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

Marta Oliveira[a,b], Klara Slezakova[a,b], Maria José Alves[c], Adília Fernandes[c], João Paulo Teixeira[d,e], Cristina Delerue-Matos[a], Maria do Carmo Pereira[b] and Simone Morais[a,*]

[a]REQUIMTE-LAQV, Instituto Superior de Engenharia, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal.

[b]LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, R. Dr. Roberto Frias, 4200-465 Porto, Portugal

[c] Escola Superior de Saúde, Instituto Politécnico de Bragança, Avenida D. Afonso V, 5300-121, Bragança, Portugal

[d] Instituto Nacional de Saúde Pública, Departamento de Saúde Ambiental, Rua Alexandre Herculano 321, 4000-055 Porto, Portugal

[e] Universidade do Porto, Instituto de Saúde Pública, Rua das Taipas 135, 4050-600 Porto, Portugal

*Corresponding author: Tel.: +351 22 834 0500 - ext 1918, Fax: +351 22 834 0500

E-mail: sbm@isep.ipp.pt

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 to 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}-0.152 μmol/mol creatinine in exposed subjects *versus* 1.21×10^{-2}-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.

Keywords: Wildland firefighters; Occupational exposure; Polycyclic aromatic hydrocarbons; Biomarkers of exposure; Urinary monohydroxyl metabolites.

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 naphthalene (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; Jongeenelen 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 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.

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.

Materials and Methods

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 Alto Douro Region of Portugal: Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL) (Figure 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 hours 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 ºC 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 hours prior the urine collection, and exposed firefighters who were actively involved in fires combat and extinction.

Urinary OH-PAHs extraction and chromatographic analysis

Extraction and quantification of OH-PAHs from urine samples were performed according to Chetiyanukornkul and colleagues (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 minutes at 37 ºC (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 ºC) 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), 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.

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.

Results

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 (Figure 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.

Figure 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 Figure 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, Figure 1S), MRD, BRG and MDL (except for 2OHFlu) 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; Figure 2). Statistically significant differences for 1OHPhen levels were only identified in individuals from MGD (253% increase; Figure 2), BRG (634% increase (Figure 2); 159% for men and 1153% for women, (Figure 1S)), and in women from TDC (113%; Figure 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)) (Figure 3). Overall (with the exception of BRG values and 1OHPy at MDL), participation in fires suppression promoted an increase of the distribution (%) of 1OHNaph and/or 1OHAce while those of 1OHPhen and 1OHPy decreased.

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 discrimination 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.

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 (Figures 2-3). 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 (Gündell 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 to 4.0 h for 2OHFlu, and 4.3 to 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 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 and coworkers (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 and colleagues (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 hours 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 (Figure 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 and coworkers (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 and coworkers (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 (Ghandi 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 (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 nº 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 to 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 to 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 to 1732 ng/L). Laitinen and coworkers (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 to 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 and colleagues (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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References

ACGIH, 2010. Documentation for a Recommended BEI of Polycyclic Aromatic Hydrocarbons. American Conference of Governmental Industrial Hygienists. Cincinatti, Ohio, USA.

Adetona, O., Simpson, C.D., Li, Z., Sjodin, A., Calafat, A.M., Naeher, L.P., 2015. Hydroxylated polycyclic aromatic hydrocarbons as biomarkers of exposure to wood smoke in wildland firefighters. J. Expo. Sci. Environ. Epidemiol. doi: 10.1038/jes.2015.75.

Alexander, B.M., Baxter, C.T., 2014. Plasticizer contamination of firefighter personal protective clothing – a potential factor in increased health risks in firefighters. J. Occup. Environ. Hyg. 11, D43-D48.

Arbuckle, T.E., 2006. Are there sex and gender differences in acute exposure to chemicals in the same setting?. Environ. Res. 101, 195-204.

ATSDR, 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Agency for Toxic Substances and Disease Registry. Atlanta. http://www.atsdr.cdc.gov/toxprofiles/tp69.html (accessed 01/04/2016).

Barbeau, D., Lutier, S., Bonneterre, V., Persoons, R., Marques, M., Herve, C., Maitre, A., 2015. Occupational exposure to polycyclic aromatic hydrocarbons: relations between atmospheric mixtures, urinary metabolites and sampling times. Int. Arch. Occup. Environ. Health. 88, 1119-1120.

Barbeau, D., Persoons, R., Marques, M., Herve, C., Laffitte-Rigaud, G., Maitre, A., 2014. Relevance of urinary 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene to assess exposure to carcinogenic polycyclic aromatic hydrocarbon mixtures in metallurgy workers. Ann. Occup. Hyg. 58, 579–590.

Baxter, C.T., Hoffman, J.D., Knipp, M.J., Reponen, T., Haynes, E.N., 2014. Exposure of firefighters to particulates and polycyclic aromatic hydrocarbons. J. Occup. Environ. Hyg. 11, D85–D91.

Boström, C.-E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T. et al., 2002. Cancer risk assessment, indicators and guidelines for polycyclic aromatic hydrocarbons in ambient air. Environ. Health Perspect. 110, 451–489.

Bouchard, M., Viau, C., 1999. Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological exposure indices for various work environments. Biomarkers. 4, 159–187.

Brzeznicki, S., Jakubowski, M., Czerski, B., 1997. Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons. Int. Arch. Occup. Environ. Health. 70, 257-260.

Buckley, T.J., Lioy, P.J., 1992. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br. J. Ind. Med. 49, 113-124.

Campo, L., Fustinoni, S., Consonni, D., Pavanello, S., Kapka, L., Siwinska, E., et al., 2014. Urinary carcinogenic 4-6 ring polycyclic aromatic hydrocarbons in coke oven workers and in subjects belonging to the general population: Role of occupational and environmental exposure. Int. J. Hyg. Environ. Health. 217, 231-238.

Caux, C., O'Brien, C., Viau, C., 2002. Determination of firefighter exposure to polycyclic aromatic hydrocarbons and benzene during fire fighting using measurement of biological indicators. Applied Occupational and Environmental Hygiene. 17, 379-386.

Chetiyanukornkul, T., Toriba, A., Kameda, T., Tang, N., Hayakawa, K., 2006. Simultaneous determination of urinary hydroxylated metabolites of naphthalene, fluorene, phenanthrene and pyrene as multiple biomarkers of exposure to polycyclic aromatic hydrocarbons. Anal. Bioanal. Chem. 386, 712-718.

Christopher, Y., van Tongeren, M., Urbanus, J., Cherrie, J.W., 2011. An assessment of dermal exposure to heavy fuel oils (HFO) in occupational settings. Ann. Occup. Hyg. 55(3), 319-328.

Ciarrocca, M., Rosati, M.V., Tomei, F., Capozzella, A., Andreozzi, G., Tomei, G. et al., 2014. Is urinary 1-hydroxypyrene a valid biomarker for exposure to air pollution in outdoor workers? A meta-analysis. J. Expo. Sci. Environ. Epidemiol. 24, 17-26.

Cirillo, T., Montuori, P., Mainardi, P., Russo, I., Triassi, M., Amodio-Cocchieri, R., 2006. Multipathway polycyclic aromatic hydrocarbon and pyrene exposure among children living in Campania (Italy). J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 41, 2089-2107.

Edelman, P., Osterloh, J., Pirkle, J., Caudill, S.P., Grainger, J., Jones, R. et al., 2003. Biomonitoring of chemical exposure among New York City firefighters responding to the World Trade Center fire and collapse. Environ. Health Perspect. 111, 1906-1911.

Estrellan, C.R, Lino, F., 2010. Toxic emissions from open burning. Chemosphere. 80, 193-207.

Fabian, T.Z., Borgerson, J.L., Gandhi, P.D., Baxter, C.T., Ross, C.S., Lockey, J.E. et al., 2014. Characterization of firefighter smoke exposure. Fire Technol. 50, 993-1019.

Fent, K.W., Eisenberg, J., Evans, D., Sammons, D., Robertson, S., Striley, C. et al., 2013. Evaluation of dermal exposure to polycyclic aromatic hydrocarbons in fire fighters, Health Hazard Evaluation Report No. 2010-0156-3196. United States Department of Health and Human Services, Centers for Disease Control and Prevention National Institute for Occupational Safety and Health.

Fent, K.W., Eisenberg, J., Snawder, J., Sammons, D., Pleil, J.D., Stiegel, M.A. et al., 2014. Systemic exposure to PAHs and benzene in firefighters suppressing controlled structure fires. Ann. Occup. Hyg. 58(7), 830-845.

Fent, K.W., Evans, D.E., 2011. Assessing the risk to firefighters from chemical vapors and gases during vehicle fire suppression. J. Environ. Monit. 13, 536-543.

Forster, K., Preuss, R., Rossbach, B., Bruning, T., Angerer, J., Simon, P., 2008. 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. Occup. Environ. Med. 65, 224–229.

Franco, S.S., Nardocci, A.C., Günther, W.M.R., 2008. PAH biomarkers for human health risk assessment: a review of the state-of-the-art. Caderno Saúde Pública. 24, 569-580.

Gandhi, M., Aweeka, F., Greenblatt, R.M., Blaschke, T.F., 2004. Sex differences in pharmacokinetics and pharmacodynamics. Annu. Rev. Pharmacol. Toxicol. 44, 499–523.

Gendre, C., Lafontaine, M., Delsaut, P., Simon, P., 2004. Exposure to polycyclic aromatic hydrocarbons and excretion of urinary 3-hydroxybenzo[a]pyrene: assessment of an appropriate sampling time. Polycycl. Aromat. Compd. 24, 433–439.

Gendre, C., Lafontaine, M., Morele, Y., Payan, J.P., Simon, P., 2002, Relationship between urinary levels of 1-hydroxypyrene and 3-hydroxybenz[a]pyrene for workers exposed to polycyclic aromatic hydrocarbons. Polycyclic. Aromat. Comp. 22, 761–769.

Gomes, F., Oliveira, M., Ramalhosa, M.J., Delerue-Matos, C., Morais, S., 2013, Polycyclic aromatic hydrocarbons in commercial squids from different geographical origins: Levels and risks for human consumption. Food Chem. Toxicol. 59, 46 – 54.

Gündel, J., Schaller, K.H., Angerer, J., 2000. Occupational exposure to polycyclic aromatic hydrocarbons in a fireproof stone producing plant: biological monitoring of 1-hydroxypyrene, 1-, 2-, 3- and 4-hydroxyphenanthrene, 3-hydroxybenz(a)anthracene and 3-hydroxybenzo(a)pyrene. Int. Arch. Occ. Env. Hea. 73, 270-274.

Hansen, A.M., Mathiesen, L., Pedersen, M., Knudsen, L.E., 2008. Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies – a review. Int. J. Hyg. Environ. Health. 211, 471-503.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46-51.

IARC, 2010a. Monographs on the Evaluation of Carcinogenic Risks to Humans. Painting, firefighting and shiftwork. International Agency for Research on Cancer. 98, Lyon, France.

IARC, 2010b. Monographs on the Evaluation of the Carcinogenic Risks to Humans: Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. International Agency for Research on Cancer. 92, 1–853.

IARC, 2002. Monographs on the Evaluation of the Carcinogenic Risks to Humans: naphthalene. World Health Organization, International Agency for Research on Cancer. 82, Lyon, France.

ICNF, 2014. Provisonal Report of Forest Fires – 2014. Instituto da Conservação da Natureza e das Florestas, Departamento de Gestão de áreas Classificadas, Públicas e de Protecção Florestal, Portugal.

JRC, 2015. Forest fires in Europe, Middle East and North Africa 2014. Joint Research Centre Technical Reports. Joint Report of JRC and Directorate-General Environment. 2015, Luxembourg.

Jongeneelen, F.J., 2014. A guidance value of 1-hydroxypyrene in urine in view of acceptable occupational exposure to polycyclic aromatic hydrocarbons. Toxicol. Lett. 231, 239-248.

Jongeneelen, F.J., 2001. Benchmark guideline for urinary 1- hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann. Occup. Hyg. 45, 3–13.

Jongeneelen, F.J., Van Leeuwen, F.E., Oosterink, S., Anzion, R.B., van Der, L.F., Bos, R.P., van Veen, H.G., 1990. Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons. Br. J. Ind. Med. 47, 454-461.

Kamal, A., Cincinelli, A., Martellini, T., Malik, R.N., 2015. A review of PAH exposure from the combustion of biomass fuel and their less surveyed effect on the blood parameters. Environ. Sci. Pollut. Res. 22, 4076-4098.

Kanagasabapathy, A.S., Kumari, S., 2000. Guidelines on standard operating procedures for clinical chemistry. World Health Organization, Regional Office for South-East Asia, New Delhi, pp. 25-28.

Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71-80.

Kirk, K.M., Logan, M.B., 2015a. Firefighting instructors' exposures to polycyclic aromatic hydrocarbons during live fire training scenarios. J. Occup. Environ. Hyg. 12, 227-234.

Kirk, K.M., Logan, M.B., 2015b. Structural fire fighting ensembles: accumulation and off-gassing of combustion products. J. Occup. Environ. Hyg. 12, 376-383.

Laitinen, J., Mäkelä, M., Mikkola, J., Huttu, I., 2012. Firefighters' multiple exposure assessment in practice. Toxicol. Lett. 213(1), 129-133.

Laitinen, J., Mäkelä, M., Mikkola, J., Huttu, I., 2010. Fire fighting trainers' exposure to carcinogenic agents in smoke diving simulators. Toxicol. Lett. 192, 61-65.

Lemieux, P.M., Lutes, C.C., Santoianni, D.A., 2004. Emissions of organic air toxics from open burning: a comprehensive review. Prog. Energ. Combust. 30, 1-32.

Lewtas, J., 2007. Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. Mutat. Res. 637, 95-133.

Li, Z., Romanoff, L., Bartell, S., Pittman, E.N., Trinidad, D.A., McClean, M. et al., 2012. Excretion profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. Chem. Res. Toxicol. 25, 1452-1461.

Li, Z., Trinidad, D., Pittman, E.N., Riley, E.A., Sjodin, A., Dills, R.L. et al., 2015. Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers to woodsmoke exposure – results from a controlled exposure study. J. Expo. Sci. Environ. Epidemiol. doi: 10.1038/jes.2014.94.

Liu, H.-H., Lin, M.-H., Chan, C.I., Chen, H.-L., 2010. Oxidative damage in foundry workers occupationally co-exposed to PAHs and metals. Int. J. Hyg. Environ. Health. 213, 93-98.

Likhachev, A.J., Beniashvili, D.Sh., Bykov, V.J., Dikun, P.P., Tyndyk, M.L., Savochkina, I.V. et al., 1992. Biomarkers for individual susceptibility to carcinogenic agents: excretion and carcinogenic risk of benzo[a]pyrene metabolites. Environ. Health Perspect. 98, 211-214.

Lutier, S., Maître, A., Bonneterre, V., Bicout, D.J., Marques, M., Persoons, R., et al., 2016. Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminium electrode production plant : Evaluation of diuresis correction methods for routine biological monitoring. Environ. Res. 147, 469-479.

Marie, C., Bouchard, M., Heredia-Ortiz, R., Viau, C., Maitre, A., 2010. A toxicokinetic study to elucidate 3-hydroxybenzo(a)pyrene atypical urinary excretion profile following intravenous injection of benzo(a)pyrene in rats. J. Appl. Toxicol. 30, 402–410.

Miller, J.N., Miller, J.C., 2000. Statistics for Analytical Chemistry, Pearson Education Ltd. Harlow.

Miranda, A.I., Martins, V., Cascão, P., Amorim, J.H., Valente, J., Borrego, C., et al., 2012. Wildland smoke exposure values and exhaled breath indicators in firefighters. J. Toxicol. Environ. Health A. 75(13-15), 831-843.

Needham, L.L., Calafat, A.M., Barr, D.B., 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210, 229-238.

NIOSH, 2007. NIOSH Pocket Guide to Chemical Hazards. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Okona-Mensah, K.B., Battershill, J., Boobis, A., Fielder, R., 2005. An approach to investigating the importance of high potency polycyclic aromatic hydrocarbons (PAHs) in the induction of lung cancer by air pollution. Food Chem. Toxicol. 43, 1103-1116.

Oliveira, M., Slezakova, K., Alves, M.J., Fernandes, A., Teixeira, J.P., Delerue-Matos, C. et al., 2016a. Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. J. Hazard. Mater. doi:10.1016/j.jhazmat.2016.03.012

Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2016b. Assessment of polycyclic aromatic hydrocarbons in indoor and outdoor air of preschool environments (3-5 years old children), Environ. Pollut. 208(B), 382-394.

Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2015. Polycyclic aromatic hydrocarbons: levels and phase distributions in preschool environments. Indoor Air 25, 557-568.

Oliveira, M., Slezakova, K., Pereira, M.C., Fernandes, A., Vaz, J., Delerue-Matos, C., et al., 2016c. Firefighter's occupational exposure to $PM_{2.5}$ and polycyclic aromatic hydrocarbons, in: Arezes, P.M., Baptista, J.P., Barroso, M.P., Carneiro, P., Cordeiro, P., Costa, N., Melo, R.B., Miguel, A.S., Perestrelo, G. (Eds.), Occupational Safety and Hygiene IV. CRC Press, Taylor & Francis Group, Boca Raton, USA, pp. 73-76.

Pleil, J.D., Stiegel, M.A., Fent, K.W., 2014. Exploratory breath analysis for assessing toxic dermal exposures of firefighters during suppression of structural burns. J. Breath Res. 8, 037107.

Portuguese Regulation, 2013. Portuguese Directive number 3974/2013. Diário da República, serie II. 53, 9522-9572.

Preuss, R., Rossbach, B., Wilhelm, M., Angerer, J., 2006. External and internal exposure to polycyclic aromatic hydrocarbons (PAH) among workers in the production of fire-proof materials – proposal of a biological monitoring guidance values. Int. J. Hyg. Environ. Health. 209(6), 575-580.

Reisen, F., Brown, S., Cheng, M., 2006. Air toxics in bushfire smoke: firefighter's exposure during prescribed burns. Forest Ecol. Manag. 234, S144.

Reisen, F., Brown, S.K., 2009. Australian firefighters' exposure to air toxics during bushfire burns of autumn 2005 and 2006. Environ. Int. 35, 342–352.

Rey-Salgueiro, L., Martínez-Carballo, E., García-Falcón, M.S., González-Barreiro, C., Simal-Gándara, J., 2009. Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. Food Chem. 115, 814-819.

Robinson, M.S., Anthony, T.R., Littau, S.R., Herckes, P., Nelson, X., Poplin, G.S. et al., 2008. Occupational PAH exposures during prescribed pile burns. Ann. Occup. Hyg. 52(6), 497-508.

Serdar, B., Lee, D., Dou, Z., 2012. Biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) and DNA damage: a cross-sectional pilot study among roofers in South Florida. BMJ Open 2(4), e001318.

Sobus, J.R., McClean, M.D., Herrick, R.F., Waidyanatha, S., Nylander-French, L.A., Kupper, L.L., et al., 2009a. Comparing urinary biomarkers of airborne and dermal exposure to polycyclic aromatic compounds in asphalt-exposed workers. Ann. Occup. Hyg. 53(6), 561-571.

Sobus, J.R., McClean, M.D., Herrick, R.F., Waidyanatha, S., Onyemauwa, F., Kupper, L.L. et al., 2009b. Investigation of PAH biomarkers in the urine of workers exposed to hot asphalt. Ann. Occup. Hyg. 53, 551–560.

USEPA, 2005. US Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F. US Environmental Protection Agency. http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.pdf. (acessed 25/04/2016).

Väänänen, V., Hämeilä, M., Kalliokoski, P., Nykyri, E., Heikkilä, P., 2005. Dermal exposure to polycyclic aromatic hydrocarbons among road pavers. Ann. Occup. Hyg. 49(2), 167-78.

Viau, C., Carrier, G., Vyskocil, A., Dodd, C., 1995. Urinary excretion kinetics of 1-hydroxypyrene in volunteers exposed to pyrene by the oral and dermal route. Sci. Total Environ. 163, 179-186.

Viau, C., Vyskocil, A., 1995. Patterns of 1-hydroxypyrene excretion in volunteers exposed to pyrene by the dermal route. Sci. Total Environ. 163, 187–190.

WHO, 1996. Biological Monitoring of Chemical exposure in Workplace-guidelines. World Health Organization, Geneva.

WHO, 2013. State of the science of endocrine disrupting chemicals 2012. United Nations Environment Programme and the World Health Organization, Geneva.

WHO, 2002. World Health Survey B – Individual Questionnaire. World Health Organization, Evidence and Information Policy. http://www.who.int/healthinfo/survey/whslongindividuala.pdf. (acessed 26/03/2016).

Yamano, Y., Hara, K., Ichiba, M., Hanaoka, T., Pan, G., Nakadate, T., 2014. Urinary 1-hydroxypyrene as a comprehensive carcinogenic biomarker of exposure to polycyclic aromatic hydrocarbons: a cross-sectional study of coke oven workers in china. Int. Arch. Occup. Environ. Health. 87, 705-713.

Yin, W.Q, Wang, L., Li, R.R., Cao, S.Y., Li, L., 2014. Personal exposure levels of PAHs in the general population in northern rural area of Jiangsu Province, China. J. Environ. Sci. Health A. Tox. Hazard. Subst. Environ. Eng. 49(3), 281-286.

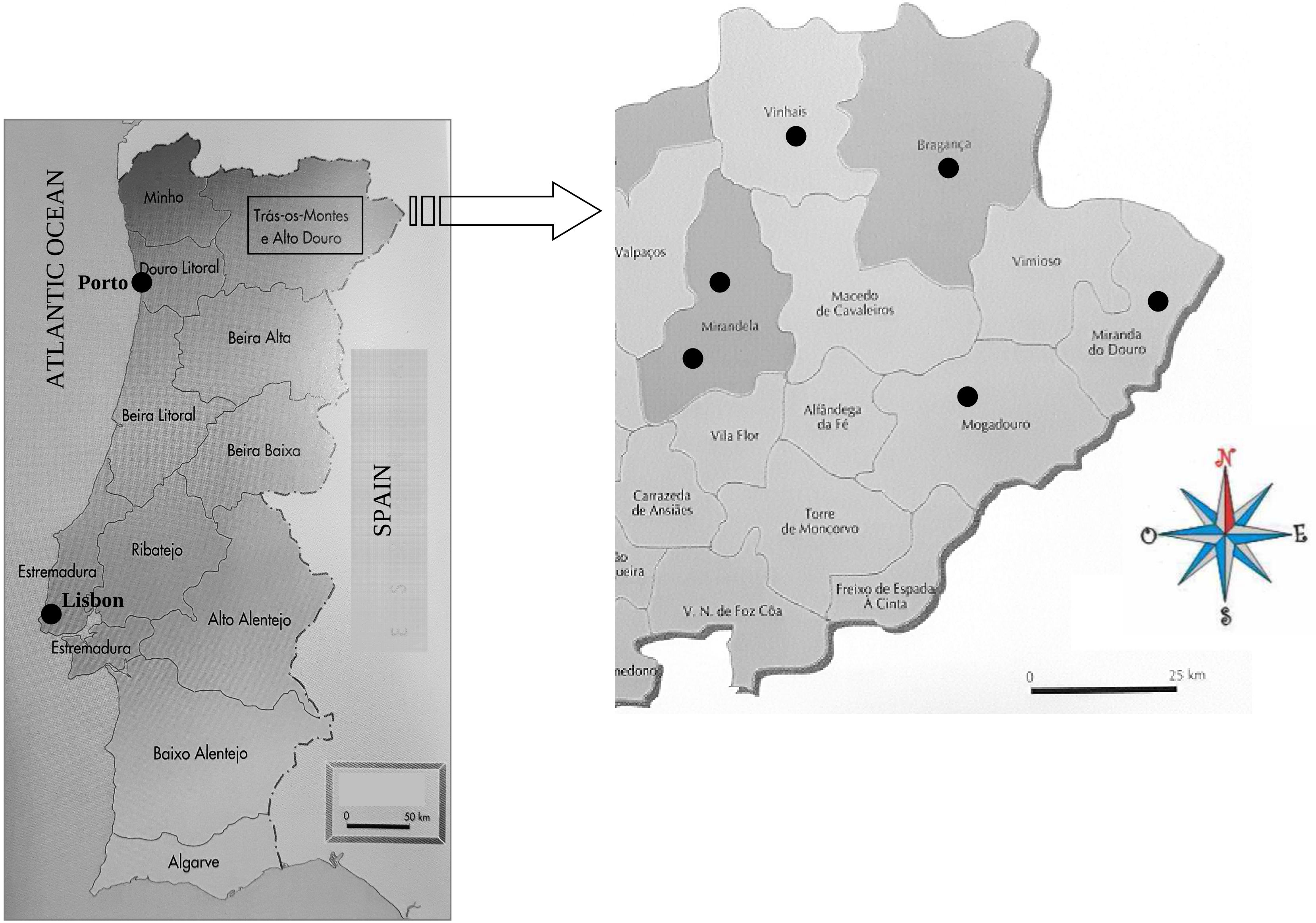
Figure Captions

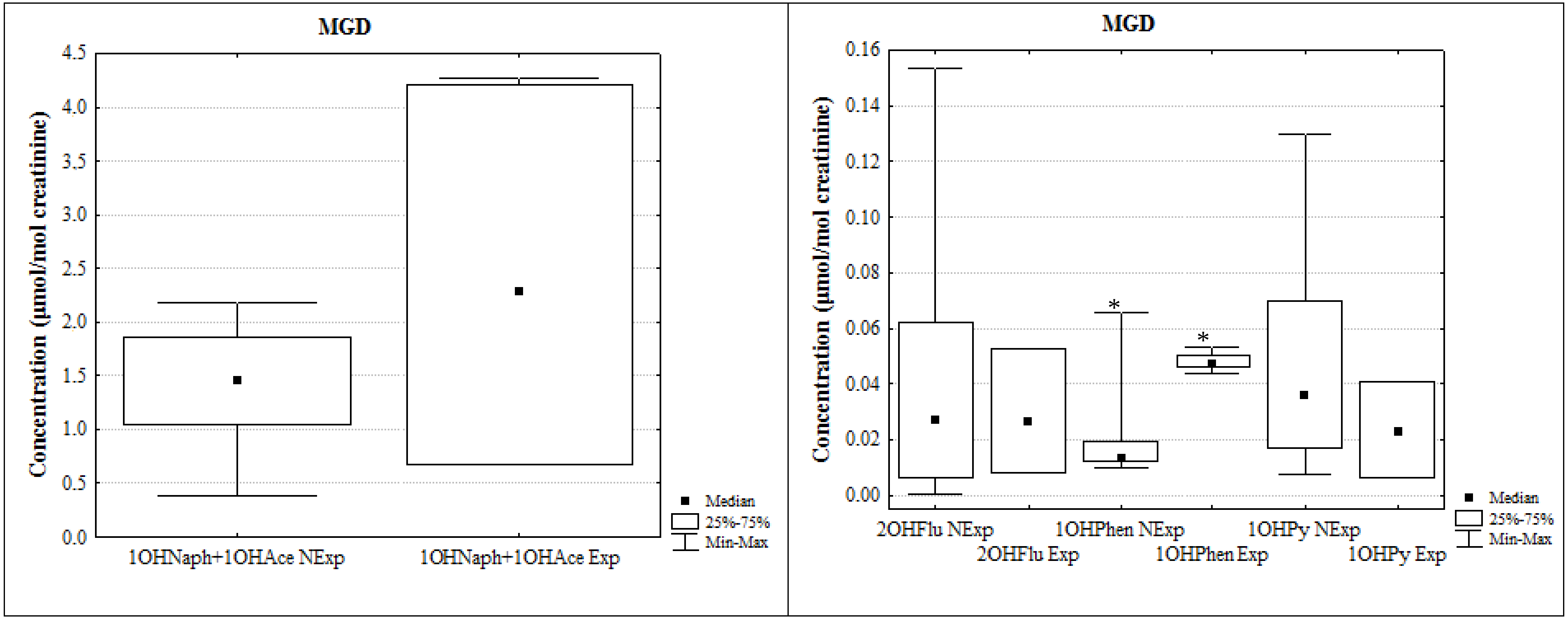
Figure 1. Geographical location of the selected fire corporations.

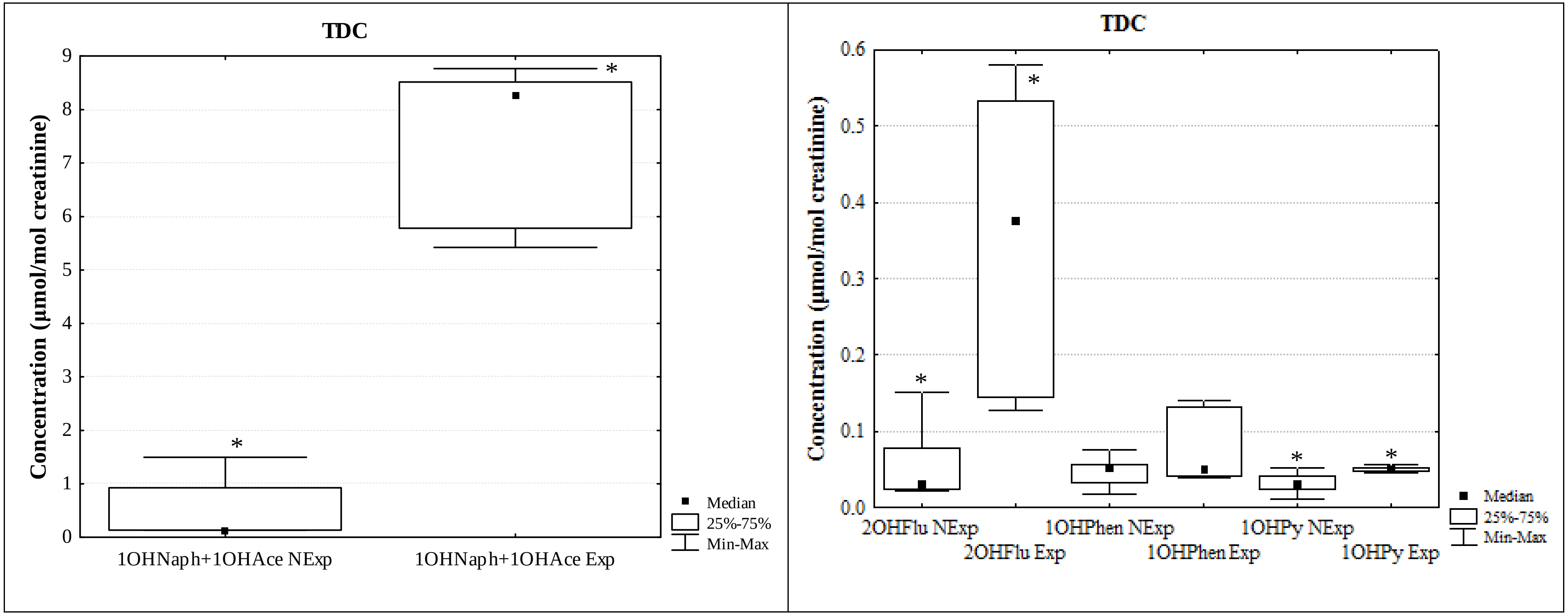
Figure 2. Concentrations of monohydroxyl-PAHs (median; 25-75% and range; μmol/mol creatinine) measured in non-exposed (NExp) and exposed (Exp) firefighters' urine from different fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela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

Figure 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).

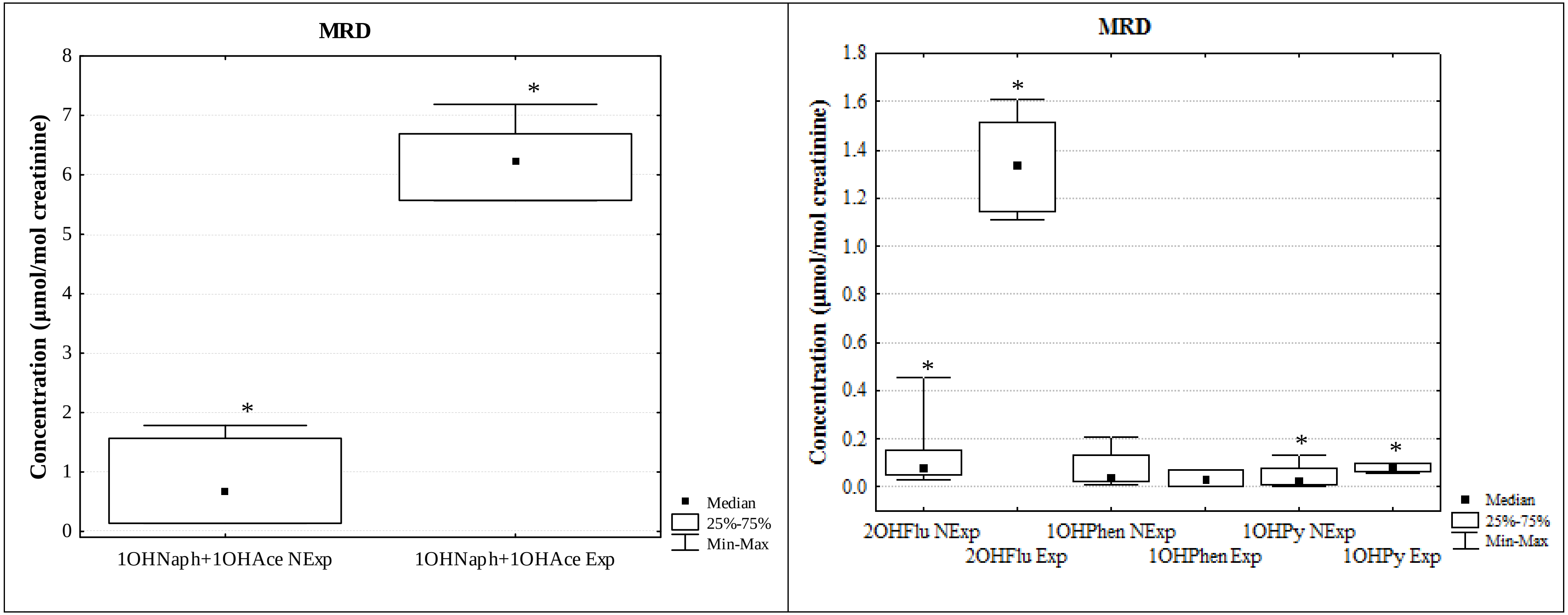
Figure 1.

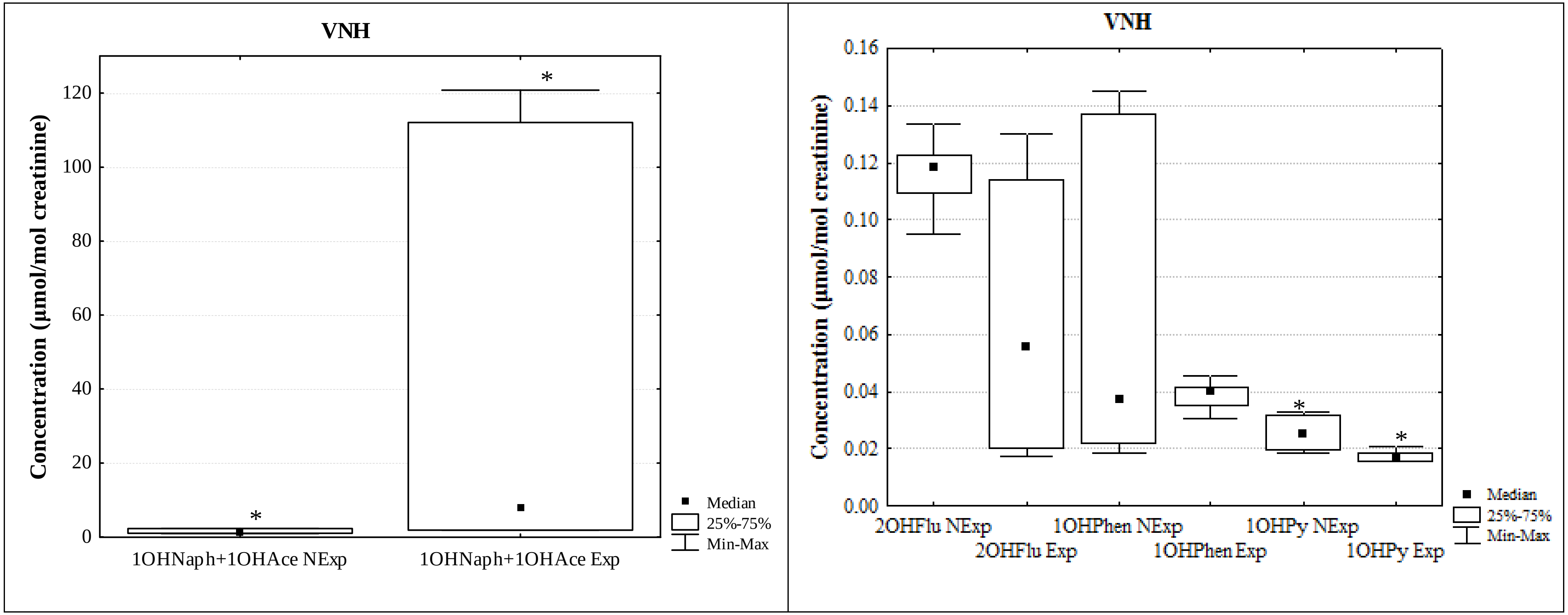

ATLANTIC OCEAN
Minho
Trás-os-Montes e Alto Douro
Porto
Douro Litoral
Beira Alta
Beira Litoral
Beira Baixa
SPAIN
Ribatejo
Estremadura
Lisbon
Alto Alentejo
Estremadura
Baixo Alentejo
0
50 km
Algarve
Vinhais
Bragança
Valpaços
Vimioso
Macedo de Cavaleiros
Mirandela
Miranda do Douro
Alfândega da Fé
Vila Flor
Mogadouro
Carrazeda de Ansiães
Torre de Moncorvo
Freixo de Espada À Cinta
V. N. de Foz Côa
N
O
E
S
0
25 km

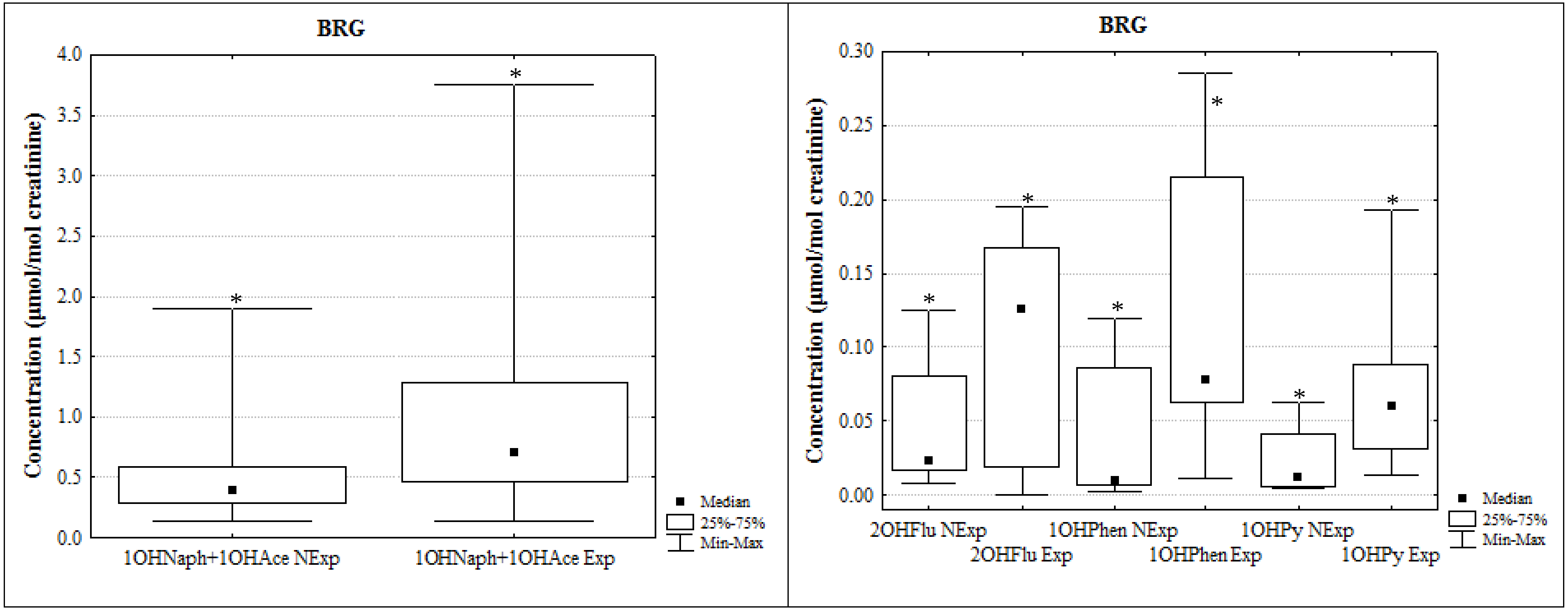

MGD
Concentration (µmol/mol creatinine)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
MGD
Concentration (µmol/mol creatinine)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
*
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max



TDC
Concentration (µmol/mol creatinine)
9
8
7
6
5
4
3
2
1
0
*
*
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
TDC
Concentration (µmol/mol creatinine)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
*
*
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max


MRD
Concentration (µmol/mol creatinine)
8
7
6
5
4
3
2
1
0
*
*
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
MRD
Concentration (µmol/mol creatinine)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
*
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max


VNH
Concentration (µmol/mol creatinine)
0
20
40
60
80
100
120
*
*
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
VNH
Concentration (µmol/mol creatinine)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
*
*
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max



BRG
Concentration (µmol/mol creatinine)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
BRG
Concentration (µmol/mol creatinine)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max

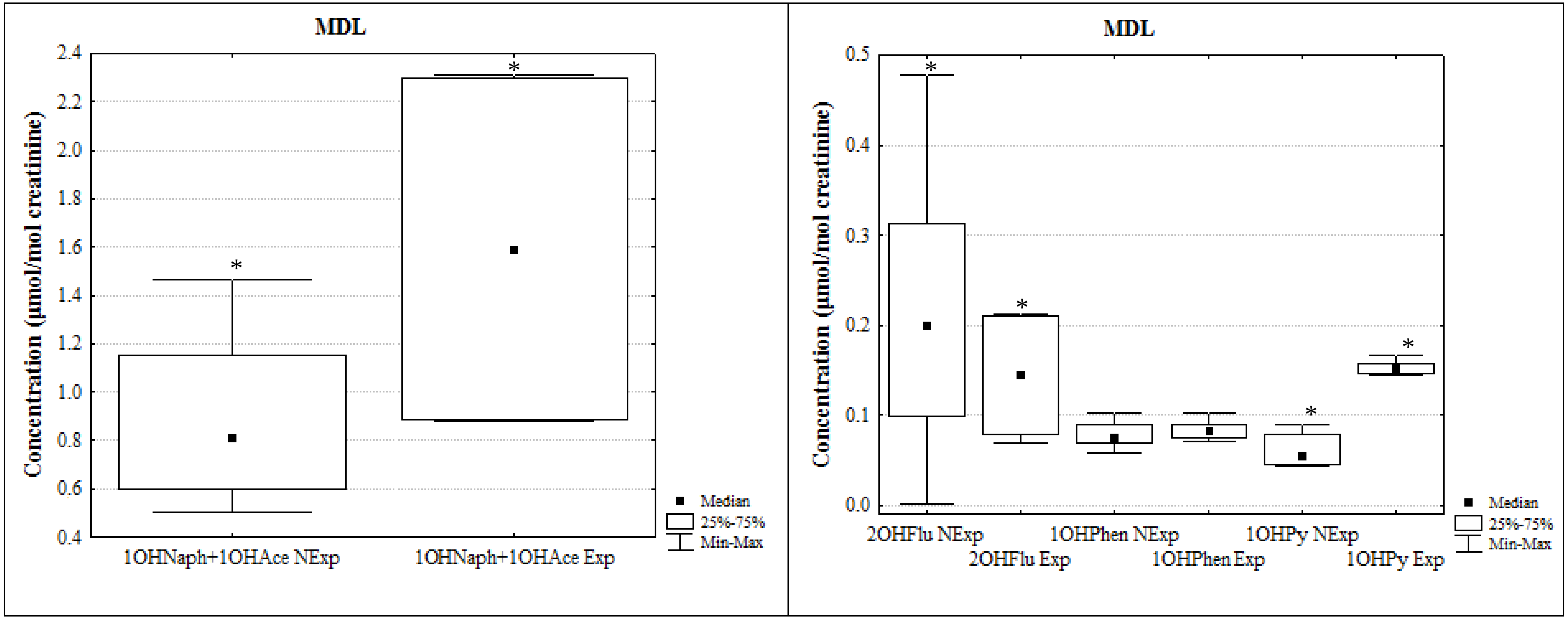
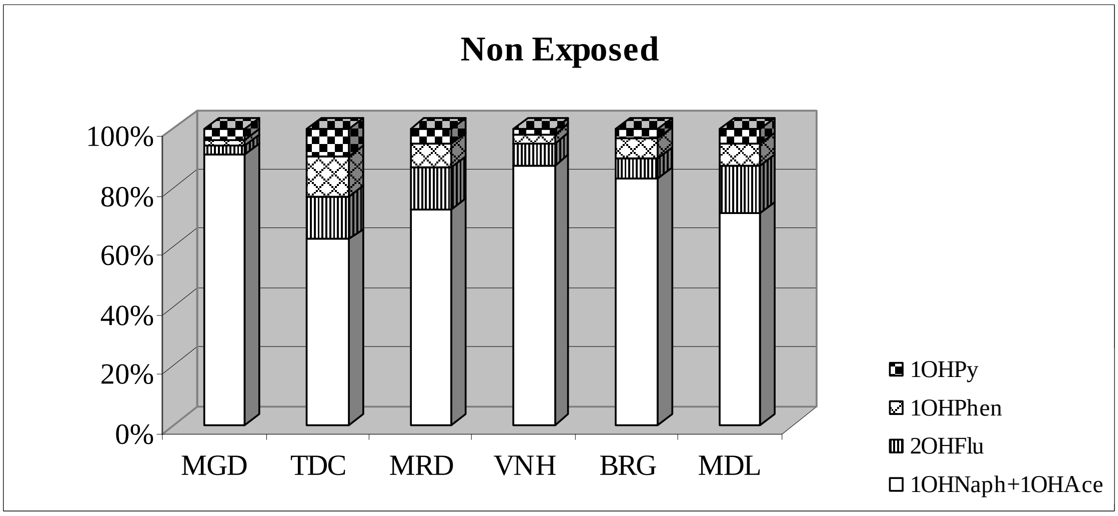
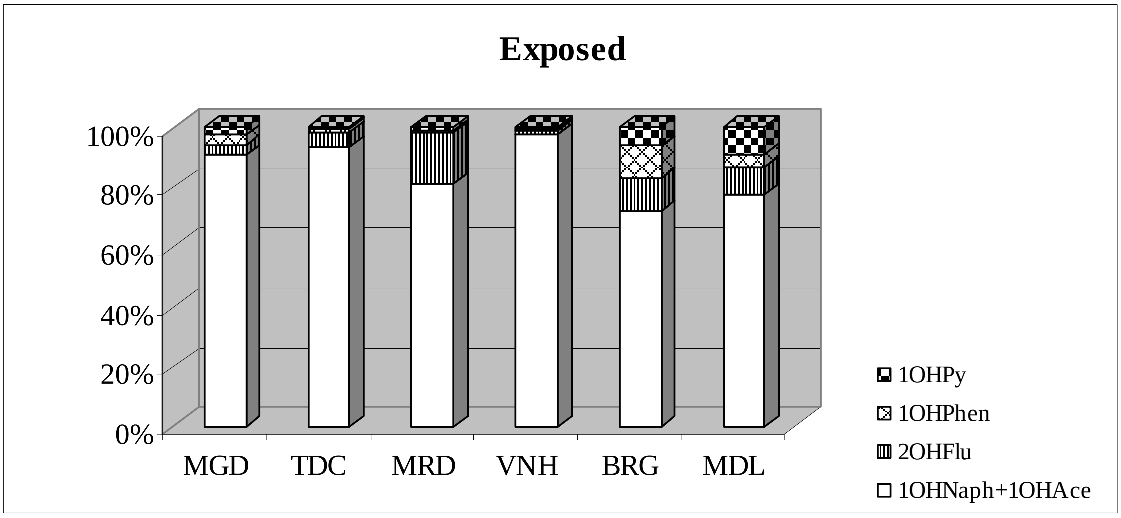

MDL
Concentration (µmol/mol creatinine)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
*
*
1OHNaph+1OHAce NExp
1OHNaph+1OHAce Exp
Median
25%-75%
Min-Max
MDL
Concentration (µmol/mol creatinine)
0.5
0.4
0.3
0.2
0.1
0.0
*
*
*
*
2OHFlu NExp
2OHFlu Exp
1OHPhen NExp
1OHPhen Exp
1OHPy NExp
1OHPy Exp
Median
25%-75%
Min-Max

Figure 2.


Non Exposed
100%
80%
60%
40%
20%
0%
MGD
TDC
MRD
VNH
BRG
MDL
1OHPy
1OHPhen
2OHFlu
1OHNaph+1OHAce

(a)


Exposed
100%
80%
60%
40%
20%
0%
MGD
TDC
MRD
VNH
BRG
MDL
1OHPy
1OHPhen
2OHFlu
1OHNaph+1OHAce

(b)

Figure 3.

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 hours before sample collection		
< 5 hours (%)	n.a.	53
5 – 10 hours (%)	n.a.	37
> 10 hours (%)	n.a.	10

n.a. – not applicable

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

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