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Firefighters' exposure biomonitoring: Impact of firefighting activities on levels of urinary monohydroxyl metabolites

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ABSTRACT

The concentrations of six urinary monohydroxyl metabolites (OH-PAHs) of polycyclic aromatic hydrocarbons, namely 1-hydroxynaphthalene, 1-hydroxyacenaphthene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, 1-hydroxypyrene (1OHPy), and 3-hydroxybenzo[a]pyrene, were assessed in the post-shift urine of wildland firefighters involved in fire combat activities at six Portuguese fire corporations, and compared with those of non-exposed subjects. Overall, median levels of urinary individual and total OH-PAHs (ΣOH-PAHs) suggest an increased exposure to polycyclic aromatic hydrocarbons during firefighting activities with ΣOH-PAH levels in exposed firefighters 1.7–35 times higher than in non-exposed ones. Urinary 1-hydroxynaphthalene and/or 1-hydroxyacenapthene were the predominant compounds, representing 63–98% of ΣOH-PAHs, followed by 2-hydroxyfluorene (1–17%), 1-hydroxyphenanthrene (1–13%), and 1OHPy (0.3–10%). A similar profile was observed when gender discrimination was considered. Participation in fire combat activities promoted an increase of the distribution percentage of 1-hydroxynaphthalene and 1-hydroxyacenaphthene, while contributions of 1-hydroxyphenanthrene and 1OHPy decreased. The detected urinary 1OHPy concentrations (1.73×10^{-2} to 0.152 μmol/mol creatinine in exposed subjects *versus* 1.21×10^{-2} to 5.44×10^{-2} μmol/mol creatinine in non-exposed individuals) were lower than the benchmark level (0.5 μmol/mol creatinine) proposed by the American Conference of Governmental Industrial Hygienists. This compound, considered the biomarker of exposure to PAHs, was the less abundant one from the six analyzed biomarkers. Thus the inclusion of other metabolites, in addition to 1OHPy, in future studies is suggested to better estimate firefighters' occupational exposure to PAHs. Moreover, strong to moderate Spearman correlations were observed between individual compounds and ΣOH-PAHs corroborating the prevalence of an emission source.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist in a large class of ubiquitous pollutants that are released during incomplete combustions (Kim et al., 2013). People are frequently exposed to PAHs in occupational and residential environments being inhalation and ingestion the most predominant routes (ATSDR, 1995; Campo et al., 2014; Laitinen et al., 2012; Liu et al., 2010; Lutier et al., 2016; Preuss et al., 2006; Yin et al., 2014). Dermal exposure also assumes a relevant contribution for some particular occupational exposure groups such as firefighters (Alexander and Baxter, 2014; Fabian et al., 2014; Kirk and Logan, 2015a, 2015b), employees from production, transport and use of heavy fuel oil (Christopher et al., 2011), roofers (Serdar et al., 2012), road pavers (Väänänen et al., 2005) and asphalt workers (Sobus et al., 2009a). Some PAHs are classified as carcinogens (probable/possible), mutagens, and teratogens and therefore these compounds represent potential health risks to the exposed populations (Boström et al., 2002; IARC, 2002, 2010b); benzo[a]pyrene (5 ring compound) is classified as carcinogenic to humans (group 1; IARC, 2010b) while naphtha-

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lene (2 rings), benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, and indeno[1,2,3-cd]pyrene (4–6 rings compounds) are considered as possible carcinogenic to humans (group 2B; IARC, 2002, 2010b). Dibenzo[a,l]pyrene and dibenz[a,h]anthracene have been also under scrutiny because they are regarded as probable carcinogens to humans (group 2A; IARC, 2010b) due to their higher carcinogenic potency than benzo[a]pyrene (Okona-Mensah et al., 2005). PAHs are known to possess reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities in humans and laboratory animals (ATSDR, 1995); recently they were included in the list of endocrine disrupting chemicals (WHO, 2013). PAHs toxicity, especially those with high molecular weight, is largely attributed to their reactive metabolites ability to react with DNA to form depurinating adducts, which results in apurinic sites and mutations (Lewtas, 2007). As a consequence DNA binding, frame-shift mutation, chromosomal aberrations, strand breaks, and mutations constitute the main products of those reactions, potentiating genetic effects that can lead to cardiovascular and reproductive damage, and the development of cancer (Kamal et al., 2015; Lewtas, 2007).

PAHs enter into the human body through the lungs, gastrointestinal track, and the skin, being then disseminated to the lipophilic tissues (Franco et al., 2008; Needham et al., 2007). PAHs metabolization occurs *via* oxidative pathways to produce a complex mixture of intermediate compounds (quinines, phenols, dihydrodiols, triols and tetrols) in order to eliminate them by conjugation with glutathione, glucuronide or sulphate conjugates. PAH metabolites can be excreted with faeces, urine and milk (Franco et al., 2008; Jongeneelen, 2001; Likhachev et al., 1992; Rey-Salgueiro et al., 2009; Kamal et al., 2015; Lewtas, 2007). Among the many different elimination routes of monohydroxyl PAHs (OH-PAHs), urine is the most described one since it is the less invasive matrix to determine biomarkers of exposure (Needham et al., 2007). Thus urinary OH-PAHs constitute a precious tool to estimate the real PAHs intake compared to environmental exposure because they reflect the internal (systemic) dose received. Pyrene is frequently classified as the marker of PAHs exposure and it is mostly eliminated in the form of 1-hydroxypyrene (1OHPy), reason why this metabolite is the most described biological indicator of internal dose of exposure to PAHs (Franco et al., 2008; Hansen et al., 2008; Bouchard and Viau, 1999). However, 1OHPy reflects only the total exposure to pyrene, which is one of the least toxic compounds included in the sixteen US Environmental Protection Agency PAHs priority contaminants (USEPA, 2005). In that regard, some authors have questioned the adequacy of 1OHPy to estimate and/or represent the total internal dose of PAHs (Hansen et al., 2008; Ciarrocca et al., 2014).

Firefighters are an occupational group that is at high risk to suffer potential health effects due to their chronic exposure to a countless number of air pollutants that are released during fire occurrences (Estrellan and Lino, 2010; Lemieux et al., 2004; Lewtas, 2007; Oliveira et al., 2016c; Reisen et al., 2006). High levels of PAHs have been described in air and on burnt surfaces after fire incidents (Kamal et al., 2015; Lewtas, 2007). Recently the International Agency for Research on Cancer (IARC) and the US National Institute for Occupational Safety and Health (NIOSH) included firefighting among the most dangerous professions and classified firefighters' occupational exposure as possible carcinogen to humans (IARC, 2010a; NIOSH, 2007). However firefighters are among the less characterized occupational groups mostly due to difficulties in the study design. Wildland firefighters are frequently engaged in heavy work activities at adverse meteorological conditions (temperature, moisture, wind speed and direction, etc.). Firefighting is an intermittent occupation and thus the number of exits related with fire occurrences and the time spent with fire suppression are mostly dependent on the accessibility of the fire location, and on the available staff (IARC, 2010a). As a consequence characterization of firefighters' occupational exposure to PAHs during fire combat activities is almost inexistent (Fent et al., 2014; Kirk and Logan, 2015a; Pleil et al., 2014; Robinson et al., 2008). Full monitoring of firefighters' exposure to PAHs *via* all exposure routes (air, food and dermal) should be performed through the quantification of their internal dose. Once again scarce information is available (Adetona et al., 2015; Edelman et al., 2003; Caux et al., 2002; Laitinen et al., 2010; Robinson et al., 2008) being the majority of the studies conducted in American cities, although it is believed that biomonitoring of internal dose is the first step toward occupational safety and prevention of potential health risks in firefighters.

Table 1
Characteristics of firefighters that participated in the study.

Characteristic	Non exposed	Exposed
n	96	57
Gender		
Male (%)	78	79
Female (%)	22	21
Age (mean ± SD; min–max; years)	36.2 ± 9.6; 22.0–55.0	36.3 ± 8,5; 21.0–52.0
Man	37.7 ± 9.4; 23.0–55.0	36.7 ± 8.4; 22.0–52.0
Woman	30.7 ± 8.7; 22.0–45.0	34.8 ± 10.0; 21.0–45.0
Weight (mean ± SD; min–max; kg)	81.5 ± 15.5; 54.0–118	79.7 ± 9.9; 59.0–98.0
Man	86.5 ± 5.1; 67.0–118	82.1 ± 8.5; 73.0–98.0
Woman	61.5 ± 5.1; 54.0–68.0	67.7 ± 7.8; 59.0–74.0
Number of years as firefighter		
≤10 years (%)	28	26
10–20 years (%)	50	42
≥20 years (%)	22	32
Time dedicated to firefighting activities within the 48 h before sample collection		
<5 h (%)	n.a.	53
5–10 h (%)	n.a.	37
>10 h (%)	n.a.	10

n.a.—not applicable.

Thus this work assesses, for the first time, the total PAHs internal dose of Portuguese wildland firefighters' by six urinary OH-PAHs, namely 1-hydroxynaphthalene (1OHNaph), 1-hydroxyacenaphthene (1OHAce), 2-hydroxyfluorene (2OHFlu), 1-hydroxyphenanthrene (1OHPhen), 1OHPy, and 3-hydroxybenzo[a]pyrene (3OHB[a]P). Urinary concentrations and distribution profiles of individual OH-PAH were determined in non-exposed and exposed firefighters serving six different Portuguese fire corporations; the gender influence was also considered in two fire stations. In addition, Spearman correlation coefficients were explored to examine the relation between urinary individual compounds and the total body burden of firefighters. Portugal has been one of the most affected Southern European countries by forest fires during the last decade (JRC, 2015), still the impact of fire combat activities on urinary OH-PAH levels of Portuguese (and European) wildland firefighters is unknown.

2. Materials and methods

2.1. Characterization of the study population and urine sampling

The study subjects were 153 healthy and no-smoking (and non-exposed to tobacco smoke) firefighters (Table 1) serving at six different firefighting corporations located in Trás-os-Montes and

Fig. 1. Geographical location of the selected fire corporations.

Alto Douro Region of Portugal: Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL) (Fig. 1). All firefighting corporations belong to cities of the district of Bragança (north of Portugal; total area of 6 608 km^2 with 139 344 inhabitants), which was one of the three most affected Portuguese regions by forest fires in 2014 (ICNF, 2014) mainly due to hot and very dry summers. During the summer of 2014, six large fires, *i.e.*, fires with a burnt area higher than 100 ha, occurred in the months of July to September and represented 45% of the total burnt area in the district of Bragança. The study proposal was reviewed and approved by Ethic Committee of University of Porto (Portugal). At each fire corporation, a descriptive explanation of the study was presented to the firefighters. All firefighters who were interested in participating in the study gave an informed consent. Each subject was requested to fill out a structured questionnaire that was adapted from a validated questionnaire (WHO, 2002). This questionnaire collected general information related with gender, age, weight, number of years as firefighter, and time dedicated to wildland firefighting activities (knockdown and/or overhaul) within the 48 h prior the urine collection (Table 1). Questions related with the use of personal protective equipment during fire combat and suppression were also included. Since PAHs are ubiquitous compounds, the questionnaire also estimated the non-occupational exposure to PAHs, namely through tobacco smoke and history, wood (or charcoal) combustion for heating at homes (although it is not usual during the selected period of sampling, May to October 2014, due to warm temperatures in Portugal) and the most consumed meals (boiled, roasted, and grilled) during the five days before urine collection. Firefighters that were exposed to tobacco smoke, wood/charcoal combustion and/or if they had consumed grilled and smoked foods within the last three days before urine collection were not considered. Subjects that fulfilled the conditions to participate in this study collected a spot urine sample, in sterilized 50 mL polycarbonate containers, in the end of their work shift. Sampling was performed in triplicate during a period of 180 days between May to October 2014. After collection, samples were coded and immediately frozen at −20 °C until analysis.

Among firefighters, two distinct groups were considered: non-exposed firefighters, *i.e.* firefighters that were not involved in fire combat activities within 48 h prior the urine collection, and exposed firefighters who were actively involved in fires combat and extinction.

2.2. Urinary OH-PAHs extraction and chromatographic analysis

Extraction and quantification of OH-PAHs from urine samples were performed according to Chetiyanukornkul et al. (2006) with some modifications. Briefly, a total volume of 10 mL of urine was buffered with acetate buffer at pH 5.0, and incubated for 120 min at 37 °C (Binder KBWF, Tuttlingen, German) with 80 μL of β-glucuronidase/arylsulfatase from *Helix pomatia* (EC 3.2.1.31/EC3.1.6.1; 5.5/2.6 U/ml) purchased from Roche Diagnostics (Indianapolis, USA). The hydrolyzed urines were loaded into Sep-Pak® Light Plus C18 (Waters; Sigma-Aldrich, Steinheim, Germany) that were preconditioned with 5.0 mL of methanol and 10.0 mL of water. After elution, cartridges were sequentially cleaned with 10.0 mL of water, and 10.0 mL of methanol/water (20:80; v/v). C18 cartridges were completely dried and eluted with 20.0 mL of methanol/ethyl acetate (10:90; v/v). Extracts were then evaporated till dryness at room temperature (Büchi R200 rotavapor and a Büchi Vac V-500 pump), redissolved in 500 μL of methanol and filtered before injection in a LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a fluorescence (FLD) detector. Chromatographic separation of the OH-PAHs was done at room temperature (20 ± 1 °C) in a C18 column (CC 150/4 Nucleosil 100-5 C18 PAH, 150 × 4.0 mm; 5 μm particle size; Macherey–Nagel, Duren, Germany). The optimum chromatographic conditions were: initial composition 50:50 methanol/water, followed by a linear gradient to 70:30 methanol/water in 3 min, holding in these conditions for 7 min; then a linear gradient to 100% of methanol in 6 min was applied with a final hold of 5 min. The total run time was 30 min with a flow rate of 1.0 mL min^{-1}. The applied optimum excitation/emission wavelength pair for each metabolite was: 232/337 nm (1OHNaph and 1OHAce), 265/335 nm (2OHFlu),

Table 2
Total urinary monohydroxyl-PAH (ΣOH-PAHs) concentrations (median; min-max; μmol/mol creatinine) measured in non-exposed and exposed firefighters from the fire stations: Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL).

	Fire station					
	MGD	TDC	MRD	VNH	BRG	MDL
Non-exposed subjects						
ΣOH-PAHs	1.54(0.438–2.24)	0.249*(0.252–1.55)	0.808*(0.240–2.39)	1.57*(1.11–2.57)	0.446*(0.208–2.20)	1.14*(0.804–2.08)
Exposed subjects						
ΣOH-PAHs	2.40(0.818–4.33)	8.75* (5.99–9.06)	7.67* (6.82–8.90)	7.86*(1.93–121)	0.973*(0.402–4.39)	1.97* (1.31–2.62)

* Statistically significant ($p \leq 0.05$; nonparametric Mann–Whitney *U* test) between non-exposed and exposed firefighters total monohydroxyl-PAH concentrations for each firefighting corporation.

263/363 nm (1OHPhen), 242/388 nm (1OHPy), and 308/432 nm (3OHB[a]P).

Calibrations, based on at least 6 calibration points, were prepared with OH-PAH mixed standards in methanol except for 1OHNaph and/or 1OHAce that were quantified with a matrix-matched calibration curve because they presented strong matrix effects. Moreover, since 1OHNaph and 1OHAce eluted at the same retention time and with the same mobile phase composition (70:30 v/v of methanol/water) for the optimum excitation/emission wavelength pair, these compounds were quantified together with mixed standards. Good correlation coefficients ($R^2 > 0.9979$) were achieved for all OH-PAHs. Precision of intra- and inter-assay were determined by replicate analysis ($n = 6$ during six consecutive days) of spiked samples (0.016 μg/L urine (2OHFlu) to 1 μg/L urine (1OHNaph + 1OHAce)). RSD values ranged from 1.3% (2OHFlu) to 6.4% (1OHPhen) and from 1.3% to 8.1% (1OHNaph and/or 1OHAce, and 1OHPy), respectively for intra- and inter-day precision. The detection (LODs) and quantification (LOQs) limits were determined as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, respectively (Miller and Miller, 2000). LODs ranged between 0.8 ng/L urine (2OHFlu) to 0.195 μg/L urine (1OHNaph and/or 1OHAce), and the respective LOQs varied from 2.8 ng/L urine (2OHFlu) to 0.650 μg/L urine (1OHNaph and/or 1OHAce).

All OH-PAH values were normalized with the urinary creatinine levels (mol/mol) that were measured by the Jaffe colorimetric method (Kanagasabapathy and Kumari, 2000).

Blanks and standards were day-to-day prepared and scanned; all determinations were performed, at least, in triplicate.

2.3. Statistical analysis

Only creatinine corrected OH-PAH concentrations were used. Statistical analysis was done using SPSS (IBM SPSS Statistics 20) and Statistica software (v. 7, StatSoft Inc., USA). OH-PAHs levels are expressed as median values and statistical significance was defined as $p \leq 0.05$. Median values were compared through the nonparametric Mann–Whitney *U* test, since normal distribution was not observed by Shapiro–Wilk's test. When the urinary OH-PAH concentration was below the LOD, the respective LOD/$\sqrt{2}$ was used (Hornung and Reed, 1990). Spearman correlation coefficients were used to estimate the relation among firefighters' urinary individual and total OH-PAHs for each fire station.

3. Results

3.1. Urinary OH-PAHs levels

Urinary OH-PAH concentrations were normalized with the creatinine values for each firefighter. Creatinine is excreted at a constant rate from the human body and thus constitutes an adequate tool to minimize the variability of individual parameters namely the fluid intake, physical exercise, and body temperature. Firefighters' creatinine levels varied between 0.706–2.90 g/L and were within the range (0.3 g/L < creatinine < 3.0 g/L) proposed by WHO (1996) for healthy people assuring sample validity.

Overall, 1OHNaph and/or 1OHAce, 2OHFlu, and 1OHPy were detected in more than 80%, 94%, and 97% of the samples, respectively. 1OHPhen was present in all urines. Considering gender discrimination, men presented slightly lower detection rates than women, with values ranging from 80% (1OHNaph and/or 1OHAce), 92% (2OHFlu) and 100% (1OHPhen) in non-exposed male subjects and between 87% (1OHNaph and/or 1OHAce) to 100% (2OHFlu, 1OHPhen, and 1OHPy) for exposed male firefighters; 1OHNaph and/or 1OHAce, 2OHFlu, 1OHPhen and 1OHPy were detected in 100% of non-exposed and exposed women. 3OHB[a]P, the metabolite of PAH marker of carcinogenicity, was never detected in non-exposed and exposed firefighters. Median concentrations and ranges of total urinary OH-PAHs (ΣOH-PAHs) measured in non-exposed and exposed firefighters working at the selected fire stations are presented in Table 2. Values varied from 0.249 (TDC) to 1.57 (VNH) μmol/mol creatinine for non-exposed individuals, and between 0.973 (BRG) to 8.75 (TDC) μmol/mol creatinine for firefighters involved in firefighting activities (Table 2). Overall, and with the exception of subjects working at MGD fire station, ΣOH-PAH concentrations were *ca.* 1.7 (MDL) to 35 (TDC) times higher ($p < 0.05$; nonparametric Mann–Whitney *U* test) in exposed comparatively with the non-exposed firefighters. Data was also analyzed by gender for TDC and BRG (insufficient data for the other fire stations under study) and the same trend was observed with levels of ΣOH-PAHs exceeding 2 (BRG) to 9 (TDC) times for men, and 2 (BRG) to 20 (TDC) times for women ($p \leq 0.024$), the concentrations observed before their active participation in fires (Table 1S). Furthermore, two more statistical comparisons were performed in order to assess if background and/or occupational exposure are different for males and females. Comparisons between non-exposed males and females' firefighters from TDC and BRG fire stations revealed that urinary 1OHNaph and/or 1OHAce levels (Fig. 1S) were significantly higher ($p = 0.036$) in males. After occupational exposure, 1OHPy was the only marker with some discriminating power ($p = 0.001$) between genders.

Fig. 2 exhibits median concentration of each individual OH-PAH detected in non-exposed and exposed firefighters serving at the selected fire corporations (data separated by gender are presented in Fig. 1S). Among the six fire corporations considered, firefighters from BRG had all OH-PAH levels (and ΣOH-PAHs) significantly higher after occupational exposure. Urinary 1OHNaph and/or 1OHAce concentrations were significantly elevated ($p < 0.05$; nonparametric Mann–Whitney *U* test) in exposed firefighters than in non-exposed ones (without and with gender discrimination) from all fire stations (57% (MGD) to 5875% (TDC)), with the exception of subjects working at MGD fire station. Higher levels, *i.e.* the same pattern of variation ($p < 0.05$; nonparametric Mann–Whitney *U* test) as for 1OHNaph and/or 1OHAce, was also observed for urinary 2OHFlu and 1OHPy concentrations in workers from TDC (except for 1OHPy in women, Fig. 1S), MRD, BRG and MDL (except for 2OHFlu)

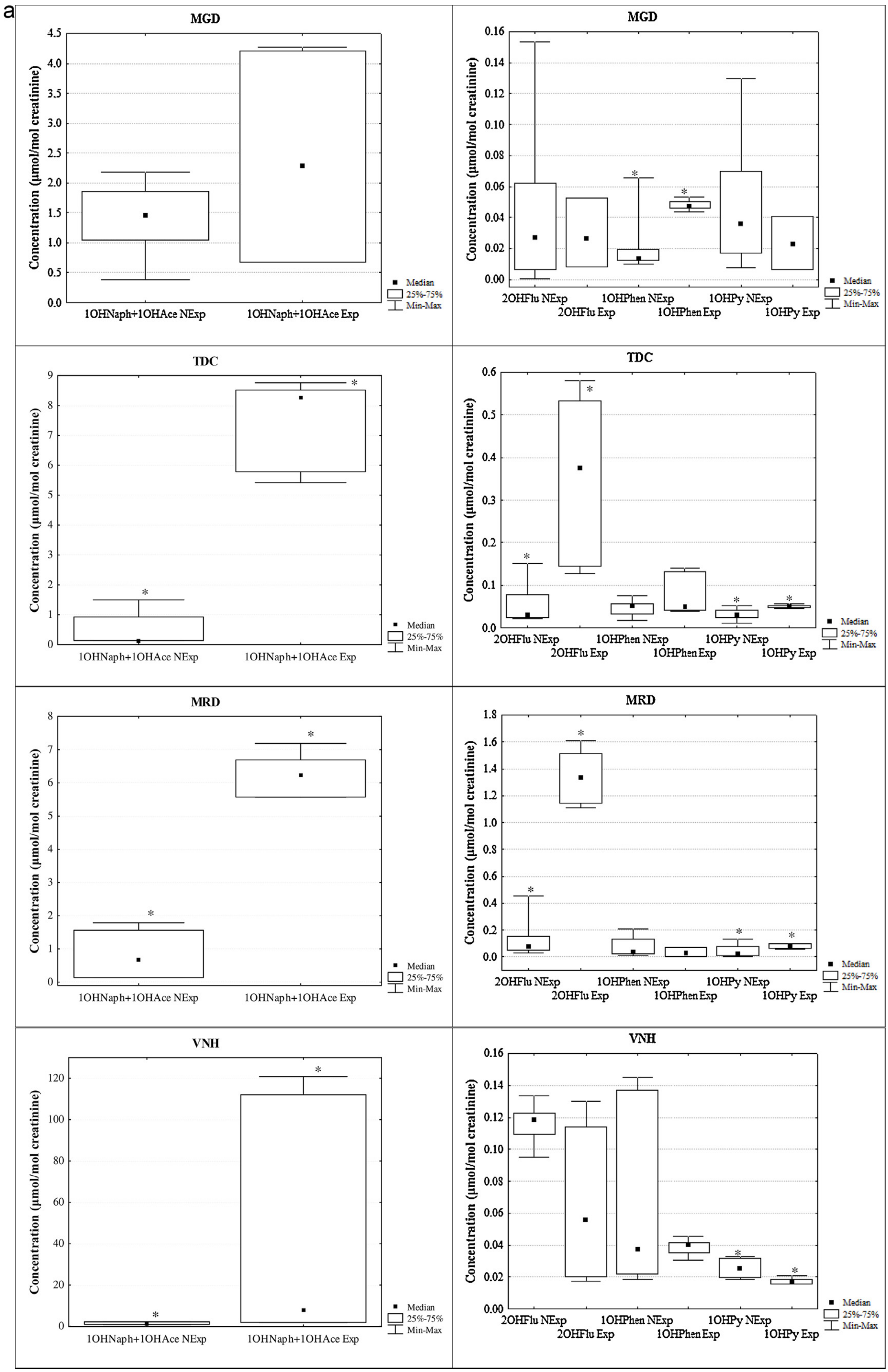

Fig. 2. Concentrations of monohydroxyl-PAHs (median; 25–75% and range; µmol/mol creatinine) measured in non-exposed (NExp) and exposed (Exp) firefighters' urine from different fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL)). *Statistically significant differences at $p \leq 0.05$ (nonparametric Mann–Whitney *U* test) between non-exposed and exposed firefighters from the same corporation.

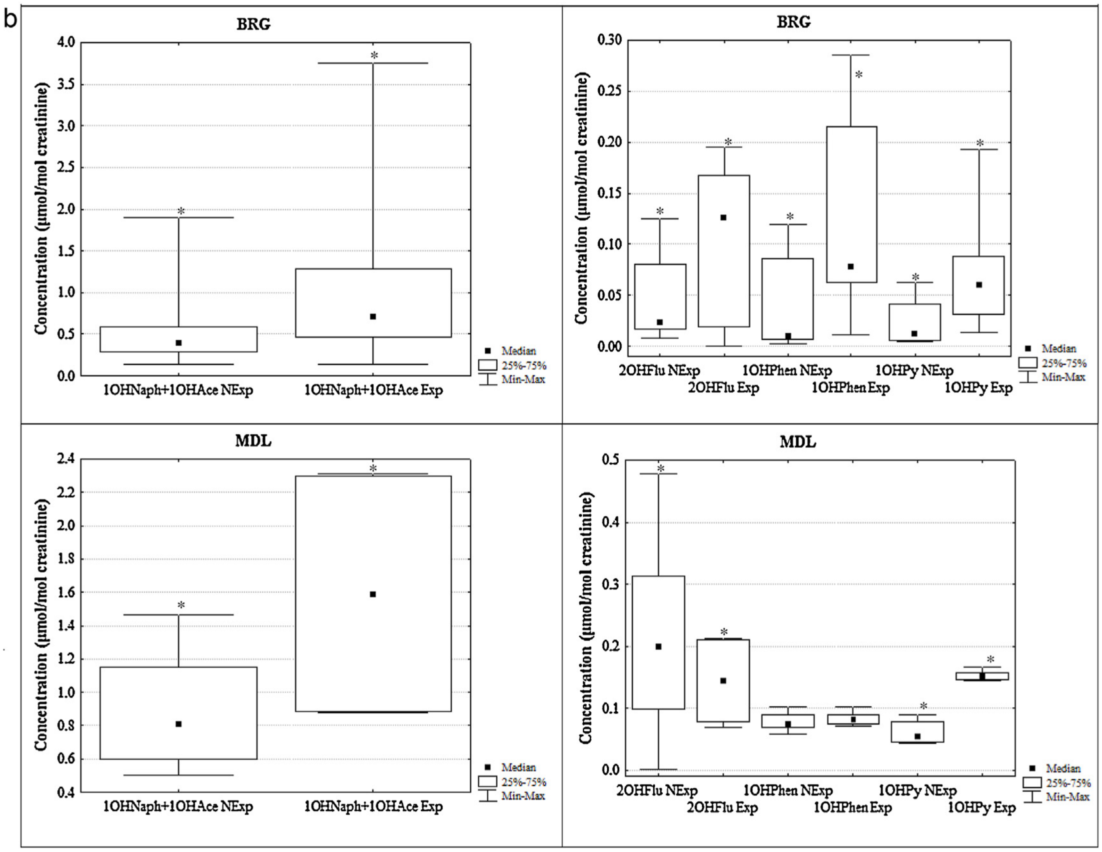

Fig. 2. (*Continued*)

fire stations with enhancements till 1179% (TDC) for 2OHFlu and 400% (BRG) for 1OHPy. Urinary 1OHPy, the established biomarker of PAHs exposure, contributed in general the less for ΣOH-PAHs (1.21×10^{-2} (BRG) – 5.44×10^{-2} µmol/mol creatinine (MDL) for non-exposed firefighters, except in MGD, and 1.73×10^{-2} (VNH) – 0.152 µmol/mol creatinine (MDL) for exposed firefighters, except in MDL; Fig. 2). Statistically significant differences for 1OHPhen levels were only identified in individuals from MGD (253% increase; Fig. 2), BRG (634% increase (Fig. 2); 159% for men and 1153% for women, (Fig. 1S)), and in women from TDC (113%; Fig. 1S) seeming to be the compound which excretion is less affected by fire combat activities. 1OHNaph and/or 1OHAce were the metabolites that contributed the most for ΣOH-PAHs (63% (TDC) to 92% (MGD) for non-exposed and 72% (BRG) to 98% (VNH) for exposed firefighters), followed by 2OHFlu (non-exposed: 4% (MGD) to 16% (MDL) *versus* exposed: 1% (VNH) to 17% (MRD)), 1OHPhen (non-exposed: 2% (MGD) to 13% (TDC) *versus* exposed: 1% (MRD) to 11% (BRG)), and 1OHPy (non-exposed: 2% (VNH) to 10% (TDC) *versus* exposed: 0.3% (VNH) to 9% (MDL)) (Fig. 3). Overall (with the exception of BRG values and 1OHPy at MDL), participation in fires suppression promoted an increase of the proportion of 1OHNaph and/or 1OHAce while those of 1OHPhen and 1OHPy decreased.

3.2. Correlations between urinary OH-PAHs

Spearman correlation coefficients (r) were determined to explore the relation between urinary individual and ΣOH-PAHs. Overall moderate to strong correlations between individual OH-PAH and ΣOH-PAHs were found for the majority of firefighters. The strongest correlations were found between urinary 1OHNaph and/or 1OHAce and ΣOH-PAHs, with coefficient varying from r = 0.840 (TDC) to r = 0.996 (MGD) for non-exposed subjects, and between r = 0.867 (TDC) to r = 0.999 (MGD, VNH, and MDL) for exposed ones ($p \leq 0.005$). A similar profile was observed when dis-

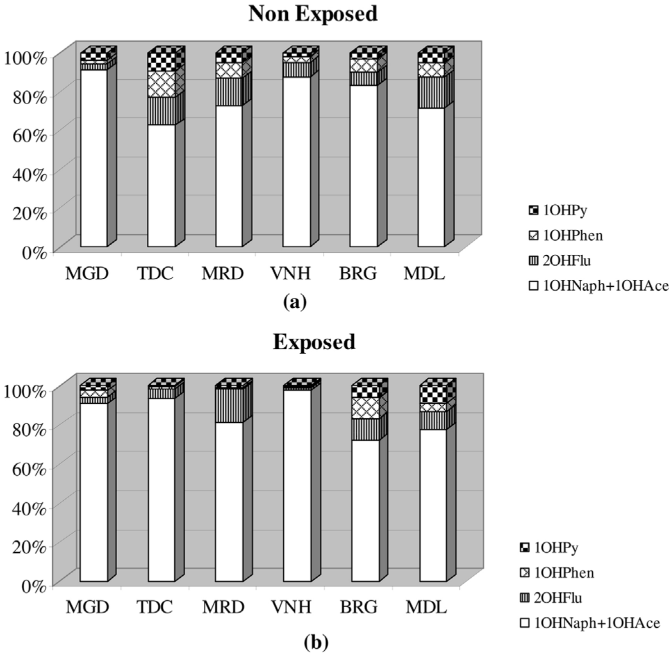

Fig. 3. Distribution (%) of urinary monohydroxyl-PAHs (1OHNaph and/or 1OHAce: 1-hydroxynaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPHen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) in a) non-exposed and b) exposed firefighters at the studied fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), Mirandela (MDL)).

crimination between gender was considered (r = 0.980 (men) and r = 0.842 (women) for non-exposed individuals ($p < 0.004$); r = 0.983 ($p < 0.001$) for exposed men and r = 0.533 ($p = 0.139$) for exposed women firefighters). Correlations with moderate to high Spearman coefficients were also found between urinary 1OHPy and ΣOH-PAHs for non-exposed ($0.480 < r < 0.746$; $p \leq 0.170$) and exposed firefighters ($0.509 < r < 0.771$; $p \leq 0.162$) attending at BRG, VNH, and MRD stations. Regarding Spearman coefficients between individual compounds, correlations between urinary 1OHPhen and 1OHPy varied from $0.390 < r < 0.851$ ($p \leq 0.222$) for all participants (non-exposed and exposed) involved in this study, with the exception of those working at MDL fire station.

4. Discussion

As previously described (Adetona et al., 2015; Edelman et al., 2003; Laitinen et al., 2010; Li et al., 2015), the urinary concentrations of OH-PAHs varied inversely with the molecular weight of their respective PAHs, *i.e.*, the lower urinary OH-PAH levels were associated with the higher molecular structures (Figs. 2 and 3). Airborne PAHs with 2–3 rings, such as naphthalene, acenaphthylene, and acenaphthene have been reported as the predominant ones (64–96% of total $PM_{2.5}$-bound PAHs) in the breathing air zone of firefighters during a regular work shift at Portuguese fire stations (Oliveira et al., 2016a). Concerning occupational exposure during firefighting activities, besides naphthalene and acenaphthylene, other low molecular weight PAHs with 3 rings (phenanthrene and fluorene) have also been identified as the most abundant compounds (Baxter et al., 2014; Fent and Evans, 2011; Kirk and Logan, 2015a; Pleil et al., 2014; Robinson et al., 2008). Moreover, it has been reported that lighter PAHs are preferentially excreted in urine as hydroxyl metabolites while compounds with five and more aromatic rings present a more complex metabolism being mostly eliminated through feces (Li et al., 2012; Marie et al., 2010). The absence of 3OHB[a]P in the urine of Portuguese (non-exposed and exposed) firefighters is in line with the very low detection rates reported by other authors (Li et al., 2012; Gündel et al., 2000; Yamano et al., 2014). Urinary 3OHB[a]P has been only found in workers with high exposures to PAHs such as those employed in electrode production (Barbeau et al., 2015; Forster et al., 2008), metallurgies (Barbeau et al., 2014), fireproof stone producing plants (Gündel et al., 2000), fireproof material in refractories, converter infeed, and coking plants (Forster et al., 2008). Limited information is available concerning the elimination kinetics of urinary OH-PAHs in humans: half-life ranging from 3.3 to 6.2 h for 1OHNaph, 2.3–4.0 h for 2OHFlu, and 4.3–6.1 h for 1OHPhen for ingestion exposure (Li et al., 2012) has been reported. For 1OHPy, a half-life excretion rate that varies from 6 to 35 h after inhalation exposure (Brzeznicki et al., 1997; Jongeneelen et al., 1990); 4 (Buckley and Lioy, 1992; Li et al., 2012) to 12 h (Viau et al., 1995) after ingestion, and reaches 13 h for skin adsorption (Sobus et al., 2009b; Viau et al., 1995; Viau and Vyskocil, 1995) were indicated. Other studies related with elimination kinetics of OH-PAHs in occupational environments have also been described. In that regard, urinary 1OHPy and 3OHB[a]P were eliminated in 3–9 h and 3–24 h, respectively, depending on the tasks performed by the workers (Bouchard and Viau, 1999; Gendre et al., 2002, 2004; Lutier et al., 2016). Marie et al. (2010) attributed the postponement in urinary excretion of 3OHB[a]P to the storage of benzo[a]pyrene in the human body being its metabolites mostly retained in the kidneys. More recently, Li et al. (2015) estimated the median half-life of 1OHNaph, 2OHFlu, 1OHPhen, and 1OHPy as 6.6, 8.4, 13.8, and 23.5 h respectively, after 2 h of exposure to woodsmoke. Therefore more studies focusing on the excretion profiles and half-lives of urinary OH-PAHs considering all the exposure routes to PAHs (inhalation, ingestion, and dermal) and the impact of the use of personal protective equipment in occupational exposed workers are needed. Also, it is very difficult to find an appropriate sampling time if exposure takes place *via* several routes and it is intended to measure a set of different biomarkers.

Overall it was observed that exposed wildland firefighters presented higher urinary concentrations of ΣOH-PAHs (except subjects from MGD) and individual OH-PAH (mainly 1OHNaph and/or 1OHAce, 2OHFlu, and 1OHPy) than non-exposed firefighters (Fig. 2), which agrees with the outcomes of other authors regarding USA firemen (Adetona et al., 2015; Edelman et al., 2003) and fire fighting trainers' exposure (Laitinen et al., 2010). Due to the very limited amount of data for women, no other major conclusion was drawn based on gender separation (with 1OHPy being the only marker with some discriminating power between genders after fire combat activities). Edelman et al. (2003) analyzed the urine of firefighters that intervened in the World Trade Center fires and collapse and found that urinary 1OHPy increased to a maximum of *ca.* 50% in firefighters involved in fire combat. Also, Laitinen et al. (2010) evaluated firefighters' occupational exposure during training activities at diving and gas simulators; authors concluded that exposure caused an increase of 50–214% and 5–159% in urinary 1OHNaph and 1OHPy concentrations, respectively. More recently, Adetona et al. (2015) assessed the occupational exposure of American firefighters during their work at prescribed burns and reported that postshift levels of all 9 characterized urinary OH-PAHs (1-, 2-OHNaph, 2-, 3-OHFlu, 1-, 2-, 3-, 4-OHPhen, and 1OHPy) were 83–323% higher than the respective pre-shift levels (Adetona et al., 2015). Regarding individuals from the MDL fire station, no justification was found for the different pattern observed and a more detailed characterization is further needed; a possible factor that contributed to this result may be the high value of ΣOH-PAHs in non-exposed firefighters from this corporation (due to elevated levels of 1OHNaph and/or 1OHAce when compared with the other workers). The variation concerning the exposure route (inhalation of gaseous *versus* particle-bound PAHs, inhalation *versus* dermal exposure) together with the variation of elimination half-life of each metabolite affects the individual levels of urinary OH-PAHs. There might also exist some interference by non-occupational exposure for some individuals (information that was not identified with the individual questionnaires). Scarce information is available regarding the influence that gender may have on exposure to occupational chemicals for men and women working at the same physical environment (Arbuckle, 2006). Concerning the firefighter's occupation, no study was found in the literature. Several factors, such as different breathing rates and respiratory volumes, as well as the degree of sweat have been reported to influence the absorption of chemicals by the human body (Arbuckle, 2006). Furthermore, women have a lower body weight and a higher percentage of body fat composition than men, which may promote the absorption of lipophilic compounds such as PAHs. Women also have a smaller plasma volume and lower average organ blood flow than men, which directly affects the rate and extent of distribution of the chemical (Gandhi et al., 2004). In addition, endocrine status may have a significant effect on women's metabolism (Arbuckle, 2006). Thus, broader studies, including men and women working at the same physical environments and conducting the same job tasks, are needed to characterize their occupational exposure.

1OHPy, the biomarker of exposure to PAHs has been widely used to assess the total burden of PAHs in occupational groups (Bouchard and Viau, 1999; Ciarrocca et al., 2014; Hansen et al., 2008). Within its pioneer study Jongeneelen (2001, 2014) proposed a no-biological effect level of 1.4 μmol/mol creatinine of urinary 1OHPy in exposed workers. The American Conference of Governmental Industrial Hygienists (ACGIH) proposed a post-shift 1OHPy benchmark level of about 0.5 μmol/mol creatinine as indicative of occupational exposure to PAHs (ACGIH, 2010). Urinary 1OHPy concentrations of both non-exposed and exposed Portuguese firefighters were well below those recommended guidelines. In addition 1OHPy was the metabolite that in general contributed the less for ΣOH-PAHs, and simultaneously presented the lowest percentage increases between non-exposed and exposed firefighters from all fire corporations. These findings are in line with previous studies (Adetona et al., 2015; Edelman et al., 2003; Laitinen et al., 2010) and may question the adequacy of using only this biomarker for evaluation of exposure to PAHs. The inter-comparison of total internal dose of non-exposed firefighters attending the different fire corporations: VNH ∼ MGD > MDL > MRD > BRG > TDC (Table 2) is overall in agreement with a preliminary study (Oliveira et al., 2016a). Regarding exposed individuals, a different profile of urinary ΣOH-PAHs was attained: TDC > VNH ∼ MRD » MGD > MDL > BRG (Table 2), which can be attributed to the active participation in firefighting activities (overhaul and/or knockdown), which is in accordance with previous reports (Adetona et al., 2015; Edelman et al., 2003; Laitinen et al., 2010). Moreover, strong to moderate Spearman correlations were observed between individual compounds and ΣOH-PAHs corroborating the prevalence of an emission source in the exposed and non-exposed groups. Based on questionnaires data and on a previous background study, which involved personal air monitoring (Oliveira et al., 2016a), inhalation may be pointed as the main route of exposure in both groups. Still, all exposed firefighters reported the use of personal protective equipment that includes the helmet with eye protection, flash hood, gloves, boots and clothes with flame-retardant characteristics, according to the Portuguese Directive n° 3974 (Portuguese Regulation, 2013). No correlation was found between the urinary ΣOH-PAHs and the number of hours dedicated to fire suppression reported by each firefighter. It is well known that firefighters occupational exposure during firefighting activities are intermittent and, even among the same fire corporation, firefighters may have different exposure profiles. The type of vegetation burnt during the fire, the nature of fuel, as well as humidity, temperature and wind conditions strongly affect the composition of fire smoke (Fent et al., 2013; Laitinen et al., 2010; Miranda et al., 2012; Reisen and Brown, 2009). Other source of firefighters' exposure to PAHs apart from fire smoke is vehicle exhaust emissions (Adetona et al., 2015; Oliveira et al., 2016a). No firefighters exposed to tobacco smoke were included in this study; however the assessment of tobacco smoke biomarkers in firefighters would be a precious tool to validate the questionnaire results.

Limited studies regarding the internal dose assessment of firefighters occupational exposure to PAHs are available (Adetona et al., 2015; Edelman et al., 2003; Caux et al., 2002; IARC, 2010a; Laitinen et al., 2010; Robinson et al., 2008). The existent studies were mostly performed in USA; none regarding European wildland firefighters during fire combat and/or suppression activities were found. However the comparison of urinary concentrations between the available studies is extremely difficult because the levels of OH-PAHs are frequently not adequately normalized with creatinine concentrations. Urinary 1OHPy is by far the most characterized OH-PAH in firefighters (Robinson et al., 2008; Laitinen et al., 2010; Caux et al., 2002; Edelman et al., 2003; Hansen et al., 2008) and only three of those studies assessed the concentrations of other urinary OH-PAHs (Adetona et al., 2015; Edelman et al., 2003; Laitinen et al., 2010). To the best of our knowledge, the urinary concentrations of 1OHAce (determined alone or together with 1OHNaph) and 3OHB[a]P were never assessed before in firefighters. Regarding the overall median and ranges of urinary 1OHPy among the exposed Portuguese firefighters (5.15×10^{-2} μmol/mol creatinine, 1.73×10^{-2} to 0.152 μmol/mol creatinine; 229 ng/L, 19.3–1078 ng/L; 1.05 nmol/L, 8.84×10^{-2} to 4.94 nmol/L), the concentrations were lower than 1OHPy levels (0.08–3.63 μmol/mol creatinine) observed in Canadian workers after firefighting operations (knockdown and overhaul; Caux et al., 2002), but greater than those reported in the end-of-shift of American firefighters (0.09 μg/L; <0.01 to 0.50 μg/L) after their active participation in prescribed pile burns (Robinson et al., 2008). 1OHPhen levels (186 ng/L) found in World Trade Center firefighters (Edelman et al., 2003) were in close range with those described in this study (197 ng/L; 1.06–1732 ng/L). Laitinen et al. (2010) determined the concentrations of urinary 1OHNaph and 1OHPy in order to estimate how the burning materials (chipboard, conifer plywood board, pure spruce and pine wood), the type of simulator and the use of personal protective equipment affected firefighting trainer's exposure. The reported mean concentrations of urinary 1OHPy for firefighters training at chipboard (4.4 nmol/L) and conifer plywood board (5.1 nmol/L) (immediately after exposure at diving simulators) were higher than the levels observed in Portuguese firefighters (Laitinen et al., 2010). Regarding urinary 1OHNaph, the concentration reported for pure spruce and pine wood diving simulator (45 nmol/L) was similar to the results achieved in this study (48.2 nmol/L; 0.485–1440 nmol/L), while levels obtained at gas simulators (135 nmol/L) were much higher than the concentrations of 1OHNaph and/or 1OHAce found in Portuguese firefighters. Also, the mean urinary concentrations of OH-PAHs, reported by Adetona et al. (2015) regarding prescribed burns, were comparatively higher than those determined in this work (8824 for 1OHNaph *versus* 5768 ng/g creatinine for 1OHNaph and/or 1OHAce, 1491 *versus* 211 ng/g creatinine for 2OHFlu, 557 *versus* 105 ng/g creatinine for 1OHPhen, and 576 *versus* 99 ng/g creatinine for 1OHPy).

In this study, consistent evidence was found that excretion of main individual urinary monohydroxyl metabolites was substantially increased in wildland firefighters that participated in fire combat (knockdown and overhaul) activities comparatively with non-exposed firefighters. Furthermore, background levels of urinary OH-PAHs in firefighters not actively exposed to fires were assessed. Preliminary data were presented, for the first time, by gender (for two corporations); however this aspect needs clearly to be more deeply explored. 1OHNaph and/or 1OHAce were the most abundant urinary metabolites in non-exposed and exposed firefighters which indicated that inhalation may be the major route of exposure; naphthalene and acenaphthene (two and three-rings PAHs) are highly volatile compounds and most of their environmental levels enter in the human body mainly in a gaseous form and thus 1OHNaph and/or 1OHAce concentrations probably reflect the contribution of air. Airborne PAHs are portioned between gas and particulate phases (Kim et al., 2013; Oliveira et al., 2015, 2016b). Low molecular weight PAHs (such as naphthalene and acenaphthene) exist almost exclusively in the gas phase while high molecular weight PAHs (5–6 rings, such as benzo[a]pyrene) are predominantly bound to particles; PAHs with 4 rings (that includes pyrene) are distributed between both gas and particulate phases (Oliveira et al., 2016b). In addition naphthalene (classified as a possible carcinogenic to humans) (group 2B; IARC 2002, 2010b) together with acenaphthene, fluorene, and phenanthrene constitute some of the most predominant PAHs in different matrices (Cirillo et al., 2006; Gomes et al., 2013; Oliveira et al., 2016a, 2016c). 1OHPy (marker of exposure to PAHs) was the less abundant one from the six analyzed biomarkers. Thus authors suggest the inclusion of other metabolites, in addition to 1OHPy, in future studies to better estimate firefighters' occupational exposure to PAHs.

Conflict of interest

The authors declare no conflict of interest.

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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.ijheh.2016.07.011.

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