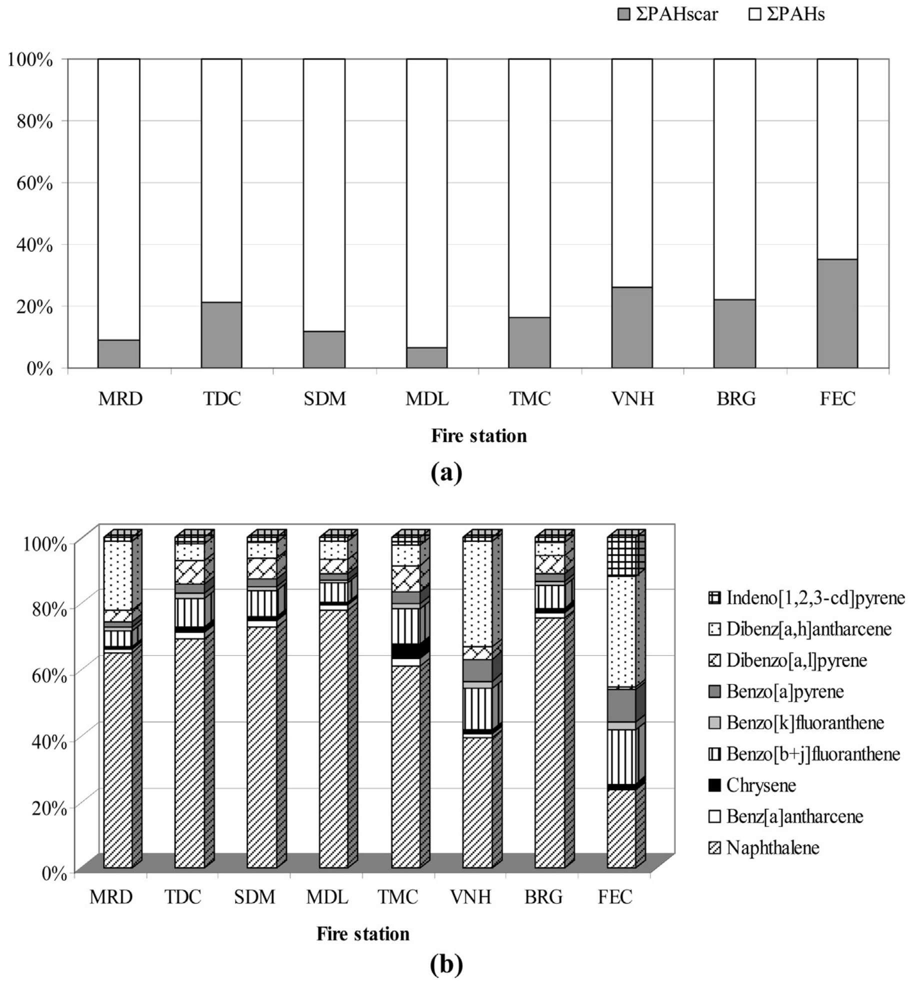

Fig. 3. PAHs distribution of a) total carcinogenic PAHs (ΣPAHs$_{car}$) among the 18 PAHs under study (ΣPAHs) at each fire station; b) individual carcinogenic PAHs among the different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

two firehouses located at rural areas with low traffic influences (TMC and TDC) presented the lowest levels of PAHs in the breathing air zone of firefighters (Table 1), followed by those situated in urban sites but constructed and/or restored more recently (BGR, VNH and SDM). Vehicular traffic emissions are one of the major sources of PAHs in urban areas [54]. In addition, the age of a building reflects its condition and affects penetration of outdoor PAH concentrations to indoor air. The older a building, the greater will be the impact of outdoor sources owing to higher air exchange through such routes as poorly fitting windows and doors [55]. The urban location (near busy roads), building age (constructed before 1984) and lay-out of FEC, MDL, and MRD contributed significantly to the elevated firefighters' environmental exposure at these firehouses. The truck bay at FEC, MDL and MRD was physically separated from the operational control center and social room (where firefighters spend most of their time) only by a door (that was frequently opened). Moreover, the determined levels at these three stations were higher than those reported (30.5–126.5 ng/m^3) for two sites located in the outskirts of the city of Porto that is a more urbanized area and has much higher traffic density than Bragança district (Metropolitan Area of Porto has 2042 km^2 with 1.7 million citizens, while Bragança district has an area of 6 608 km^2 with 139 344 citizens); Porto district corresponds to the nearest geographical region (Fig. 1) that was previously characterized in terms of atmospheric PAH levels [56].

More detailed analysis was performed concerning the carcinogenic PAHs (ΣPAHs$_{car}$; Table 1). The inter-fire stations comparison of the content of ΣPAHs$_{car}$ in the breathing air zone of firefighters was FEC » MRD ≈ VNH > MDL > BRG ≈ SDM ≈ TDC ≈ TMC. In accordance with the ΣPAH levels, workers from FEC exhibited significantly higher ($p \leq 0.002$) ΣPAHs$_{car}$ (Fig. 3a). Globally the compound that contributed the most to ΣPAHs$_{car}$ was naphthalene (39.4% at VNH to 78.1% at MDL), except for firefighters from FEC firehouse. For that fire station, dibenz[a,h]anthracene (with a toxicity equivalent factor 5 times higher than benzo[a]pyrene; [9] was the predominant PAH accounting with 33.8% of ΣPAHs$_{car}$, followed by naphthalene (23.5%) (Fig. 3b). In addition, firefighters working at VNH firehouse also presented a relevant contribution of dibenz[a,h]anthracene (32.1% of ΣPAHs$_{car}$) in their breathing air zone; for the other sites, values varied from 4.1% (BRG) to 21.1% (MRD) of ΣPAHs$_{car}$. The maximum concentrations of benzo[a]pyrene, the PAHs marker of carcinogenicity, accounted with 10% at FEC, 6.7% at VNH, 1.5–3.4% of ΣPAHs$_{car}$ for the other firehouses (Fig. 3b). Furthermore, it is important to mention that the median concentrations of benzo[a]pyrene of FEC firefighters exceeded 10 to 20 times the existent limit value of 1 ng/m^3 (annual mean total content in PM$_{10}$ fraction) for ambient air [40]. Dibenzo[a,l]pyrene, which carcinogenic potency has been estimated to be approximately 100 times that of benzo[a]pyrene [9], presented concentrations lower than 0.671 ng/m^3 (0.44–8.2% of ΣPAHs$_{car}$) in the breathing air zone of firefighters.

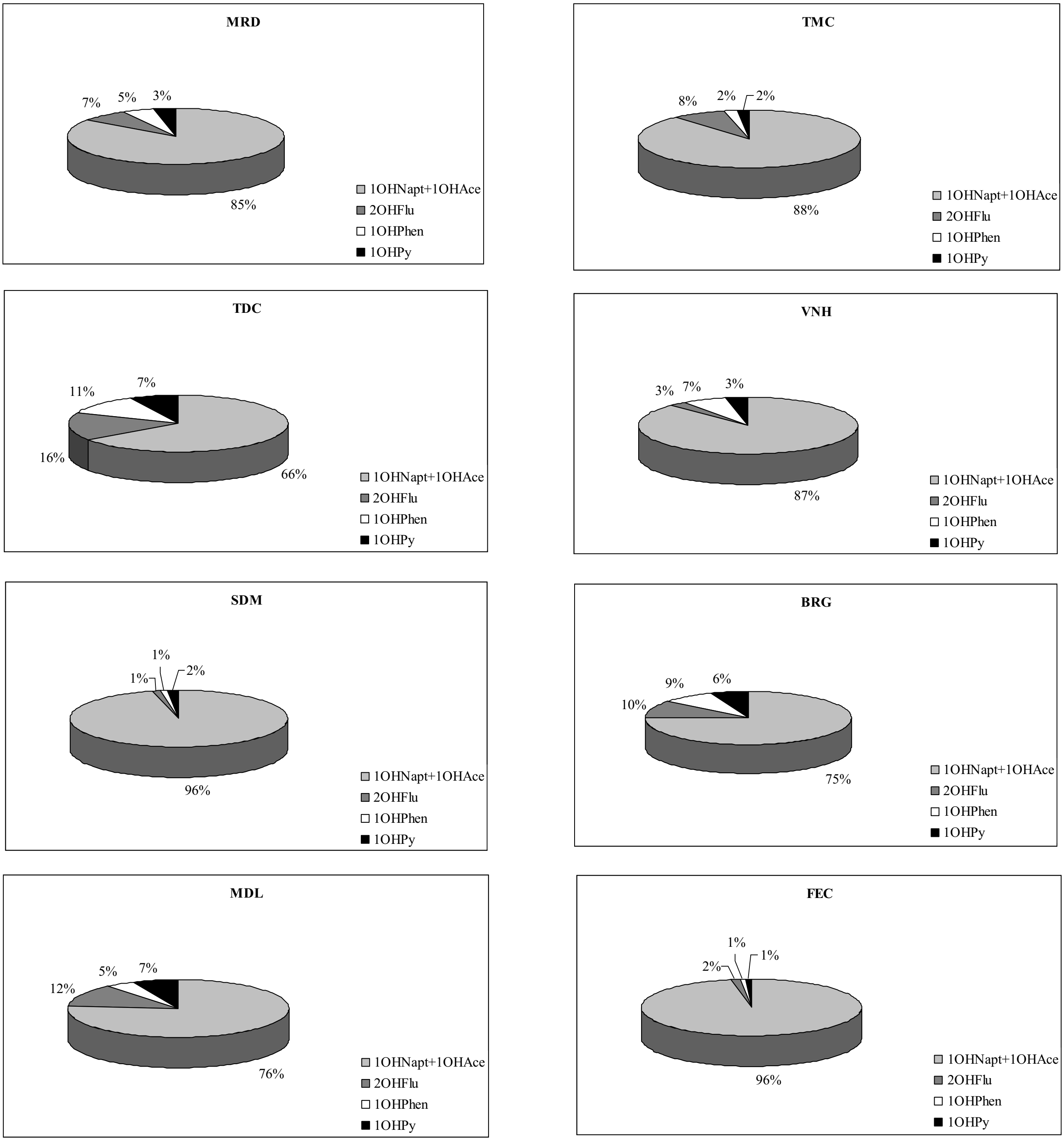

Fig. 4. Distribution of monohydroxyl-PAHs (1OHNaph + 1OHAce: 1-hydroxynaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPHen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) in the urine samples of firefighters at different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

Firefighters personal exposure to PAHs at Portuguese fire stations were well below the existent PAHs occupational exposure limits proposed by the American Conference of Governmental Industrial Hygienists (ACGIH), the NIOSH and the US Occupational Safety and Health Administration (OSHA) (Table 3S, Supplementary material).

Studies regarding firefighters' occupational exposure to PAHs at fire stations are very scarce [23,32]; none in European facilities. Moreover, comparison between various studies is hampered by different designs (analysed particulate fraction or/and number of determined PAHs). Shen and coworkers [32] collected dust samples from vacuum cleaner bags used to routinely clean twenty American firehouses. Median total PAHs concentration in dust was 4124 ng/g. Among the 10 compounds under study, pyrene was the predominant PAH measured in these dust samples, followed, in decreasing order, by benzo[ghi]perylene, fluoranthene and chrysene. Baxter and colleagues [23] conducted an air monitoring study in three different environments, including in the fire truck bay and equipment storage area, the kitchen/common area, and the sleeping quarters of two American fire stations [23]. Surprisingly, out of 16 quantified PAHs, only naphthalene (at 9.22–9.24 $\mu g/m^3$) was detected in $PM_{2.5}$-bound PAHs samples collected in the kitchen and truck bay of one firehouse. Median concentrations of naphthalene (4.95 at TMC to 12.2 ng/m^3 at MRD, Table 1) in the breathing air zone of Portuguese firefighters were lower than those reported levels [23]. Furthermore, some recent studies [20,30] have reported that a quantifiable deposition of PAHs outside and inside firefighters' ensembles (helmets, gloves, hoods, and turnout gear) occurs which may significantly contribute to total PAHs body burden. Further research is required to investigate this aspect.

3.2. Biomonitoring

Biomonitoring of firefighters is an effective assessment tool to understand total exposures, especially to compounds such as PAHs that are not absorbed exclusively by inhalation, but also through dermal contact and food ingestion [18–20]. Median concentrations of individual and total OH-PAHs (ΣOH-PAHs) in the urine samples of firefighters are presented in Table 2. Urinary OH-PAH levels were normalized by the creatinine levels of each firefighter. Globally creatinine concentrations in the urine of firefighters ranged between 0.82 to 2.74 g/L, being within the accepted creatinine range for healthy people (> 0.3 and < 3 g/L) [57]. Since PAHs are ubiquitous pollutants, each participating firefighter was requested to fill a questionnaire regarding the existence of possible exposure to PAHs outside the occupational environment. Only non-smoking firefighters with dietary exclusive of barbecue and deep-fried foods within five days before urine samples collection were considered. Thus, it was assumed that firefighters had similar (non-significant) levels of PAHs exposure through food consumption and that tobacco smoke contribution was negligible. Still, the assessment of tobacco biomarker data, and the use of pre-exposure urine samples, would be precious to validate these assumptions. Knowledge of the elimination kinetics of the urinary OH-PAHs is essential to define the urine sampling strategy. Limited studies regarding elimination kinetics of OH-PAHs in humans are available: half-life of urinary 1OHPy excretion rates varies between 6 to 35 h after inhalation exposure [58,59] and 4.4 [60] to 12 h [61] after ingestion exposure; half-life ranging from 3.3 to 6.2 h for 1OHNaph, 2.3 to 4.0 h for 2OHFlu, and 4.3 to 6.1 h for 1-OHPhen were also reported for ingestion exposure [37]. 1OHNaph + 1OHAce were detected in more than 87% of samples, 2-OHFlu and 1OHPy presented detection rates above 96% and 1OHPhen was detected in all urine samples. In agreement with some previous studies, urinary 3OHB[a]P (metabolite of the PAH marker of carcinogenicity) presented null or very low detection rates [37,62,63]. Some studies with animals revealed that urinary concentrations of 3OHB[a]P represent only 0.1–0.2% of the benzo[a]pyrene dose, due to a complex metabolism that produces several different metabolites which are mainly excreted in feces [64].

Overall the inter-comparison of urinary ΣOH-PAHs of firefighters from fire stations: FEC » VNH > MRD ≈ SDM > MDL > BRG > TMC » TDC, followed the same trend as the content of airborne ΣPAHs with the exception of workers from VNH and MDL that exchanged position. Accordingly, urinary ΣOH-PAHs in individuals from FEC were significantly elevated ($p \leq 0.002$) (2 (MRD) to 14 (TDC) times higher) than at other firehouses. The concentrations of urinary OH-PAHs in firefighters were inversely related to the size of compounds, i.e., the higher the molecular weight the lower the detected OH-PAH concentrations (Table 2). Urinary 1OHNaph + 1OHAce were by far the predominant compounds accounting with 66% (TDC) to 96% of ΣOH-PAHs (FEC, SDM), being followed by 2OHFlu (1% at SDM to 16% at TDC), 1OHPhen (1% at SDM and FEC to 11% at TDC), and 1OHPy (1% at FEC to 7% at TDC) (Fig. 4). This distribution profile follows the same pattern as the PAHs distribution in the breathing air zone of firefighters while working at fire stations (Table 1); airborne PAHs with two and three aromatic rings were the most abundant ones in all fire stations (63.9% at FEC to 95.7% of ΣPAHs at MDL, Fig. 2). Urinary 1OHNaph + 1OHAce concentrations were significantly different ($p \leq 0.002$) from the other metabolites for all groups of individuals (Table 2). The median concentrations of urinary 1OHPy in Portuguese firefighters ranged between 1.36×10^{-2} (SDM) to 0.146 (MDL) μmol/mol creatinine (Table 2). Nowadays, measurements of urinary 1OHPy are routinely applied to control industrial exposure to PAHs in coke ovens and primary aluminum production and to control exposure of professionals when handling coal tar derived products [34]. Although no reference standard guidelines are established for urinary OH-PAHs, Jongeneelen [33,34] proposed a no-biological effect level of 1.4 μmol/mol creatinine of 1-hydroxypyrene in urine of exposed workers, i.e. the lowest reported level at which no genotoxic effects were found. In addition the Biological Exposure Index Committee of ACGIH stated that the presence of urinary 1OHPy above a benchmark level of about 1 μg/L (0.5 μmol/mol) indicates occupational exposure to PAHs, since very few non-occupationally exposed persons, smokers or non-smokers, will excrete this amount of 1OHPy [65]. ACGIH also recommends that the benchmark should be considered as a post-shift level. The quantified concentrations of post-shift urinary 1OHPy in firefighters from all fire stations were well below those recommended values. This observation is important since firefighters participating at firefighting activities are heavily exposed to PAHs [18–20] and thus may present high background levels of 1OHPy.

Regarding characterization of firefighters' exposure, 1OHPy and 1OHNaph are the more investigated metabolites [27,31,38,39]. The comparison of the detected levels with previous reports is extremely difficult since, in the majority of the studies, concentrations are not adequately normalized with the personal urinary creatinine levels. Creatinine is eliminated from the human body at a constant rate, and thus is widely used to minimize the variability of parameters such as individual fluid intake, body temperature, physical exercise and ambient temperature, which changes from person to person. Still, urinary 1OHPy concentrations in Portuguese firefighters (overall range: 0.078–3.28 nmol/L) were slightly higher than levels found in firefighters before their participation in gas simulators (0.6–1.2 nmol/L) but lower than concentrations at diving simulator fires (0.6–9.2 nmol/L) [32]. In addition 1OHPy levels were similar with the levels reported in firefighters before their participation in prescribed pile burns (< 0.01–0.56 μg/L [27]) but slightly higher than the concentrations observed in the control group of firefighters that were present at the World Trade Center Collapse in 2001 (157 ng/L in this study *versus* 62.5 ng/L [39]). Only two studies reported concentrations of other metabolites, namely several OHPhen [39] and 1OHNaph [31] compounds among firefighter's urine. Urinary 1OHPhen levels were similar with those observed in the control group of firefighters (170 ng/L in this study *versus* 158 ng/L [39]). In firefighters, urinary 1OHAce, 2OHFlu, and 3OHB[a]P were never assessed before. Urinary OH-PAHs excretion in firefighters during their regular work at fire stations was lower than levels reported for other industrial workers with known PAHs exposure [63,66–70].

3.3. Correlations between airborne PAHs and urinary OH-PAHs

The possible contribution of airborne individual (naphthalene, acenaphthene, naphthalene + acenaphthene, fluorene, phenanthrene, and pyrene) and ΣPAHs to respective individual (1OHNaph + 1OHAce, 2OHFlu, 1OHPhen, and 1OHPy) and ΣOH-PAHs excretion in the post-shift urine was estimated. Moderate to strong correlations were observed between ΣPAHs and urinary ΣOH-PAHs (ranging from $r = 0.367$, $p = 0.332$ at TMC to $r = 0.886$, $p = 0.019$ at FEC) for firefighters from six fire stations (FEC, BRG, MDL, MRD, TDC, TMC); statistical significance was reached for workers from MRD ($r = 0.733$, $p = 0.025$), MDL ($r = 0.786$, $p = 0.021$), BRG ($r = 0.839$, $p = 0.001$) and FEC ($r = 0.886$, $p = 0.019$) which were three of the firehouses where the highest environmental exposure were detected (Table 1).

These findings are in agreement with those described for coke oven workers ($r = 0.680$, $p \leq 0.01$ between ΣPAHs of 15 compounds and ΣOH-PAHs of 10 metabolites) [61]. The positive correlations confirm that OH-PAH excretion increases with an increasing exposure of PAHs at fire stations. It seems that, at these sites, firefighters had fire station indoor air as their major exposure source of PAHs. Also, ΣPAHs correlated well with 1OHPy, the biomarker of PAHs exposure, for individuals from MRD ($r = 0.703$, $p = 0.035$), SDM ($r = 0.941$, $p = 0.005$), MDL ($r = 0.262$, $p = 0.531$) and VNH ($r = 0.944$, $p \leq 0.001$). These urban sites are in the list of the 5 firehouses where environmental PAHs exposure were the highest (FEC » MDL > MRD » SDM > VNH > BRG > TMC ≈ TDC; Table 1); the only observed exception was FEC. These results suggest that 1OHPy may be a suitable biological indicator of internal dose of exposure to those PAHs that are emitted by the predominant source. These correlations are higher than those reported in coke oven workers ($r = 0.456$, $p < 0.01$; [63]) but similar with the relationships described in workers employed in coke production, production of graphite electrodes, special carbon products, and production of refractory materials ($r = 0.717$, $p < 0.01$) [70]. Other significant relationships were reached between naphthalene + acenaphthene with urinary 1OHNaph + 1OHAce for firefighters at MDL ($r = 0.833$, $p = 0.010$), fluorene and 2OHFlu at FEC ($r = 0.771$, $p = 0.072$) and BRG ($r = 0.736$, $p = 0.006$), phenanthrene and 1OHPhen at FEC($r = 0.971$, $p = 0.001$); and pyrene with 1OHPy at TMC ($r = 0.878$, $p = 0.002$) and VNH ($r = 0.745$, $p = 0.005$). Yamano et al. [63] and Rossbach et al. [70] also reported moderate to strong correlations between urinary metabolite concentration and personal exposure to the respective PAH.

Globally these results suggest that airborne PAHs at fire stations may contribute to firefighters' total PAHs body burden; the influence is more evident in firefighters exposed to the highest levels. Still the existence of other significant common sources (food, home air and/or outdoor air) cannot be discarded. Naphthalene and acenaphthene are the more volatile compounds and most of their environmental levels enter in the human body mainly through air. Fluorene, phenanthrene and pyrene are absorbed through the respiratory tract but also by the gastrointestinal tracts, and by the skin.

4. Conclusions

This work characterized firefightersí exposure to PAHs during their work shift at eight Portuguese fire stations by personal exposure monitoring and biomonitoring. Overall, $PM_{2.5}$-bound ΣPAH concentrations ranged from 46.4–428 ng/m^3, with 2–3 aromatic rings accounting with 63.9–95.7% of ΣPAHs. PAHs with 4–5 or more aromatic rings corresponded to 0.789–2.05% and 2.54–34.7% of ΣPAHs, respectively. The obtained data highlighted the importance of site specificity and poor building construction/conservation, as well as inappropriate building layout on environmental PAH levels at the studied fire stations. Still, firefighter's personal exposure to PAHs at Portuguese fire stations was well below the existent occupational exposure limits.

In accordance with the airborne PAHs profile, urinary 1-hydroxynaphthalene and 1-hydroxyacenaphthene were the predominant metabolites (66–96% ΣOH-PAHs). Thus, it is recommended that total body burden of PAHs should not be based exclusively on 1OHPy biomonitoring, as it has been performed in the large majority of studies. In addition, the contribution of some selected airborne individual compounds and ΣPAHs to respective individual and ΣOH-PAHs excretion in the post-shift urine was estimated for the first time in firefighters. The attained significant positive correlations for firefighters from four fire stations indicated the influence of occupational exposure on metabolite levels. Results for a larger number of fire stations, participants and number of (air and urine) samples are needed to draw more meaningful and statistically more significant conclusions. Also, a more comprehensive monitoring that includes all possible sources (food, home and outdoor air, *etc.*) would be precious to give more quantitative support to the impact of occupational exposure on firefighter's total PAHs internal dose.

Conflict of interest

The authors declare that there are no conflicts of interest.

Statement of novelty

This work characterized firefighters' exposure to PAHs during their work shift at eight fire stations by personal monitoring (18 PAHs) and biomonitoring (six urinary metabolites). Only two studies were found regarding firefighters' environmental exposure to PAHs at fire stations, both performed in USA. No information exists concerning other countries. Moreover, no study was found that simultaneously assessed airborne PAH exposure levels at fire stations and urinary levels of one or more PAH metabolites. Also, contribution of personal airborne individual and total PAHs exposure to respective individual and total OH-PAHs excretion in urine was explored for the first time in firefighters.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2016.03.012.

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