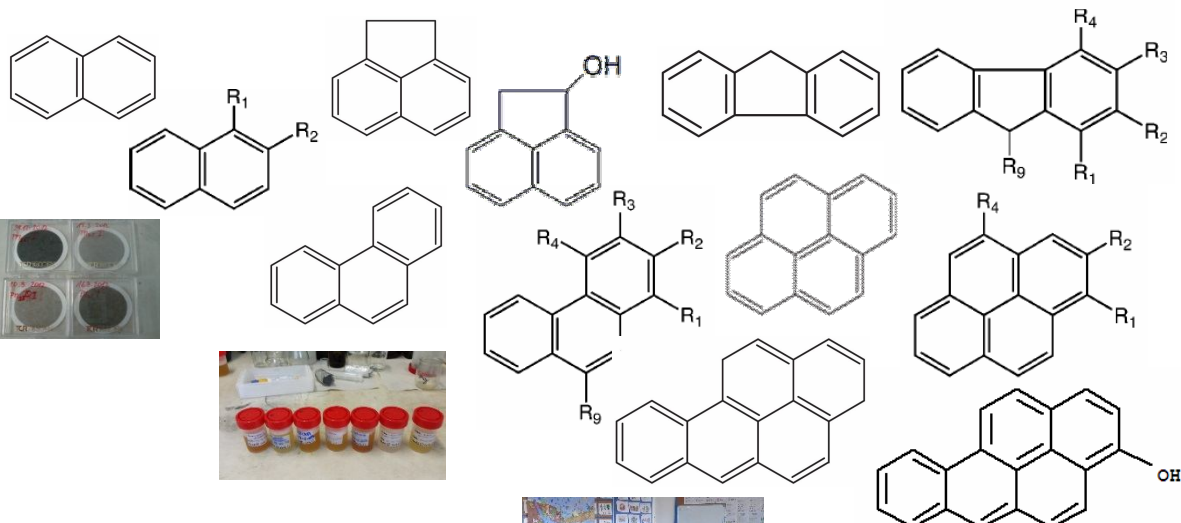
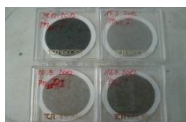




Particulate matter and polycyclic aromatic hydrocarbons from forest fires: impacts on air quality and occupational risks assessment



Marta Madalena Marques de Oliveira

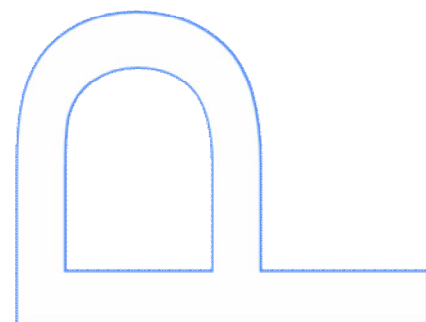
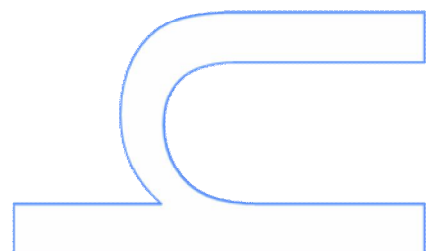
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ABSTRACT

Particulate matter (PM), some metals (cadmium, hexavalent chromium, and nickel) and metalloids (arsenic), and some polycyclic aromatic hydrocarbons (PAHs) are classified as carcinogenic (or possible/probable carcinogenic) air pollutants by the International Agency for Research on Cancer. Thus, this work assessed the exposure to PM (including its elemental content), and 18 PAHs (16 US Environmental Protection Agency (USEPA) priority PAHs, dibenzo[a,l]pyrene, and benzo[j]fluoranthene) by both external (air) and internal dose (by biomarkers of exposure, namely 1-hydroxynaphthalene (1OHNaph), 1-hydroxyacenaphthene (1OHAc), 2-hydroxyfluorene (2OHFlu), 1-hydroxyphenanthrene (1OHPhen), 1-hydroxypyrene (1OHPy), and 3-hydroxybenzo[a]pyrene (3OHB[a]P)). This work is focused on children environmental exposure (3-5 and 8-10 years old attending preschools and primary schools, respectively) and on firefighter's occupational exposure (at fire stations and after fire combat activities).

Regarding (pre)school (micro)environments, PM concentrations were below the available recommendations. Calcium, sodium, aluminium, and potassium were the most abundant elements in indoor PM (82–84%) resulting mainly from crustal sources. Carcinogenic elements (1–2% of the indoor content) were mostly PM_{2.5}-bound (83–91%). Indoor-to-outdoor ratios of individual elements indicated contributions of indoor origin and from penetration of outdoor emissions; trace metals were associated with ambient anthropogenic emissions. Risks from overall preschool exposure to carcinogenic elements were negligible for children; for adults carcinogenic risks exceeded (4–11 times) the USEPA recommended value of 10^{-6} being 8–40 times higher than for children.

Particulate and gaseous PAHs were concurrently (indoors and outdoors) collected at classrooms and (pre)schools' yard in north of Portugal. The total concentrations of the analysed PAHs (Σ PAHs) were higher indoors than outdoors. Gaseous PAHs accounted with more than 85% (indoors) and 77-97% (outdoors) of total air Σ PAHs. Particulate PAHs were predominantly associated with PM₁ (54-76%). Compounds with 5 and 6 aromatic rings (benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene) were predominantly present in PM, whereas PAHs with 2 and 3 aromatic rings (naphthalene, fluorene and phenanthrene) existed almost entirely in the gas phase (76-94% indoors and 42-79% outdoors). Mean PM_{2.5}-benzo[a]pyrene concentrations at preschools were always below the guideline of 1 ng/m³ define for PM₁₀ content (Directive 2004/107/EC),

however at some primary schools that guideline was exceeded. Indoor/outdoor ratios of lighter PAHs (2–3 rings) indicated a contribution from indoor sources whereas compounds with 4–6 rings resulted mainly from penetration of outdoor emissions; vehicular traffic (both from diesel and gasoline combustions) was the main source of indoor PAHs. A similar distribution profile was observed between airborne PAHs and urinary monohydroxyl metabolites (OH-PAHs). 1OHNaph+1OHAce represented more than 78% of Σ OH-PAHs, being followed by 2OHFlu, 1OHPy, and 1OHPhen. 3OHB[a]P (PAH biomarker of carcinogenicity) was not detected. The contribution of indoor airborne PAHs to the urinary levels of OH-PAHs was explored by Spearman correlation coefficients. Results suggest that children had preschool indoor air as their major exposure source of naphthalene and acenaphthene, while no conclusion was reached regarding fluorene, phenanthrene and pyrene. Total carcinogenic risks due to exposure to PAHs in the indoor air of pre- and primary schools were higher than in outdoor air, which was attributed to the longer periods that children spend indoors. Overall cancer risks of children and staff attending at preschools and primary schools exceeded 5-37 and 7-87 times the World Health Organization health-based guideline of 10^{-5} , respectively.

Concerning firefighter's occupational exposure, Σ PAHs varied widely (46.4–428 ng/m³) in the breathing air zone of firefighters during their regular work shift mainly due to site specificity (urban/rural) and characteristics (age and layout) of fire station buildings. Airborne PAHs with 2–3 rings were the most abundant (63.9–95.7% Σ PAHs). Consistent evidences were found that excretion of main individual urinary OH-PAHs was substantially increased in wildland firefighters that participated in fire combat (knockdown and overhaul) activities comparatively with non-exposed firefighters. Similarly with the findings obtained for schoolchildren, urinary 1OHNaph and 1OHAce were the predominant metabolites in non-exposed and exposed firefighters (63-98%). Also, participation in fire combat activities promoted an increase of the distribution percentage of 1OHNaph and 1OHAce, while contributions of 1OHPhen and 1OHPy decreased. The detected urinary 1OHPy concentrations were lower than the benchmark level (0.5 μ mol/mol creatinine) proposed by the American Conference of Governmental Industrial Hygienists. Moreover, strong to moderate Spearman correlations were observed between Σ PAHs, individual compounds and Σ OH-PAHs corroborating the prevalence of an emission source. Overall, it is expected that the attained results may help to develop mitigation strategies. Also, the collected data indicated a clear need to establish air quality international guidelines or limits, particularly in (pre)schools (micro)environments.

Keywords: Schoolchildren; Firefighters; Environmental and occupational exposure; Particulate matter (PM); Elemental composition; Polycyclic aromatic hydrocarbons (PAHs); Biomarkers of exposure; Health risk assessment.

RESUMO

A matéria particulada (PM), alguns metais (cádmio, crómio hexavalente e níquel), metalóides (arsénio) e alguns dos hidrocarbonetos aromáticos policíclicos (PAHs) são classificados como poluentes carcinogénicos (ou possível/provavelmente carcinogénicos) do ar pela Agência Internacional para a Investigação do Cancro (IARC). Este estudo avaliou a exposição a PM (incluindo o seu teor em metais/metalóides) e 18 PAHs (16 PAHs considerados como poluentes prioritários pela Agência de Proteção Ambiental Americana (USEPA), dibenzo[a,l]pireno e o benzo[j]fluoranteno) através da medida da dose externa (ar) e interna (por biomarcadores de exposição, nomeadamente o 1-hidroxinaftaleno (1OHNaph), 1-hidroxi-acenafteno (1OHAce), 2-hidroxifluoreno (2OHFlu), 1-hidroxifenantreno (1OHPhen), 1-hidroxipireno (1OHPy) e o 3-hidroxibenzo[a]pireno (3OHB[a]P)). Este trabalho caracteriza a exposição ambiental de crianças (com 3-5 e 8-10 anos de idade que frequentam pré-escolas e escolas primárias, respetivamente) e a exposição ocupacional de bombeiros (nas corporações e após as atividades de combate a fogos).

No que respeita aos (micro)ambientes (pré)escolares, as concentrações de PM foram inferiores às recomendações existentes. O cálcio, sódio, alumínio e potássio foram os elementos mais abundantes na PM do ar interior (82-84%) sendo resultantes principalmente de fontes da crosta terrestre. Os elementos carcinogénicos (1-2% do teor analisado no ar interior) encontraram-se maioritariamente adsorvidos à fração PM_{2.5} (83-91%). Os rácios para as concentrações elementares no ar interior/ar exterior (I/O), determinados para cada elemento, indicaram contribuições do ar interior e da penetração das emissões exteriores no ar interior estando os metais vestigiais associados a emissões antropogénicas. Os riscos resultantes da exposição global a elementos cancerígenos em crianças do ensino pré-escolar foram desprezáveis; para os adultos os riscos carcinogénicos excederam (4-11 vezes) o valor de 10⁻⁶ recomendado pela USEPA, sendo estes riscos 8-40 vezes superiores aos encontrados para as crianças.

Os PAHs presentes nas fases particulada e gasosa do ar foram simultaneamente (no ar interior e exterior) recolhidos em salas de aula, nos jardins e em recreios de (pré)escolas no norte de Portugal. As concentrações totais dos PAHs (ΣPAHs) detetados no ar interior foram superiores às do ar exterior. Os compostos presentes na fase gasosa representaram mais de 85% (ar interior) e 77-97% (ar exterior) do ΣPAHs no ar total. Os PAHs presentes na PM encontraram-se

predominantemente associados à fração PM₁ (54-76% do ΣPAHs). Os compostos com 5 e 6 anéis aromáticos (benzo[b+j]fluoranteno, benzo[k]fluoranteno, benzo[a]pireno, dibenz[a,h]antraceno, benzo[ghi]perileno e o indeno[1,2,3-cd]pireno) foram maioritariamente encontrados na PM, enquanto que os PAHs com 2 e 3 anéis aromáticos (naftaleno, fluoreno e fenantreno) estavam quase exclusivamente na fase gasosa (76-94% no ar interior e 42-79% no ar exterior). As concentrações médias de PM_{2.5}-benzo[a]pireno determinadas no ar das pré-escolas foram sempre inferiores a 1 ng/m³ definida para o teor anual na PM₁₀ (Directiva 2004/107/EC), contudo em algumas escolas primárias esse limite foi pontualmente excedido. Os rácios I/O dos PAHs mais leves (2-3 anéis) indicaram a contribuição de fontes interiores enquanto os compostos com 4-6 anéis resultaram maioritariamente da penetração de emissões exteriores sendo, neste caso, o tráfego de veículos (tanto com motores diesel ou gasolina) a fonte principal. Foi encontrado um perfil de distribuição semelhante entre os níveis de PAHs no ar e os metabolitos de PAHs (OH-PAHs) na urina. 1OHNaph+1OHAcce representaram mais de 78% do ΣOH-PAHs, seguidos pelo 2OHFlu, 1OHPhy e 1OHPhen. O 3OHB[a]P (biomarcador de carcinogenicidade dos PAHs) não foi detetado. A contribuição dos PAHs presentes no ar interior para os níveis de OH-PAHs na urina foi explorada com coeficientes de correlação de Spearman. Os resultados sugeriram que o ar interior da pré-escola constituía a maior fonte de exposição a naftaleno e acenafteno das crianças; não foi possível chegar a nenhuma conclusão para o fluoreno, fenantreno e pireno. O total de riscos carcinogénicos devido à exposição a PAHs no ar interior de pré-escolas e de escolas primárias foram superiores aos encontrados para o ar exterior, o que foi atribuído aos períodos longos que as crianças passam em ambientes interiores. Globalmente os riscos de cancro em crianças e adultos presentes em pré-escolas e escolas primárias excederam respetivamente 5-37 e 7-87 vezes a diretriz de 10⁻⁵ estabelecida pela Organização Mundial de Saúde.

Relativamente à exposição ocupacional de bombeiros, os níveis de ΣPAHs variaram amplamente (46.4-428 ng/m³) na zona de ar respirável dos bombeiros durante o seu turno de trabalho regular, o que se deveu principalmente à especificidade da localização (urbana/rural) das corporações estudadas e às características (idade e estrutura) dos edifícios. Os PAHs com 2-3 anéis foram os mais abundantes (63.9-95.7% ΣPAHs). Foram encontradas evidências consistentes quanto ao aumento substancial da excreção de cada um dos OH-PAHs nos bombeiros que participaram em atividades de combate a fogos (combate direto às chamas e fase de rescaldo) comparativamente aos bombeiros não expostos. Em linha com as evidências encontradas para as crianças em idade escolar, os metabolitos 1OHNaph e 1OHAcce

foram os predominantes na urina dos bombeiros não expostos e expostos (63-98%). Além disso, a participação em atividades de combate a incêndios promoveu um aumento da percentagem de distribuição do 1OHNaph e 1OHAce enquanto as contribuições do 1OHPhen e 1OHPy diminuíram. As concentrações urinárias de 1OHPy detetadas foram inferiores ao nível de referência (0.5 $\mu\text{mol/mol}$ creatinina), proposto pela Conferência Americana e Governamental de Higienistas Industriais. Foram ainda observadas correlações de Spearman moderadas a fortes entre ΣPAHs , os compostos individuais e $\Sigma\text{OH-PAHs}$, corroborando a prevalência de uma fonte emissora.

Espera-se que os resultados obtidos possam contribuir para o desenvolvimento de estratégias de mitigação. Os dados recolhidos sugerem a necessidade de estabelecer diretrizes ou limites internacionais para a qualidade do ar, particularmente em (micro)ambientes (pré)escolares.

Palavras-chave: Crianças em idade escolar; Bombeiros; Exposição ambiental e ocupacional; Matéria particulada (PM); Composição elementar; Hidrocarbonetos aromáticos policíclicos (PAHs); Biomarcadores de exposição; Avaliação de riscos para a saúde.

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Health Issues, Nova Science Publishers, Hauppauge, New York, USA, 2013, pp. 289-307; ISBN: 978-1-62257-473-5.

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7. M. Oliveira, K. Slezakova, C. Delerue-Matos, M.C. Pereira, S. Morais, Environmental and internal dose assessment of exposure to PAHs in preschool children: levels and impact of indoor air pollution on excretion of main monohydroxyl metabolites, submitted.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ace	Acenaphthene
Acen	Acenaphthylene
ACGIH	American Conference of Governmental Industrial Hygienists
Ant	Anthracene
AT	Averaging time
ATSDR	Agency for Toxicity Substances and Disease Registry
B[a]A	Benz[a]anthracene
B[a]P	Benzo[a]pyrene
B[a]P _{eq}	Benzo[a]pyrene equivalent
B[b+j]Fln	Benzo[b+j]fluoranthene
B[ghi]P	Benzo[ghi]perylene
B[k]Fln	Benzo[k]fluoranthene
BRG	Bragança fire station
BW	Body weight
CO	Carbon monoxide
CO ₂	Carbon dioxide
CVD	Cardiovascular disease
D	Age-specific dose rate
DB[a,h]A	Dibenz[a,h]anthracene
DB[a,l]P	Dibenzo[a,l]pyrene
DNA	Desoxyribonucleic acid
ED	Exposure duration
EEA	Environmental European Agency
EF	Enrichment factor
E _{Fr}	Exposure frequency
ET	Exposure time
EU	European Union
FEC	Freixo de Espada à Cinta fire station
Flu	Fluorene
Fln	Fluoranthene
FLD	Fluorescence Detection
HPLC	High Pressure Liquid Chromatography
IAQ	Indoor air quality

IARC	International Agency for Research on Cancer
Ind	Indeno[1,2,3-cd]pyrene
I/O	Indoor – to – outdoor ratio
IR	Inhalation rate
ISAAC	International Study of Asthma and Allergies in Childhood
IUR	Chronic inhalation unit risk
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave assisted extraction
MDL	Mirandela fire station
MGD	Mogadouro fire station
MRD	Miranda do Douro fire station
Naph	Naphthalene
NIOSH	National Institute for Occupational Safety and Health
NO	Nitrogen monoxide
NO ₂	Nitrogen dioxide
NO _x	Nitrogen oxides
O ₃	Ozone
OH-PAHs	Monohydroxyl-polycyclic aromatic hydrocarbons
OSHA	Occupational Safety and Health Administration
PAH/PAHs	Polycyclic aromatic hydrocarbon / hydrocarbons
PAHs _{car}	Carcinogenic polycyclic aromatic hydrocarbons
PAD	Photodiode array detection
PEL	Permissible exposure limits
Phen	Phenanthrene
PVC	Polyvinyl chloride
PEL	Permissible airborne exposure limit
PM	Particulate matter
PM ₁₀	Particulate matter which passes through a size-selective inlet with a 50% efficiency cut-off at 10 µm aerodynamic diameter
PM _{2.5}	Particulate matter which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 µm aerodynamic diameter
PM ₁	Particulate matter which passes through a size-selective inlet with a 50% efficiency cut-off at 1.0 µm aerodynamic diameter
PS1	Preschool one (Oporto)

PS2	Preschool two (Chaves)
PTFE	Polytetrafluoroethylene
Py	pyrene
r	Spearman correlation coefficient
RfC	Reference concentration
RfD	Inhalation reference dose
RH	Relative humidity
ROS	Reactive oxygen species
RSD	Relative standard deviation
SCBA	Self-containing breathing apparatus
SD	Standard deviation
SDM	Sendim fire station
SO ₂	Sulphur dioxide
T	Temperature
TDC	Torre Dona Chama fire station
TEF	Toxicity equivalency factor
THQ	Non carcinogenic risk
TMC	Torre Moncorvo fire station
TVOCs	Total volatile organic compounds
TR	Carcinogenic risks
TLV	Threshold limit value
TSP	Total suspended particles
UV-Vis	Ultraviolet – visible detection
UNECE	United Nations Economic Commission for Europe
USA	United States of America
USEPA	United States Environmental Protection Agency
VNH	Vinhais fire station
WHO	World Health Organization
WTC	World Trade Center
1OHNaph	1-hydroxynaphthalene
1OHAce	1-hydroxyacenaphthene
2OHFlu	2-hydroxyfluorene
1OHPhen	1-hydroxyphenanthrene
1OHPy	1-hydroxypyrene
3OHB[a]P	3-hydroxybenzo[a]pyrene

Greek symbols

Å	Angström
µm	micrometer
µg	microgram
µmol	micromole
Σ	Sum

Roman symbols

Al	Aluminium
As	Arsenic
B	Boron
Ba	Barium
Be	Beryllium
Ca	Calcium
Cd	Cadmium
Ce	Cerium
Co	Cobalt
Cr	Chromium
Cs	Cesium
Cu	Copper
In	Indium
K	Potassium
La	Lanthanum
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Nd	Neodymium
Ni	Nickel
P	Phosphorus
Pb	Lead
Pr	Praseodymium
Rb	Rubidium
Sb	Antimony
Se	Selenium

Si	Silicium
Sn	Tin
Sr	Strontium
Tb	Terbium
Zn	Zinc

CHAPTER 1 – PREFACE

1.1 Relevance and motivation

Air pollution is both an environmental and a social problem, as it leads to a multitude of adverse effects on human health, ecosystems, the built environment, and the climate (EEA 2015). According to a World Health Organization (WHO) assessment of the burden of disease due to air pollution, more than two million premature deaths each year can be attributed to the effects of urban outdoor and indoor air (WHO 2006). Air pollution as a whole and particulate matter (PM) was recently classified as carcinogenic by the International Agency for Research on Cancer (IARC) (IARC 2013). Upon inhalation, particles with aerodynamic diameter smaller than 10 μm (PM_{10}) enter the lower respiratory tract, while gas exchange regions of lungs are penetrated by particles smaller than 2-3 μm ($\text{PM}_{2.5}$). Despite the mechanisms of adverse health effects are not fully understood, epidemiological studies have consistently shown an association between atmospheric PM and the number of respiratory and cardiovascular diseases including lung cancer (Brunekreef et al., 2009; Hamra et al., 2014; Kim et al., 2015; Pope et al., 2002). Human exposure occurs in outdoor environments but also indoors by all routes (inhalation, ingestion, and skin contact), regardless of the source. In addition people spend considerable amount of their time indoors (homes, schools, offices, restaurants, etc.), and the pollutants generated in these environments may lead to higher levels of harmful exposures when compared to the respective outdoor concentrations. During the last three decades much of the scientific attention was focused on ambient air pollution and its impacts on public health. Consequently, the knowledge of health impacts of indoor air pollution has been hindered. Understanding the complexity of indoor air exposure and the respective health implications, and consequently, the development of protective guidelines are actually the key focuses of many international organisations. Thus, the awareness about indoor air exposure and its significance for human health has been increasing with growing attention on susceptible groups (WHO 2010).

The available information on PM chemical composition (metals, polycyclic aromatic hydrocarbons (PAHs), etc.) is still limited, particularly in educational settings and at occupational environments. PAHs are a large group of organic compounds that are formed during incomplete combustion of organic matter and originated from natural but mostly anthropogenic sources (Kim et al., 2013). Many PAHs are cytotoxic and mutagenic to lower and higher organisms (WHO 1998) being some of them considered carcinogenic or probable/possible carcinogenic to humans (IARC 2002, 2010b). As a consequence some PAHs are included in the European Union (EU) and US Environmental Protection Agency (USEPA) priority pollutant list (Directive

2004/107/CE; USEPA 2005; WHO 2010). However, PAHs monitoring is complicated by the mixed aerosol/vapor composition of airborne compounds and by the absorption of these chemicals from inhalation, food ingestion, and dermal contact. These compounds are absorbed into the blood stream and following possible metabolism (biotransformation) are distributed within the body. For these reasons, researchers have turned to biological monitoring to assess internal dose of PAHs, i.e., total exposure to PAHs. Urinary metabolites of PAHs, monohydroxyl-PAHs (OH-PAHs), reflect a more accurate estimation of the actual PAHs intake compared to environmental monitoring because they estimate the internal dose. Thus biomarkers are relevant indices in human health studies (Aitio et al., 2007). Data obtained with this approach should be combined with information reached by environmental monitoring to attain a more comprehensive characterization of exposure.

Young children represent one of the most susceptible subpopulations with regard to potentially harmful effects induced by air pollution (Pongpiachan and Paowa 2014; Schüepp and Sly 2012; WHO 2010) mainly because their respiratory, immune, reproductive, central nervous, and digestive systems are not fully developed (Burtcher and Schüepp 2012; Madureira et al., 2015; Makri et al. 2004; Salvi 2007). Children spend per day approximately 7–8 h of their time (i.e. 30%) at premises of the education settings (i.e. pre-schools and schools) which raise interest in characterizing health relevant pollutants in these microenvironments.

Firefighting, along with construction, mining, and agriculture, ranks among the most hazardous professions, with its occupational exposure being regarded as possible carcinogen to humans by IARC and the US National Institute for Occupational Safety and Health (NIOSH) (IARC 2010a; NIOSH 2007). However, firefighting is one of the least studied occupations in terms of exposures and their relationship to occupational diseases.

Thus, the work reported in this thesis is related with assessment of exposure to PM and PAHs by both external (air) and internal dose (by biomarkers of exposure, namely urinary OH-PAHs) (bio)monitoring. Children attending preschools (3-5 years old) and primary schools (8-10 years old), as well as firefighters were the selected groups. The contribution of environmental and occupational exposure to total internal dose and the potential health risks were also estimated.

1.2 Objectives

In order to contribute to the knowledge of children and firefighters exposure to PM and PAHs in occupational settings, and to the potential health risks, the main objectives of this work were:

- To characterize PM (including elemental composition) and PAH levels in indoor and/or outdoor air of Portuguese school microenvironments and fire stations;
- To evaluate PAHs internal body burden through the assessment of urinary OH-PAHs in preschool children and in firemen (background values and after participation in firefighting activities);
- To explore the contribution of indoor/breathing air to total internal exposure to PAHs in preschool children and firemen;
- To assess potential health risks for the selected groups due to PM elemental composition and/or airborne PAH exposures.

It is believed that the concretization of these objectives allowed a better understanding of children and firefighters environmental and occupational exposure and will also support epidemiological studies dealing with PM and PAH-exposed populations. This work also contributes with data that may help to develop and implement strategies to reduce potential health risks due to the environmental and occupational exposures.

1.3 Thesis outline

This work is divided into two main parts: Part I - Children environmental exposure at schools and Part II - Firefighters' occupational exposure. This thesis has a total of 10 chapters, being the content of each chapter summarized below.

Chapter 1 (present chapter) is the preface of the thesis. It explains the motivation of the work as well as the main objectives, and thesis organization.

Chapter 2 provides a global introduction to the issue of air pollution, with particular emphasis on indoor air quality (IAQ). In addition, the definitions, main sources and health risks of PM, metals, and PAHs exposure are described. The importance of PAHs biomonitoring is also briefly introduced.

Chapters 3 to 6 are related with Part I – Children environmental exposure at schools. Chapter 3 presents the first assessment of air quality in preschool environments with emphasis on elemental composition of PM₁₀ and PM_{2.5}. Chapter 4 is dedicated to children exposure to PM and PAHs (gas and particulate phase) in Portuguese pre- and primary schools. Firstly a comprehensive study was conducted at ten primary schools situated in the Oporto Metropolitan Area (north of Portugal). The selected schools were representative of various urban environments (city center, residential, industrial). Regarding preschools, a preliminary study was performed in order to establish a first contact with preschool microenvironments and to estimate contribution of traffic emissions to the IAQ of preschool. Then, a more complete study was conducted at two urban preschools from different geographical regions (Oporto and Chaves). Health risks, through the assessment of toxicity equivalent factors (TEF), non carcinogenic (THQ) and carcinogenic (TR) risks, in children with 3-5 and 8-10 years old and in school's staff, were also estimated. Chapter 5 presents assessment results of environmental exposure (to PM and PAHs) of preschool children complemented with data from six urinary biomarkers of PAHs exposure; the impact of indoor air pollution on excretion of main urinary OH-PAHs was characterized. The main findings concerning schoolchildren exposure to PM, a set of 29 elements and PAHs are displayed in Chapter 6.

Chapters 7 to 9 are dedicated to Part II – Firefighters' occupational exposure. Chapter 7 is dedicated to firefighters' occupational exposure (without participation in fire combat activities) to PM and PAHs in their breathing air zone at Portuguese fire stations and also estimates the contribution of inhaled PAH concentrations to the urinary concentrations of individual and total OH-PAHs. Chapter 8 reports the impact of firefighting activities on levels of urinary OH-PAHs. Chapter 9 presents the major findings related with firefighters' occupational exposure.

Finally, suggestions for future work are addressed in Chapter 10.

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CHAPTER 2 – AIR POLLUTION

Air pollutants are emitted from anthropogenic and natural sources and may be transported or formed over long distances. Some air pollutants can persist in the environment for long periods of time and accumulate in the environment and food chain, affecting humans and animals via multi-route exposure (inhalation, dermal, ingestion). At its last report, European Environment Agency (EEA) pointed transports and industries, energy, power plants, agriculture, households and waste management as the main source sectors of air pollution in Europe (EEA 2015).

The quality of air inside homes, offices, schools, day care centres, public buildings, health care facilities or other private and public buildings where people spend a large part of their life is an essential determinant of healthy life and people's well-being. IAQ may significantly impact on the respective health outcomes and thus requires particular attention. The factors that affect IAQ the most are the presence of indoor air contaminant sources, outdoor air quality and deficiencies in ventilation (Fernández et al., 2013, WHO 2010). Special attention needs to be given to hazardous substances emitted from buildings, construction materials and indoor equipment, as well as some human activities performed indoors that may originate a broad range of health problems, especially in developing countries (WHO 2010). Protection against detrimental action of air pollutants requires, first of all, reliable information about their levels of exposure. This assessment (and consequently the potential interventions) is needed at international level.

2.1 Particulate Matter

PM is one of the most relevant air pollutants (WHO 2006), consisting in a complex mixture of solid or solid/liquid particles suspended in air and varying in size, shape, origin, and with components having diverse chemical characteristics (WHO 2006). Particles are usually classified according to their aerodynamic diameter and can be generally separated into three major fractions: coarse particles larger than 2.5 μm (in aerodynamic diameter), fine particles smaller than 2.5 μm ($\text{PM}_{2.5}$) and ultrafine particles (smaller than 0.1 μm) (WHO 2006). The size of particles is an important factor since it determines the place where particle deposition occurs in the respiratory system. Particles with an aerodynamic diameter of 10 μm or less are naturally inhaled by humans, while larger particles are less likely to enter the human respiratory tract. After inhalation, coarse particles are deposited in the nose and mouth whereas particles smaller than 1 μm (PM_1) penetrate deeply into the lungs and deposit in the smaller conducting air ways and gas exchange regions of lungs where they can cause severe damages (Bosetti et al., 2010; Brugha and Grigg 2014; Kim et al., 2015).

Among PM, the most commonly studied fractions are PM₁₀ and PM_{2.5}; information concerning PM₁ is still limited. The smaller the particles, such as PM_{2.5} and PM₁, deeply they can penetrate in the lungs, exacerbating pulmonary diseases, lung cancer and premature mortality and morbidity (WHO 2006). However PM adverse health effects depend not only on particle size but also on its chemical composition and number (Cakmak et al., 2014; Grahame et al., 2014; Michael et al., 2013; Lippmann 2014). Chemical composition of PM is diverse and includes nitrates, sulphates, elemental and organic carbon, organic compounds (such as PAHs), biological compounds, metals and metalloids (arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb)); these compounds can cause eventually more adverse effects for human health (Kim et al., 2015; Rohr and Wyzga 2012). Although there is no general rule, it seems that some of these chemical species are more associated with fine smaller particles, i.e. PM_{2.5} and lower fractions (Castro et al., 2010, 2011; Slezakova et al., 2010). Coarse fraction, i.e. PM_{2.5-10} is more attributed to minerals from dust and sea salt, however, some anthropogenic species were also found in this fraction, although in much smaller proportions (Maenhaut et al., 1996; Slezakova et al., 2007).

PM is originated from a variety of outdoor natural (sea salt, pollen, spores, bacteria, plant, animal debris, and suspended crustal materials) and anthropogenic (combustion of fossil fuels, incineration, biomass burning, fires, domestic heating for households, road wear, and ammonia emissions from agricultural operations, etc.) sources (EEA 2015; WHO 2006). Recently the EEA reported that coal and biomass combustion in households, commercial and institutional buildings strongly contributed to increase PM_{2.5} concentrations during the past decade in Europe (EEA 2015). Traffic emissions have been considered as the main outdoor source of PM, particularly at urban areas (Amato et al., 2014). Continuous re-suspension of soil particles, cloth fibers, calcium-rich particles released from building deterioration, cleaning and cooking activities, combustion processes such as wood and fossil fuel burning, tobacco smoke and the emissions from printers and photocopy machines are important PM indoor sources (Amato et al., 2014; Blondeau et al., 2005; Destailats et al., 2008). Still, the introduction of legislation prohibiting smoking in public buildings and areas which have been introduced in the last decade in many states of United States of America (USA), Canada, and in Europe, have let to decrease by 80 to 90% the exposure to particulates (Goodman et al., 2009).

The available limits for the different fractions of PM in the indoor and outdoor air are presented in Table 2.1. EEA reported that PM₁₀ and PM_{2.5} EU limits were exceeded in 22 and 7 of the 28 EU Member States, respectively (EEA 2015). Overall 17% of the total European urban population was exposed to PM₁₀ levels above the daily limit value

(50 $\mu\text{g}/\text{m}^3$) and almost 61% to levels exceeding the stricter WHO air quality guideline (20 $\mu\text{g}/\text{m}^3$) (EEA 2015). Regarding $\text{PM}_{2.5}$, 9% and 87% of EU population was exposed to concentrations above the available limits (EU target of 25 $\mu\text{g}/\text{m}^3$ and the WHO guideline of 10 $\mu\text{g}/\text{m}^3$, respectively). WHO and USEPA do not provide any guidelines concerning indoor PM levels. Even among the developed countries, the existence of some national IAQ guidelines is rare. Among the few countries with established indoor air legislation is Portugal which government updated in 2013 its existent guidelines for the indoor air in public buildings (Portuguese Regulation 2013). The Portuguese indoor PM limits were re-defined with values of 25 and 50 $\mu\text{g}/\text{m}^3$ for $\text{PM}_{2.5}$ and PM_{10} , respectively (8-h mean; Table 2.1).

It is estimated that ambient $\text{PM}_{2.5}$ was responsible for 3.1 million deaths worldwide and for about 403 000 premature deaths in Europe (EEA 2015; Brugha and Grigg 2014; Kim et al., 2015). The life expectancy of Europeans is estimated to be decreased, on average, almost 9 months due to PM exposure (Brugha and Grigg 2014). In that regard, high emphasis has been given to the increase of hospital admissions, emergency room visits, respiratory symptoms, and exacerbation of chronic respiratory and cardiovascular diseases that can be attributed to PM exposure, especially in the most vulnerable groups of the population (Kim et al., 2015). As a consequence premature death in people suffering from heart and lung diseases, irregular heartbeat and nonfatal heart attacks, irritation of airways, coughing, difficulty breathing, and aggravated asthma have been associated with PM exposure (Atkinson et al., 2010; Correia et al., 2013; Fang et al., 2013; Meister et al., 2012). Chronic exposure to PM has been related with cardio respiratory diseases (myocardial infarctions, stroke, and chronic obstructive pulmonary disease), cystic fibrosis, exacerbations of asthma, respiratory infections and lung cancer (Brugha and Grigg 2014; WHO 2013a). Some evidences suggest that prenatal exposure to air pollution including PM may increase vulnerability of infants to respiratory infections (Brugha and Grigg 2014).

Table 2.1. Summary of the existent guidelines and limits (in $\mu\text{g}/\text{m}^3$) for pollutants in indoor and outdoor (ambient) air.

Pollutant	Indoor air		Outdoor air	
	Averaging time	Limit value	Averaging time	Limit value
PM ₁₀	8 h mean ^(a)	50 ^(b)	annual mean	20 ^(c)
			annual mean	40 ^(d, h)
			24 h (70% not to be exceeded more than 35 times over a calendar year)	50 ^(c, d, h)
			24 h (not to be exceeded more than once per year on average over 3 years)	150 ^(e)
PM _{2.5}	8 h mean ^(a)	25 ^(b)	annual mean	10 ^(c)
			annual (averaged over 3 years)	12 ^(e)
			24 h	25 ^(c)
			24 h (averaged over 3 years)	35 ^(e)
Metals				
Arsenium			Annual mean	0.006 ^(g, h, i)
Cadmium			Annual mean	0.005 ^(g, h, i)
Nickel			Annual mean	0.020 ^(g, h, i)
Lead			Annual mean	0.5 ^(h, i)
Polycyclic aromatic hydrocarbons				
Naphthalene	annual mean	10 ^(f)	annual mean	1 ng/m ³ ^(g, h, i)
Benzo[a]pyrene				

^(a) as air suspended particles
^(b) Portuguese Regulation (2013)
^(c) WHO 2006
^(d) Directive 2008/50/EC
^(e) USEPA 2010
^(f) WHO 2010
^(g) Directive 2004/107/EC
^(h) European Union (2016)
⁽ⁱ⁾ total content in PM₁₀ fraction.

2.2 Elemental composition of PM

Most metals exist in the solid phase and thus occur almost exclusively in the particle phase of the atmosphere, where they are ubiquitous in both fine and coarse fractions (EEA 2015; Hu et al., 2012; Singh et al., 2011). According to IARC, As, Cd, hexavalent Cr, and Ni compounds are classified as carcinogenic to humans (IARC 2012) whereas inorganic Pb compounds are classified as probable carcinogens (IARC 2006).

Some airborne trace metals and metalloids may derive from natural crustal sources (volcanic dust, oceanic aerosols, and soil crust), but the majority results from anthropogenic activities with main sources including vehicle emissions (primary source for Cr, Pb, Cd, and barium (Ba)), industrial and construction processes (responsible for manganese (Mn), aluminium (Al), and silicon (Si)), oil (responsible for Ni) and coal combustions, and metal industry (metal specific) (Fang et al., 2010; Okuda et al., 2008; Slezakova et al., 2014; Susaya et al., 2010). Waste incinerations, power plants, and combustion of fossil fuels, with particular emphasis on traffic emissions from motor vehicles are also relevant sources of airborne trace metals (EEA 2015). With respect to indoor environments, the abundance of trace elements results mostly from infiltration of outdoor emissions (EEA 2015) and from various indoor sources such as wall paints, indoor equipment and utensils (Chattopadhyay et al., 2003; Kebede et al., 2013; Paoletti et al., 2006; Taner et al., 2013).

In most environments PM-bound metals/metalloids exist in low concentrations and it has not been fully established if those levels are sufficient/significant to cause adverse health effects (Dominici et al., 2007; Slezakova et al., 2013). The mechanisms involved in adverse health effects caused by inhaled particles containing metals are not well understood. Smaller fractions of PM can enter deeper into alveolar region of the lungs where they can interact with the lung liquid fluid and once released trace metals are absorbed into human physiological systems, exerting adverse toxic effects (Mukhtar and Limbeck 2013). Therefore it has been proposed that the damage to health can be derived from metal-mediated generation of reactive oxygen species (ROS), which can cause severe oxidative stress within cells or tissues through the oxidation of nucleic acids, proteins and lipids (da Silva et al., 2015). In that regard some transition metals such as cobalt (Co), copper (Cu), Cr, iron (Fe), Ni and vanadium (V) have been known to play a role in redox reactions under certain conditions causing oxidative damage to biomolecules (da Silva et al., 2015). However the potential health effects of PM-bound elements depend upon the bonding and hence solubility of metallic components in addition to particle size, shape, total human

exposure and the health status of the exposed population (Mukhtar and Limbeck 2013). In addition some toxicological studies have suggested that several elements including Al, Si, V, and Ni are most closely associated with health impacts (Rohr and Wyzga 2012). More recently Shuster-Meiseles et al. (2016) found that As, zinc (Zn), and Fe were the elements that best predicted the reactive oxygen species-generating and the antioxidant response element activating capacity of the near roadway PM in the pulmonary cells. Thus lung injuries and inflammation have been associated with exposure to PM-bound metals/metalloids (da Silva et al., 2015; Hirshon et al., 2008; Prieditis and Adamson 2002).

2.3 Polycyclic aromatic hydrocarbons

PAHs are ubiquitous compounds formed during pyrolysis or incomplete combustion of organic matter with air and food as major routes of exposure (ACGIH 2005). PAHs are classified as persistent organic pollutants by United Nations Economic Commission for Europe (UNECE 1998) and some of them classified as endocrine disrupting chemicals (WHO 2013b). PAHs are a large class of organic compounds that are constituted of two or more fused aromatic rings, with molecules containing only carbon and hydrogen atoms. These compounds exist with a great number of structures (and in large number of isomers) that influence their chemical-physical properties. PAHs are non-polar compounds with low water solubility and marked lipophilicity; the higher the molecular weight, the higher the lipophilicity (Table 2.2).

Although there are several hundreds of PAHs, the scientific attention has been typically focused on sixteen PAHs recommended by USEPA as priority pollutants (USEPA 2005), Table 2.2; Figure 2.1. Due to the presence of aromatic rings, some PAHs present high toxicity, mutagenic and carcinogenic characteristics. Among those is benzo[a]pyrene, which is classified as carcinogenic to humans by IARC (group 1; IARC 2010), and naphthalene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, and indeno[1,2,3-cd]pyrene that are considered as possible carcinogenic to humans (group 2B; IARC 2002, 2010). 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 2010) due to their higher carcinogenic potency than benzo[a]pyrene (Okona-Mensah et al., 2005; Slezakova et al., 2013).

Table 2.2. Sixteen United States Environmental Protection Agency priority PAHs: physical-chemical properties and carcinogenicity classification

Compound	Molecular weight g/mol	Melting point (°C)	Boiling point (°C)	Vapor pressure at 25 °C (Pa)	Classification	
					IARC ^a	USEPA ^b
Naphthalene	128.17	81	218	10.4	2B	C
Acenaphthylene	152.19	92-93	265	8.9 × 10 ⁻¹	-	D
Acenaphthene	154.21	95	279	2.9 × 10 ⁻¹	3	-
Fluorene	166.22	115-116	295	8.0 × 10 ⁻²	3	D
Anthracene	178.23	216	342	8.0 × 10 ⁻⁴	3	D
Phenanthrene	178.23	100	340	1.6 × 10 ⁻²	3	D
Fluoranthene	202.25	109	375	1.2 × 10 ⁻³	3	D
Pyrene	202.25	150	393	6.0 × 10 ⁻⁴	3	D
Benz[a]anthracene	228.29	161	400	2.8 × 10 ⁻⁵	2B	B ₂
Chrysene	228.29	254	448	8.4 × 10 ⁻⁵	2B	B ₂
Benzo[b]fluoranthene	252.31	167	357	1.3 × 10 ⁻⁷	2B	B ₂
Benzo[k]fluoranthene	252.31	216	480	7.3 × 10 ⁻⁷	2B	B ₂
Benzof[a]pyrene	252.31	178	496	1.3 × 10 ⁻⁸ (20 °C)	1	B ₂
Dibenz[a,h]anthracene	278.35	267	524	1.3 × 10 ⁻⁸ (20 °C)	2A	D
Indeno[1,2,3-cd]pyrene	276.33	164	536	1.4 × 10 ⁻⁸	2B	B ₂
Benzofghi]perylene	276.33	278	545	1.4 × 10 ⁻⁸	3	-

^aGroup 1 - carcinogenic to humans; Group 2A - probably carcinogenic to humans; Group 2B - possible carcinogenic to humans; Group 3 - unclassifiable as to carcinogenicity in humans; Group 4 - probably not carcinogenic to humans (IARC 2002, 2010)

^bGroup A - human carcinogens; Group B - probable human carcinogens (B1: based on limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals; B2: based on sufficient evidence of carcinogenicity in animals); Group C - possible human carcinogens; Group D - not classifiable as to human carcinogenicity; Group E - evidence of non-carcinogenicity for humans (USEPA 1986, 2005)

^c(WHO 1998).

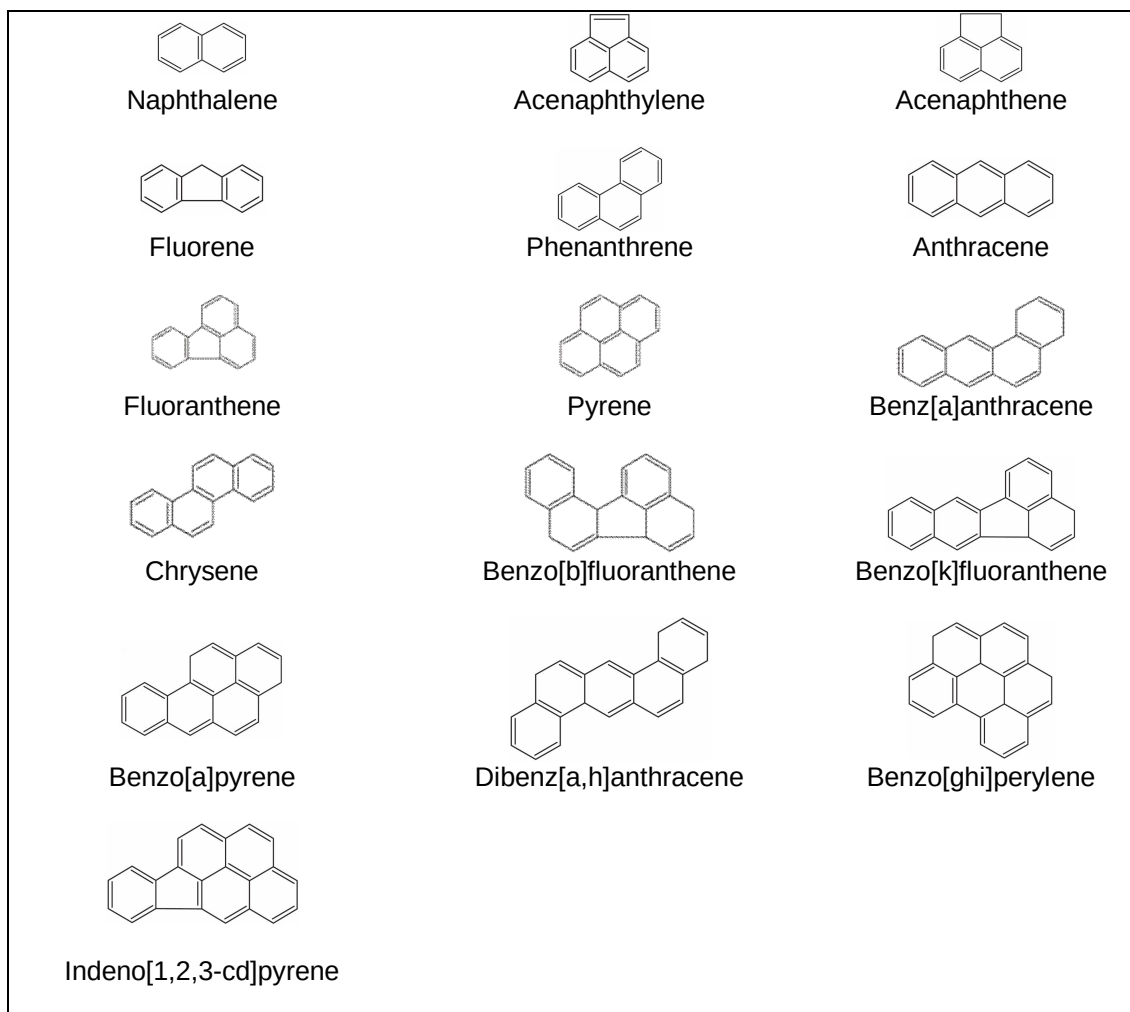


Figure 2.1. Molecular structures of sixteen United States Environmental Protection Agency priority PAHs.

Despite airborne PAHs be portioned between particulate and vapor phases, the more harmful PAHs are associated with particulate phase, being predominantly present in particles of smaller sizes (i.e. $PM_{2.5}$ and PM_1) (Slezakova et al., 2010). PAHs adsorbed/absorbed on PM can travel long distances before their deposition; the long-range transport through the atmosphere makes PAHs a transboundary environmental problem. However the persistence and transformation of atmospheric PAHs depends on various atmospheric parameters, such as temperature, relative humidity, intensity of solar radiation, and precipitation (Fang et al., 2006; Tham et al., 2008; Tsapakis and Stephanou 2005, 2007); in the presence of atmospheric oxidants (such as ozone, hydroxyl radical and nitrate radical) atmospheric PAHs undergo chemical and photochemical degradations (Park et al., 2002). As a consequence at the convention on long-range trans-boundary air pollution on persistent organic pollutants, the United Nations Economic Commission for Europe (UNECE) included PAHs in the list of substances in which total annual emissions should be reduced (UNECE 2015). The

major anthropogenic atmospheric emission sources of PAHs include biomass burning, coal and petroleum combustion, coke and metal production and road traffic (Kamal et al., 2015). Benzo[a]pyrene is usually emitted from the incomplete combustion of various fuels, being the domestic home-heating, in particular wood, coal, and waste burning, but also coke and steel production, and road traffic the most predominant sources in European countries (EEA 2015). Other relevant sources of benzo[a]pyrene include fires and rubber-tyre wear. Tobacco smoke is one of the major indoor sources of PAHs (Castro et al., 2011; Slezakova et al., 2009, 2014) particularly in the industrialized countries whereas in the developing ones open fires of wood and/or other combustion materials (dung, agricultural residues, or coal) for cooking or heating purposes are typically more dominant. The penetration of emissions from outdoor air, use of heating systems, and some electronic equipment (such as printers, computers) are also some relevant indoor sources of PAHs (Destailats et al., 2008; Heinrich 2011).

Benzo[a]pyrene is often used as a surrogate for other carcinogenic PAHs in studies estimating human cancer risks. There have been also efforts to regulate PAHs levels in air. Using benzo[a]pyrene as indicator of carcinogenic PAHs, current European legislation on ambient air (Directive 2004/107/EC) sets annual target value of 1 ng/m^3 for benzo[a]pyrene in PM_{10} (Table 2.1). However, findings about the presence of more potent PAHs, such as dibenzo[a,l]pyrene and dibenz[a,h]anthracene (Okona–Mensah et al., 2005) have raised some questions concerning the suitability of this approach (Pufulete et al., 2004). Within its last report EEA (2015) reported that benzo[a]pyrene pollution was significant and widespread in EU, particularly in central and eastern countries. In 2013, about 25% of the urban population from the European 28 member states was exposed to benzo[a]pyrene levels above the target value of 1 ng/m^3 (EEA 2015). The existent Air Quality Directive (Directive 2004/107/EC) prescribes that benzo[a]pyrene levels should be made in the PM_{10} fraction, however since benzo[a]pyrene is present in $\text{PM}_{2.5}$ and fractions of lower size, data available in any PM fraction is relevant. In that regard the evaluation of PAHs in the smallest particulate fraction, such as $\text{PM}_{2.5}$ and PM_{1} , is health–relevant because of the ability of these particles to deposit deeply in the respiratory airways; the epidemiological evidence indicates that these smaller particles may have a greater potency in causing adverse health effects than larger particles (Kim et al., 2015). Despite no IAQ guideline being defined for benzo[a]pyrene or PAHs, WHO included PAHs and naphthalene as priority indoor air pollutants (WHO 2010) and defined an annual naphthalene exposure limit of $10 \text{ } \mu\text{g/m}^3$ (Table 2.1, WHO 2010). In the last report, WHO (2010) also stated

that no threshold could be defined for PAHs, pointing out that all indoor exposures are considered relevant to human health.

PAHs are reported to possess reproductive, developmental, hemato-, cardio-, neuro-, and immuno-toxicities in humans and laboratory animals (ATSDR 1995). PAHs can cause vomiting, dysfunction of respiratory symptoms and during pregnancy they contribute to reduce fetal birth weight and growth (Kamal et al., 2015). However PAH health effects will depend mainly on the extent and concentration of PAHs during exposure, the toxicity of the mixture of PAHs, and the route of exposure (ACGIH 2005). Airborne particulate-bound PAHs cause significant oxidative stress and thus are capable of inducing the synthesis of ROS that promotes cell damage by lipid peroxidation or enzymatic disruption. As a consequence PAHs play an undeniable role in the inflammation induction, contributing to carcinogenicity as well as to worsening of respiratory and cardiovascular diseases (Bae et al., 2010; Kamal et al., 2015; Kim et al., 2013). A growing body of evidence suggests that exposure to benzo[a]pyrene at levels over 1.0 ng/m³ induces DNA damage (WHO 2010). However, these issues need to be further addressed; until now it was not possible to distinguish between the toxic effects of airborne PAHs alone or in combination with particles to which they are bound.

2.4 Polycyclic aromatic hydrocarbon biomarkers

Comprehensive exposure assessment requires measurement of pollutants in external media and of internal dose by biomarkers. Since air monitoring of PAHs is difficult and does not capture total exposure, making development of suitable biological exposure markers is highly desirable. Biomonitoring is defined in the environmental public health field as “the assessment of human exposure to an environmental chemical via the measurement of that chemical, its metabolite(s), or reaction product(s) in human exhaled air, blood, urine, milk, saliva, adipose or other tissue in individuals taken separately but generally taken together to constitute a population” (Cavallo et al., 2009; Franco et al., 2008; Hansen et al., 2008; Needham et al., 2007). Furthermore, WHO defines “biomarker as any substance, structure, or process that can be measured within an organism, or its products, and influences or predicts the incidence of harmful effects or disease” (WHO 2001). Thus, biomarkers are powerful tools that have received the greatest attention in studying populations exposed to chemical contaminants. Biomarkers are classified in three categories: (i) biomarkers of exposure, which involve detection and measurement of an exogenous substance, metabolite, or product of interaction between the xenobiotic, or product of interaction between the

xenobiotic and some molecule or target cell; (ii) biomarkers of effect, including measurement of biochemical and physiological alterations that can be related to the occurrence of disease or harm to health; and (iii) biomarkers of susceptibility, indicative of an organism's inherent or acquired ability to respond to the challenge of exposure to xenobiotics (Franco et al., 2008). Thus biomarkers are relevant indices in human health studies and may be used to identify exposed individuals or groups, quantify the exposure, assess the health risks, or assist in diagnosis of occupational or environmental disease (Aitio et al., 2007).

In nature, PAHs are chemically inert compounds, and they only cause toxicity after being metabolized in the human body. PAHs are absorbed into the human body through the skin, lungs and gastrointestinal track, being distributed by blood route to several tissues, especially the lipophilic ones due to their non polar character (Franco et al., 2008; Kamal el al., 2015; Needham et al., 2007). The lipophilic nature of PAHs strongly contributes to their transport across biologic cell membrane, and thus their toxicity is expected to be tissue specific. It is known that PAHs are primarily metabolized by different enzymes in phase I and II metabolic pathways, which are widely distributed in human organs (nasal tissues, red and white blood cells, platelets, uterus, hair follicle, brain, spleen placenta, liver, lungs or kidneys) (Kamal et al., 2015). Liver presents the highest capacity to metabolize PAHs, followed by lungs, intestinal mucosa, skin and kidneys (Kamal et al., 2015). PAHs metabolism occurs via oxidative pathways to produce a complex mixture of quinines, phenols, dihydrodiols, triols and tetrols in the biological system. The aim of the metabolization is to increase these intermediates polarity to obtain hydrophilic substances in order to expedite their excretion. Hydroxylated metabolites may be biotransformed to phase 2 metabolites by conjugation with glutathione, glucuronide or sulphate conjugated (Kamal et al., 2015; Lewtas 2007; Toriba and Hayakawa 2007).

Figure 2.2 present the chemical structure of the principal OH-PAHs. Pyrene is one of the most abundant hydrocarbons in all PAH mixtures (Cirillo et al., 2006; Gomes et al., 2013; Slezakova et al., 2009, 2010) and about 90% of the total urinary excretion of pyrene is in the form of 1-hydroxypyrene (1OHPy) (Tsai et al., 2003) making this metabolite the most widely used biological indicator of internal dose of exposure to PAHs. 3-hydroxybenzo[a]pyrene (3OHB[a]P) is the main metabolite of the known human carcinogenic benzo[a]pyrene and is more representative of PAHs carcinogenic risk than 1OHPy (Lafontaine et al., 2006). Acenaphthene, fluorene, and phenanthrene are common PAHs in different matrices (Cirillo et al., 2006; Gomes et al., 2013; Slezakova et al., 2009; Wilson et al., 2003) and their major urinary metabolites are 1-hydroxyacenaphthene (1OHAc), 2-hydroxyfluorene (2OHFlu), and 1-

hydroxyphenanthrene (1OHPhen), respectively. Concerning naphthalene, among more than thirty identified metabolites, 1-hydroxynaphthalene (1OHNaph) and 2-hydroxynaphthalene are the main ones (Li et al., 2012). It is worth to mention that high molecular weight PAHs may react with DNA to form depurinating adducts resulting 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, initiating a multistage process leading to genetic effects, including cancer, cardiovascular damage, and adverse reproductive outcomes (Kamal et al., 2015; Lewtas 2007).

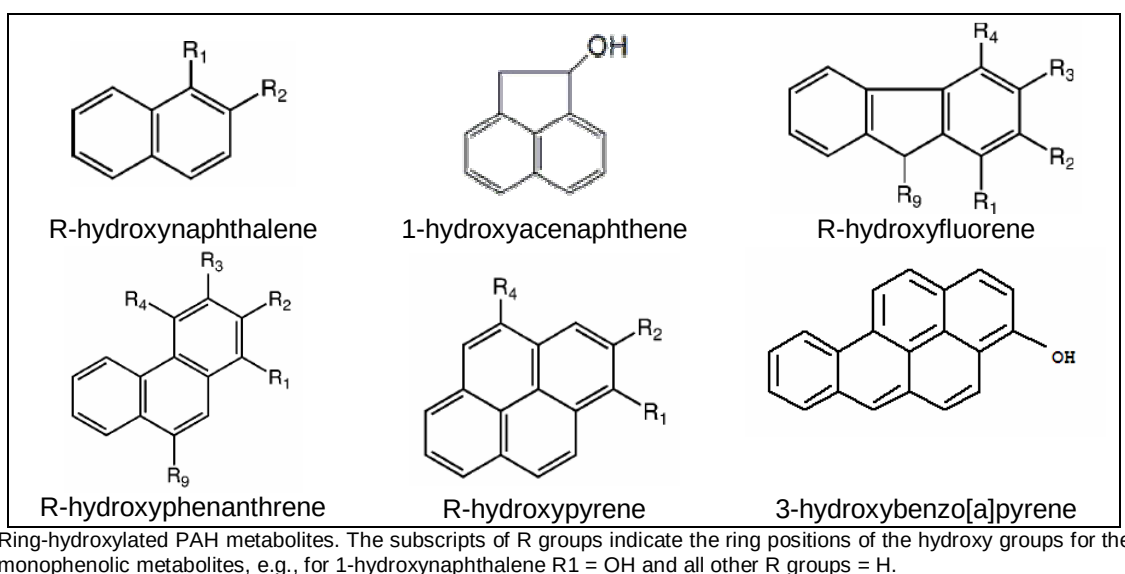


Figure 2.2. Molecular structures of the most common monohydroxyl-PAHs.

Bile, faeces, urine, milk, and exhaled breath condensate are the principal elimination routes of PAHs and/or their metabolites in human organisms (Franco et al., 2008; Gube et al., 2010; Jongeenelen 2001; Likhachev et al., 1992; Rey-Salgueiro et al., 2009). Among the many different elimination routes of OH-PAHs, urine is the easiest, cheapest and less invasive matrix to determine biomarkers of exposure. Globally OH-PAHs are excreted in human urine as free hydroxylated metabolites in free extent and mostly conjugated with sulfuric and/or glucuronic acids or with glutathione (Toriba and Hayakawa 2007). Thus urinary OH-PAHs reflect a more accurate estimation of the actual PAHs intake compared to environmental exposure because they estimate the internal dose. Data obtained from urinary biomarkers of exposure should be combined with information reached by environmental monitoring since a more comprehensive assessment can be attained.

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**PART I – CHILDREN ENVIRONMENTAL EXPOSURE AT
SCHOOLS**

**CHAPTER 3 – ASSESSMENT OF AIR QUALITY IN PRESCHOOL
ENVIRONMENTS WITH EMPHASIS ON ELEMENTAL
COMPOSITION OF PARTICULATE MATTER AND POTENTIAL
HEALTH RISKS**

Chapter 3. Assessment of air quality in preschool environments with emphasis on elemental composition of particulate matter and potential health risks

This study evaluated concentrations of main air pollutants in a Portuguese preschool (indoors/outdoors) environment, with emphasis on elemental characterization of different PM fractions, and estimated risks for the pupils (aged 3 to 5 years). With exception to total volatile organic compounds (TVOCs), levels of PM₁₀, PM_{2.5}, carbon monoxide (CO) and dioxide (CO₂), and formaldehyde were below legislative guidelines. Calcium (Ca), sodium (Na), aluminium (Al), and potassium (K) were the most abundant elements in indoor PM (82–84% of the analysed content) resulting mainly from crustal sources. Carcinogenic elements (1–2% of the indoor analysed content) were mostly PM_{2.5}-bound (83–91%). Indoor-to-outdoor (I/O) ratios of individual elements indicated contributions of indoor origin and from penetration of outdoor emissions indoors; trace metals were associated with ambient anthropogenic emissions (namely traffic). Non-carcinogenic and carcinogenic risks from overall preschool exposure were acceptable for children; for adults carcinogenic risks exceeded (4–11 times) the USEPA recommend value of 10⁻⁶ being 8–40 times higher than for children.

3.1 Introduction

Children are particularly vulnerable to air pollution because they breathe more rapidly than adults, and thus they inhaled more pollutants per kilogram of body weight (Gilliland 2009; Pohl and Abadin 2008). In the first years of life, children spent much of their time indoors, either at homes or at educational settings; the latter represents especially important microenvironment for child exposure due the large amount of time spent there on a weekly basis (Lupoli et al., 2009; Slezakova et al., 2015; Yang et al., 2009). Children can be exposed to high levels of pollutants indoors, either due to the poor building constructions and/or maintenance or lack of proper hygiene procedures (i.e., poor cleaning, ventilation) (Annesi-Maesano et al., 2013; Morawska et al., 2013). Several studies have been conducted during the last decade, which demonstrated the importance of IAQ on children cognitive development, performance, and school attendance (Annavarapu and Kathi 2015; Haverinen-Shaughnessy et al., 2015; Mendell and Heath 2005; Mohai et al., 2011; Sunyer et al., 2015). Associations between poor indoor air and increase risk of allergic sensitization, irritation phenomena, acute and chronic respiratory disorders, and lung function impairment in infants or children have been demonstrated (Annesi-Maesano et al., 2013; Deng et al., 2015; Hulin et al., 2012; Mendel 2007). Furthermore, exposure to genotoxic carcinogenic compounds at a young age may represent further health risks, i.e. causing genetic damage (mutation, sister chromatid exchanges and other genetic disruption) (Merlo et al., 2007; Neri et al., 2006; Wigle et al., 2007) that may increase the risk of cancer later in life (Carpente et al., 2013; Vedham et al., 2015). Some studies show that health impact of air pollutants is much higher on pupils than on adults in similar environments (Guo et al., 2010). Thus, children are more susceptible to indoor pollution (Alves et al., 2014).

With regard to children exposure to PM, much of the current research is focused on fine fraction, PM_{2.5} due to its ability to penetrate deep into the human lungs. However, since children also tend to breathe through their mouths, both coarse and fine fractions should be included in order to correctly assess the respective health risks in children. Table 3.1 presents the published studies during 2003 to 2014 (organized by geographical region) on PM in indoor and outdoor school environments, specifically reporting PM₁₀, PM_{2.5} and PM₁ levels. Regarding PM₁ scarce information is available (Table 3.1).

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments.

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Europe	Austria, Linz	Elementary school	164	7-10				23.1 ± 20.1	14.6 ± 10.8	10.8 ± 9.3	Moshhammer and Neuberger (2003)
	Austria	Elementary schools	596	6-10	29 [#]	12 [#]					Wallner et al. (2012)
	Belgium, Antwerp	Primary schools		6-14							Stranger et al. (2008)
		Urban									
		Suburban									
	Belgium, Antwerp	Primary schools									
	Belgium, Antwerp	Primary schools									Stranger et al. (2007)
	Czech Republic, Prague	Elementary school gym									Braniš et al. (2009)
	Denmark, France, Italy, Norway, Sweden				112 ± 69			63.6			Simoni et al. (2010)
	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims	Primary schools	4901	9-11				18.0 - 23.8			Pénard-Morand et al. (2005)
	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims	Primary school	5338	9-11					8.7 - 23.0		Annesi-Maesano et al. (2007)
	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims	Primary schools	6683	9-11				21.5 ± 3.6			Pénard-Morand et al. (2006, 2010)
	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims	Primary schools	149	9-11			8.9; 13.4		6.1; 12.2		Flamant-Hulin et al. (2010)

n.d. – not detected

[#] Median values;

* Average concentration ± confidence limit at 95%;

** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Europe	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims	Primary schools	7780	9-10		(12.2 - 17.5)**					Annesi-Maesano et al. (2012)
	France, Bordeaux, Clermont-Ferrand, Créteil, Marseille, Strasbourg, Reims		9315	9-11		15.7 ± 7.1					Banerjee and Annesi-Maesano (2012)
	Greece, Athens	Primary schools			229 ± 182 (14.9 - 166)	82.0 ± 56.0 (3.2 - 31.3)		166 ± 133	56.0 ± 26.0		Diapouli et al. (2008)
	Greece, Athens						(0.7 - 9.0)				Dorizas et al. (2013)
	Germany, Munich	Primary and secondary schools			105; 71.7	23.0; 13.5					Fromme et al. (2007)
	Germany, Munich	Elementary schools			117 ± 48			21 ± 15			Oeder et al. (2012)
	Germany, Munich	Primary school			118 [#]	37.4 [#]		24.2 [#]	17.0 [#]		Fromme et al. (2008)
	Italy, Burcei		214	6-14				24.5			Rusconi et al. (2011)
	Italy, Milan	Elementary and secondary schools			(130 - 136)	(26.7 - 39.0)	(16.3 - 23.7)		(30.9 - 60.5)		Rovelli et al. (2014)
	Italy, Rome	Elementary schoolchildren Summer Winter	5	8-11		16 ± 8 20 ± 13			14 ± 5 33 ± 21		Gatto et al. (2013)
	Poland, Dublin	Secondary schools			(39 - 191)	(23 - 119)	(22 - 118)				Polednik (2013)
	Poland, Upper Silesia	Nursery schools	360			73.9 (53.1 - 96.7)			38.4 (19.4 - 66.4)		Mainka et al. (2015)
	Portugal, Bragança	Nursery schools Urban Rural		<1-6	(34.3 - 45.0) (12.1 - 58.3)	(24.7 - 30.1) (9.03 - 31.7)	(24.4 - 29.8) (9.0 - 31.0)				Nunes et al. (2015)
	Portugal, Aveiro	Elementary schools City center Suburban			49.2 ± 9.46 72.8 ± 28.8			23.4 ± 10.3 43.4 ± 11.5			Pegas et al. (2012)

n.d. – not detected

[#] Median values;

* Average concentration ± confidence limit at 95%;

** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Europe	Portugal, Aveiro	Elementary schools and a kindergarten			(62.2 - 362)	(44 - 117)			(37 - 42)		Alves et al. (2013)
	Portugal, Aveiro	Primary school, city center		107				36			Alves et al. (2014)
	Portugal, Coimbra	Elementary schools									Ferreira and Cardoso (2013)
		Autumn/winter			110 ± 35	80 ± 34		80 ± 33	70 ± 28		
		Spring/summer			100 ± 24	70 ± 23		120 ± 59	90 ± 28		
	Portugal, Coimbra	Elementary schools									Ferreira and Cardoso (2014)
		Fall/winter		5-10	120 ± 50	80 ± 40					
		Spring/summer			110 ± 30	100 ± 30					
	Portugal, Lisbon	Primary schools	1175	5-10	73	10		(24.9 - 56.5)	(9.4 - 30.8)		Freitas et al. (2009)
	Portugal, Lisbon	Primary schools						(8 - 47)	(3 - 10)		Almeida et al. (2011)
	Portugal, Maia	Primary schools			140 ± 70			120 ± 130			Macedo et al. (2013)
	Portugal, Porto	Primary schools			140 ± 109	95 ± 60	91 ± 59	125 ± 73	115 ± 68	114 ± 68	Madureira et al. (2012)
	Portugal, Porto	Urban nurseries		3-5	(22.3 - 56.8)	(18.2 - 48.9)	(16.8 - 47.8)				Branco et al. (2014)
	Serbia	Primary school			70.6 ± 19.8	43.6 ± 12.9		40.5	26.5		Jovanović et al. (2014)
	Spain, Barcelona	Primary schools				(7 - 105)			(1 - 192)		Amato et al. (2014)
	Sweden, Stockholm	Schools and preschools		6-11		8 (3.3 - 19.0)			8 (3.2 - 26)		Molnár et al. (2007); Witchmann et al. (2010)
	The Netherlands, Beusichem	Primary school	34	11-12	33			86	41		Scheepers et al. (2012)
	The Netherlands, Utrecht	Schools located near busy roads	54	10-12					19		Van Roosbroeck et al. (2007)

n.d. – not detected

Median values;

* Average concentration ± confidence limit at 95%;

** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Europe	The Netherlands	Schools located near busy motorways		7-12				20.5 ± 2.20			Janssen et al. (2003)
	Turkey, Eskişehir	Primary schools									Demircigil et al. (2014)
		Winter						45.7 ± 17.2			
		Urban						47.6 ± 17.1			
		Suburban									
		Summer						75.0 ± 17.3	25.2 ± 6.8		Ekmekcioglu and Keskin (2007)
		Urban						85.9 ± 22.6	22.6 ± 7.1		
		Suburban									
	Turkey, Istanbul	Elementary schools			221 ± 11.1 (132 - 290)	70.9 ± 3.6 (45.6 - 95.2)		24 - 130			
Africa	United Kingdom, Kent	Primary schools									Peacock et al. (2003)
		Rural	179	7-13				18.7 ± 11.3			Reddy et al. (2012)
								21.2 ± 11.5			
								18.4 ± 9.8			
		Urban						22.7 ± 10.6			
Asia	South Africa, Durban	Primary schools	129	9-11				86.8 ± 1.1			Naidoo et al. (2013)
	South Africa, Durban	Primary schools	422	9-11	48.5 ± 35.4 51.3 ± 28.0	20.0 ± 23.6 20.7 ± 15.0					
	China, Ala Shan		120	9-12				409 ± 217	242 ± 135		
	China, Beijing							279 ± 80.9	147 ± 66.0		
	China, Guangzhou	Primary schools	1983	9-11							He et al. (2010)
		High polluted area						96.1			Zhang et al. (2014)
		Moderately polluted area						80.3			
		Low polluted area						80.0			
	China, Taiyuan	Junior high schools	2134	11-15	118 ± 103			188 ± 220			
	China, Taiwan	Middle schools	331686	12-14				69.2			Lee et al. (2003)

n.d. – not detected

Median values

* Average concentration ± confidence limit at 95%;

** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Asia	China, Taiwan, Taipei	Elementary and middle schools	32143	6-15				55.6 ± 16.6			Hwang et al. (2005, 2006)
	China, Taiwan, Taipei	Elementary and middle schools	4221	8-14				40.7	28.2		Chen et al. (2011)
	China, Taiwan, Taipei	Elementary and middle schools	100	8-13				39.0	25.1		Chen B-Y et al. (2012)
	China, Taiwan, Taipei	Junior high schools	2919	12-16				(35.3 - 98.3)			Chang et al. (2012)
	China, Taiwan				(81 - 97)	(30 - 36)					Hsu et al. (2012)
	India, Delhi	School located in proximity of industrial and heavy traffic roads						222 ± 111			Jyethi et al. (2014)
	Iran, Sari	Primary schools			401 (266 - 568)	46.6 (29.1 - 69.1)	17.6 (12.1 - 28.9)		36.9 (15.5 - 116)		Mohammadyan and Shabankhani (2013)
	Korea, Choongnam				46 (6.2 - 135)						Son et al. (2006)
	Korea, Incheon	Elementary school	41	8-12				74.0 ± 36.5 (15.6 - 182)	57.8 ± 27.5 (12.7 - 131)	50.8 ± 25.1 (10.9 - 115.8)	Song et al. (2011)
	Korea, Jeju							31.0 ± 9.4	15.3 ± 4.5		Bae et al. (2010)
	Korea, Seoul							52.9 ± 27.1	35.9 ± 23.5		Yang et al. (2009)
	Korea	Kindergarten Elementary school Middle school High school Classrooms Laboratory room Computer room kindergarten			94.4 ± 50.1 77.9 ± 46.3 83.5 ± 50.8 86.0 ± 43.9 107 ± 90.6 71.1 ± 51.8 77.9 ± 68.9 94.9 ± 50.1						Kabir et al. (2012)

n.d. – not detected

Median values;

* Average concentration ± confidence limit at 95%;

** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
Asia	Korea, Seoul, Daejeon, Incheon, Sihung	Elementary schools Residential area Traffic-pollution area Complex-pollution area	4545					(53.8 - 67.9) (39.8 - 55.8) (50.0 - 51.9)			Kim et al. (2013)
	Malaysia	Primary schools	1952	10-11							Zainal Abidin et al. (2014)
		Urban Semi-rural				19.8 ± 6.9 23.8 ± 5.4			22.5 ± 9.2 28.2 ± 4.7		
	Pakistan, Lahore	School located at a low pollution area School located at a high pollution area	179	8-12	223 ± 119 591 ± 219	29.1 ± 15.1 163 ± 61.6	8.4 ± 5.5 52.7 ± 12.1	223 ± 93.5 729 ± 53.5	28.5 ± 10.3 183 ± 30.2	7.3 ± 5.3 58.2 ± 9.6	Sughis et al. (2012)
	Pakistan, Lahore				(54.8 - 294)	(154 - 1433)					Yousaf and Khan (2013)
North America	Palestine, Gaza				349 ± 197	104 ± 85.0		149 ± 98.3	60.5 ± 50.7		Elbayoumi et al. (2013)
	USA, Montana	Elementary school Middle school				(14.2 - 54.0) (4.6 - 10.8)					Ward et al. (2007)
	USA, New York								27.5 ± 19.0		Richmond-Bryant et al. (2009)
	USA, South Texas					(2.8 - 7.9)			(6.5 - 23.2)		Zhang and Zhu (2012)
	USA, Texas	Elementary schools			(15 - 37)	(8.2 - 15)		(21 - 35)	(8.4 - 14)		Raysoni et al. (2013)
	USA, Texas, El Paso	Elementary schools Low exposure zone High exposure zone	38	6-12	(8.3 - 33) (6.5 - 100) (3.7 - 37)	(3.4 - 17) (3.7 - 37)		(5.9 - 100) (15 - 120) (5.9 - 38)	(3.9 - 30) (5.9 - 38)		Li et al. (2011)
	USA, Texas, El Paso	Light traffic zone Heavy traffic zone	29	6-12	17.2 ± 8.0 22.1 ± 21.2	7.6 ± 3.6 10.2 ± 8.0		18.8 ± 11.0 41.0 ± 22.3	8.8 ± 5.0 15.6 ± 9.5		Samat et al. (2012)

n.d. – not detected
Median values;

* Average concentration ± confidence limit at 95%;
** Values represent the range of 25-75% percentiles.

Table 3.1. PM₁₀, PM_{2.5} and PM₁ levels (mean ± SD or range, expressed as µg/m³ unless indicated otherwise) reported for indoor and outdoor school environments (continued).

Continent	Country, city	Note	Children n	age	Indoor pollutants			Outdoor pollutants			Reference
					PM ₁₀	PM _{2.5}	PM ₁	PM ₁₀	PM _{2.5}	PM ₁	
North America	USA, Texas, El Paso	Elementary schools	36	6-11				35.2 ± 12.1 (16.7 - 66.1)	13.8 ± 4.4 (7.1 - 24.9)		Zora et al. (2013)
		High-traffic area						20.4 ± 14.9 (6.6 - 59.3)	9.9 ± 4.8 (4.0 - 18.5)		
		Low-traffic area									
Central America	Mexico, Iztapalapa, Iztacalco, Netzahualcoyotl		95	6-14					25.5 ± 13.7		Barraza-Villarreal et al. (2011)
			196	6-12							
			29	6-12	68.2 ± 37.1 47.1 ± 17.9	26.7 ± 16.2 20.2 ± 8.5		87.7 ± 30.3 54.8 ± 25.3	31.1 ± 14.0 20.4 ± 9.9		
South America	Brazil, Mato Grosso		309	6-15					24.3 ± 19.2		Jacobson et al. (2012)
			1076	10-14					17.1 ± 7.4		
			4064	13-14				124 35			
Oceania	Brazil, Rio Janeiro	Duque de Caxias Seropédica	118	6-15				84.7 ± 29.5 (55 - 91)			Castro et al. (2009) Franco et al. (2009) Zhang et al. (2006)
Oceania	Australia	Primary schools			(21.8 - 27.1) (24.9 - 34.0)						Guo et al. (2010) Cavanagh et al. (2007)
		Winter									
		Summer									
Oceania	Australia, Queensland, New Zealand, Christchurch	Primary schools	89	12-18	(7 - 64)	6.7 ± 0.2			11.6 ± 0.8		Guo et al. (2010) Cavanagh et al. (2007)

n.d. – not detected

* Median values

** Average concentration ± confidence limit at 95%.

*** Values represent the range of 25-75% percentiles.

However, fewer studies focused on assessment of PM concentrations in preschool environments (i.e. children 3–5 years old; Mainka et al., 2015; Molnár et al., 2007; Nunes et al., 2015; Yang et al., 2009; Wichmann et al., 2010); yet preschools represent the first place for child social activity. The majority of the studies were conducted in Europe (52%) and Asia (26%) whereas in North (USA, 7%), Central (Mexico, 3%) and South America (Brazil and Colombia, 6%) there were a total of 16% of the studies; Africa and Oceania are the continents with the least information on PM in schools (6%).

Among all the studies that concerns schoolchildren exposure to PM, 84% exceeded the defined PM_{10} ($50 \mu g/m^3$; 8-h mean) guideline for the indoor air (Portuguese Regulation 2013). Only few studies reported indoor PM_{10} concentrations lower than $50 \mu g/m^3$; four studies performed in European (Nunes et al., 2015; Pegas et al., 2012; Scheepers et al., 2012; Wallner et al., 2012), and in American countries (Li et al., 2011; Sarnat et al., 2012; Raysoni et al., 2013), one study conducted in Asian (Son et al., 2005) and in Australian schools (Zhang et al., 2006).

A similar trend (65% of exceedances of the indicated $PM_{2.5}$ guideline, $25 \mu g/m^3$; 8-h mean; (Portuguese Regulation 2013) was observed for $PM_{2.5}$ concentrations in the indoor air of the majority of European [except in some French (Annesi-Maesano et al., 2012; Flamant-Hulin et al., 2010; Banarjee and Annesi-Maesano 2012), Austrian (Wallner et al., 2012), German (Fromme et al., 2007), Italian (Gatto et al., 2013); Portuguese (Almeida et al., 2011) and Swedish (Molnár et al., 2007; Wichmann et al., 2010 schools], and American schools [except for schools located in Montana (Ward et al., 2007) and Texas (Li et al., 2011; Raysoni et al., 2013; Sarnat et al., 2012; Zhang and Zhu 2012)]. Regarding Asian countries only one study fulfilled the guideline of $25 \mu g/m^3$ (Zainal Abidin et al., 2014 for Malaysian primary schools).

It is noteworthy that among all the studies that presented PM_{10} levels for the outdoor air (Table 3.1), 89% studies reported concentration ranges lower than $150 \mu g/m^3$ (i.e. the USEPA 24-h limit; Table 2.1). In addition, the levels retrieved from 41% of the studies were lower than $50 \mu g/m^3$, i.e. the PM_{10} guideline value for 24-h set by WHO (Table 3.1). Concerning levels of $PM_{2.5}$ outdoors, 60% of the studies reported concentrations that were predominantly lower than the existent guidelines of 25 and $35 \mu g/m^3$, defined by WHO (2006) and USEPA (2010), respectively. Among the 40% of studies that exceeded those limits, 60% (Stranger et al., 2007, 2008; Diapouli et al., 2008; Rovelli et al., 2014; Alves et al., 2013; Ferreira and Cardoso 2013; Madureira et al., 2012; Amato et al., 2014; Scheepers et al., 2012) were European, 34% were Asian (Bae et al., 2010 in Seoul, Korea and China; Moshammadyan and Shabankhan 2013;

Song et al., 2011; Sughis et al., 2012 in the high pollution area; Elbayoumi et al., 2013) and the remaining 6% characterized American schools (Li et al., 2011 in schools located in a high exposure zone).

Occupancy through re-suspension of deposited PM and possible particle generation strongly influences the PM levels measured indoors. Oeder et al. (2012) found that PM_{10} concentrations during teaching hours were 5 to 6 times higher than outdoors. In addition, early morning and late afternoon $PM_{2.5}$ peaks are usually observed on weekdays, which are consistent with traffic rush hours and revealing the predominant effect of vehicular emissions (Guo et al., 2010) in the indoor PM levels. Almost 46% of the studies regarding school environments present indoor PM_{10} and/or $PM_{2.5}$ levels higher than the respective outdoor concentrations. These evidences are more pronounced in European countries probably because of the higher number of studies available for this continent during the last ten years. To support these facts a higher number of studies, particularly from Africa, America, and Oceania continents are needed. Madureira et al. (2012) and Sughis et al. (2012) are the only studies that reported simultaneously PM_{10} , $PM_{2.5}$ and PM_1 concentrations in school outdoor and indoor air. Thus more studies including measurements in the indoor and outdoors air of school microenvironments as well as the inclusion of PM_1 concentrations are also needed in order to better understand the relationship between PM_{10} , $PM_{2.5}$ and PM_1 indoors. It is also necessary to point out that the studies from Asian schools (as well as from South America) were predominantly focused on analyzing the PM levels in outdoor air of school microenvironments (such as playgrounds, school vicinity, and surroundings), and did not consider the many indoor sources that may be present in the indoor areas. Therefore, more information on PM levels of school microenvironments is necessary in order to better understand the respective exposure of children.

Over the last decade, the information concerning elemental composition of PM in preschools is still almost inexistent. Up to this date only two studies reported limited data on chemical properties of $PM_{2.5}$ in preschools omitting information on coarse fractions or further elemental composition (Mainka et al., 2015; Molnár et al., 2007). Molnár et al. (2007) assessed the indoor and outdoor concentrations of 13 $PM_{2.5}$ trace elements at 40 sampling sites, including ten classrooms in five schools and ten preschools located in Stockholm, Sweden. Most of the elements showed lower levels in the indoor areas comparatively to the outdoors ones, including the carcinogens Ni and Pb. Mainka and coworkers (2015) also evaluated the levels of $PM_{2.5}$ and the

composition on 11 trace elements at naturally ventilated preschools located in the area of Gliwice, Poland. Four nurseries were selected in order to characterize areas with different degrees of urbanization and traffic densities. Fe, Cr and Zn were the most abundant elements at the urban and rural nurseries, accounting for over 85% of total elements. Mainka et al. (2015) observed that Fe was the predominant element in the indoor and outdoor air of both urban and rural nurseries during the winter season, while during the spring Cr was the dominant element at rural nurseries. These findings were attributed to the soil particles that can be tracked back on shoes or clothing from the outdoors since Fe is the major element in the Earth's crust. Regarding Cr, soil re-suspension was reported as the most influential source. However, authors also suggested emissions from sewage sludge dumping on agricultural fields as probable sources since it took place at the rural sites during the sampling campaigns (Mainka et al., 2015). No assessment of the potential health risks was performed in the previous studies.

The main aims of this study were to investigate levels and elemental composition of particulate matter (both PM_{10} and $PM_{2.5}$) at a preschool environment, and to assess non-carcinogenic and carcinogenic risks from overall preschool exposure. Indoor and outdoor PM elemental compositions were characterized and the impacts of outdoor emissions to indoor environment were assessed. Levels of gaseous pollutants, namely TVOCs, CO, CO_2 , ozone (O_3), and formaldehyde were also concurrently measured in ambient air and indoors in order to better characterize the air quality of the studied preschool.

3.2 Material and Methods

3.2.1 Sampling site characterization

PM₁₀ and PM_{2.5} were continuously collected in indoor and outdoor air during 7 weeks of May–July 2013 at a Portuguese preschool. Preschool is the first program for child learning and social development (typical enrollment between the ages of three and five) being voluntary attended before the obligatory school education (from 6 years onwards). The studied preschool was located in the zone of Paranhos in Oporto city (2nd largest metropolitan area, situated in north of the country). Some sources of local air pollution were identified in the surrounding area of the preschool: vehicle traffic emissions (Slezakova et al., 2013a, 2013b), petrochemical complex that includes an oil refinery, an international shipping port, an incineration unit and a power plant (Pereira et al., 2007). The selected preschool was founded in 1940 and enrolled 173 children with 3–5 years. Further details regarding the studied place are in Table 3.2.

3.2.2 Sample collection

Both PM fractions were collected each day (from Mondays to Fridays) using the 24 h sampling duration. Indoors the collection of PM samples was performed in a room which was representative of the different types of activities carried out by the children in the preschool. It was continuously occupied during the days, by different students, and used for education, playing and entertainment, and also for exercising and physical education. Figure 3.1 and Table 3.2 show further details of this indoor environment.



Figure 3.1. Characterization of the indoor environment at the sampled preschool.

Table 3.2. Characteristics of the studied preschool and the indoor environment.

Preschool	
Location	Private establishment
	Built in 1940, two floors building
	173 enrolled students: 3–5 years old
Traffic density	Urban - traffic
	Situated on moderately trafficked streets
	Mean:16 cars min ⁻¹ Peak hours: 8:30h (27 cars min ⁻¹) 18:30h (25 cars min ⁻¹)
Room characteristics	
Room height (m)	2.90
Floor surface (m ²)	101
Volume (m ³)	292
Heating system	Non-existent
Main materials	Concrete, plastic and wood
Floor coating	Plastic (PVC)
Wall coating	White paint
Ceiling coating	White paint
Door area (m ²)	4.1
Type of door	Double, wood and glass
Doors number	2
Window areas (m ²)	5.7
Type of window	Single glass layer, wood
Windows number	10
Ventilation	
Throughout day	as occupants thought necessary
Morning (8.00 a.m.)	15–20 min
After classes (6.00 p.m.)	15–20 min
	Doors maintained always closed during the educational activities (classes) and physical activities;
	room always opened during recesses and periods with recreational activities

PM masses were collected on polytetrafluoroethylene (PTFE) membrane disks (Ø47 mm, SKC Ltd., UK). The sampling setups consisted of low-flow (38.3 L min⁻¹) pumps (models Bravo H2; TCR TECORA, Italy) that were connected to PM EN LVS sampling heads (in accordance with norm EN14907 for PM_{2.5} and EN12341 for PM₁₀). The samplers' orifices were positioned at height of 1.5 m and at least 1 m far from any obstacles or walls. All necessary precautions were taken during the sampling campaign

to provide suitable and safe environment for the respective students. Simultaneously, sampling of both PM fractions was performed in the outdoor schoolyard. In total 138 samples (70 indoor and 68 outdoor ones) were obtained. Outdoor samples were collected in inner yard (approximately 15 m far from the neighboring street). Special attention was given to any obstacles such as vegetation and/or fences which might hamper the PM sampling. The preschool grounds were bordered by fence (4 m high), brick wall (up to 4 m), and in some places combination of both (brick-wall base with fence above). The inner yard had asphalt surface, whereas the playground (surrounded with lower fence approximately 1 m high) and gardens (included bushes and flower beds) consisted of unpaved soil. In addition to sampling, main meteorological parameters were retrieved from the station located approximately 600 m from the preschool: temperature, precipitation, wind speed as well as solar radiation.

Indoor and outdoor sampling of the gaseous pollutants (TVOCs, CO, CO₂ and O₃), temperature (T) and relative humidity (RH) was conducted with a multiparametric probe by (model TG 502; GrayWolf Sensing Solutions, Shelton, USA); equipment precision was daily checked according to manufacturer's instructions. The sampling was done twice to three times (during mornings as well as in the afternoons of each day), for 20 to 30 min long periods (logging interval of 15 s). Additionally, concentrations of formaldehyde in indoor air were recorded by Formaldemeter™ (model htV-M; PPM Technology, Caernarfon, UK) in the same conditions as previously described for the other pollutants.

Throughout whole sampling campaign additional information (such as room occupancy, frequency and type of ventilations, existence of any relevant activity/emissions source) was registered. Furthermore, each day preschool personnel was asked to provide information concerning existence of any emission source and/or unusual event.

3.2.3 PM_{2.5} and PM₁₀ masses

Gravimetric mass determinations of both PM fractions was done in agreement with Slezakova et al. (2014). The steps of gravimetric mass determination were the following: 24 h to equilibrate filters before weighing at room temperature (Mettler Toledo AG245 analytical balance weighing with accuracy of 10 µg) followed by weighing during the following 24–48 h. If the measurements for one sample differed more than 10 µg, they were discarded and the filters were repeatedly weighed until

three reproducible values were obtained. $PM_{2.5-10}$ (coarse fraction; $2.5 \mu m < \text{particle aerodynamic diameter} < 10 \mu m$) was determined by subtracting PM_{10} and $PM_{2.5}$. After sampling filters were stored inside polyethylene containers that were protected with aluminium foil.

3.2.4 Elemental characterization

A set of elements including beryllium (Be), boron (B), Na, magnesium (Mg), Al, phosphorus (P), K, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, selenium (Se), strontium (Sr), rubidium (Rb), molybdenum (Mo), Cd, tin (Sn), antimony (Sb), cesium (Cs), Ba, Pb and four rare earth elements, namely lanthanum (La), cerium (Ce), praseodymium (Pr), and neodymium (Nd) were determined in PM_{10} and $PM_{2.5}$ by inductively coupled plasma mass spectrometry (ICP-MS; X Series, Thermo Scientific, USA) adapted from method of NIOSH 7300 (NIOSH, 2003) and USEPA method 6020 (USEPA 1990) with major modifications. The digestion of the collected PM was performed using mixture of acids (4 ml HNO_3 and 1 ml $HClO_4$) with linear ramp up to $140^\circ C$ in 10 min, and then for another 15 min at $140^\circ C$ by a microwave digestion equipment (MARS-X 1500W; CEM, Mathews, NC, USA). During each set of microwave digestion, a filter blank was included. After digestion, all extracts were diluted to 100 ml solution in ultra-pure water.

Quantitative analysis was conducted using external calibrations that were prepared with at least 6 calibration points by the dilution of multi-element standards (IV-ICP-MS 71B for Mo, Sn, Sb and IN-ICP-MS 71A for the remaining elements; Inorganic Ventures, Christiansburg, Virginia, USA), and using as internal standards indium (In; isotope 115) and terbium (Tb; isotope 157). Each element was analysed at its most abundant isotope, namely ^9Be , ^{11}B , ^{23}Na , ^{24}Mg , ^{27}Al , ^{31}P , ^{39}K , ^{44}Ca , ^{52}Cr , ^{55}Mn , ^{59}Co , ^{60}Ni , ^{65}Cu , ^{66}Zn , ^{75}As , ^{82}Se , ^{88}Sr , ^{85}Rb , ^{95}Mo , ^{111}Cd , ^{118}Sn , ^{121}Sb , ^{133}Cs , ^{137}Ba , ^{206}Pb + ^{207}Pb + ^{208}Pb , ^{139}La , ^{140}Ce , ^{141}Pr , and ^{146}Nd . Limits of detection (LODs) were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 (Miller and Miller 2000). Globally LODs ranged from 0.091 ng m^{-3} (La) to 45.3 ng m^{-3} (P). Analytical precision evaluated as relative standard deviation (RSD) ranged between 1.8 (Mg) and 9.4% (Mo). Calibration standards and blank filters were analysed on regular basis. Each analysis was repeated at least three times.

3.2.5 Enrichment factor analysis

Enrichment factor (EF) of a given element in relation to the earth's crust was calculated to assess anthropogenic or natural type of a metal enrichment. EF was computed for each element using the following equation (3.1):

$$EF = (C_E/C_X)_{\text{Aerosol}} / (C_E/C_X)_{\text{Crust}} \quad (3.1)$$

where $(C_E/C_X)_{\text{Aerosol}}$ represents the ratio between mass concentrations of the elements E and X (reference element) in atmospheric PM, and $(C_E/C_X)_{\text{Crust}}$ is the ratio of the element E to the reference element X in average upper continental crust (Mason 1958). If $EF < 10$, then element E is thought to originate mostly from crustal sources, if $EF > 10$ then E is thought mostly of non-crustal (i.e. man-made) origin (Torfs and Van Grieken 1997); higher EF implies higher contribution from non-crustal sources. Furthermore, factor values in a range between 10 and 100 are regarded as moderately enriched, whereas elements with factors higher than 100 are considered as highly enriched (Latif et al., 2014). Typically, Al (Hsu et al., 2010; Slezakova et al., 2009) or Fe (Buczyńska et al., 2014; Contini et al., 2010; Latif et al., 2014) are used as reference elements assuming their anthropogenic atmospheric contribution as negligible. Still, no criteria are defined to select a specific reference element. Thus aluminum was chosen as a reference element in present work because of its stability to most anthropogenic contaminants (Zhang et al., 2013a) but enrichment method using La as reference element was also applied in order to confirm atmospheric PM origin from natural or anthropogenic emission sources.

3.2.6 Risk estimation

Calculations of non-carcinogenic (THQ) and carcinogenic (TR) risks was done based on USEPA guidelines (USEPA 2015a, 2015b). Table 3.3 summarizes details regarding the exposure (durations, frequencies) and child physiology (weight, rates of inhalation).

Table 3.3. Timetable and child activity patterns: an example for 3–years old children.

Time	Environment	Observed activities	Activity intensity
8:30-9:00	Indoor	Playing (calm, seated, TV)	Sedentary
9:03-10:29	Indoor	Seated only (talking)	Sedentary
10:30-11:15	Playground	Running, jumping, swings	High intensity
11:17-11:40	Indoor	Sedentary and other (painting, walking)	Sedentary
11:45–13:00	Indoor	Seated (eating, drinking, talking)	Light
13:05-15:00	Indoor	Sleeping	Sleep
15:04-16:00	Indoor	Seated, and other	Sedentary
16:00–17:30	Indoor	Organized activities (singing, dancing), running	High intensity

Tables 3.4 – 3.6 encapsulate the default parameters and provide example for the calculations. The following equations were applied to compute THQ and TR (USEPA 2015a):

$$\text{THQ} = (\text{EFr} \times \text{ED} \times \text{ET} \times \text{C} \times \text{IR}) / (\text{RfD} \times \text{BW} \times \text{AT}) \quad (3.2)$$

$$\text{TR} = (\text{EFr} \times \text{ED} \times \text{ET} \times \text{C} \times \text{IUR}) / \text{AT} \quad (3.3)$$

$$\text{RfD} = (\text{RfC} \times \text{IR}_A \times \text{AR}) / (\text{BW}_A \times 100) \quad (3.4)$$

The definition of defaults factors and used values are shown in Tables 3.5 and 3.6, respectively. In order not to over-estimate target risks, all were calculated for the lowest possible exposure durations (1 year for 3–years old pupils, 2 years for 4–5–years old ones).

Table 3.4. Calculation of non–carcinogenic (THQ; dimensionless) and carcinogenic (TR; dimensionless) risks: example for As in indoor PM_{2.5} for 3–years old children.

Parameter		THQ	TR
EFr (days year ⁻¹)	250	1.51×10 ⁻¹	6.59×10 ⁻⁸
ED _{3–years old} (years)	1		
ET _{3–years old} (h day ⁻¹)	7.25 per 24 h, i.e. 0.302		
AT (days)	25 500		
IR _{3–years old} (m ³ day ⁻¹)	8.5		
BW _{3–years old} (kg)	14		
C _{As} (μg m ⁻³)	5.19 ×10 ⁻³		
RfD _{As} (mg kg ⁻¹ day ⁻¹)	4.29×10 ⁻⁶		
IUR _{As} (μg m ⁻³) ⁻¹	4.30×10 ⁻³		

Table 3.5. Standard default factors and values used for different age categories for non-carcinogenic (THQ) and carcinogenic (TR) calculations.

Defaults factor		Children			Adults		
Symbol	Definition	Unit	3 years old	4–5 years old	25–54 years old	55–64 years old	
EF ^r	Exposure frequency	(days year ⁻¹)	250 ^a	250	250	250	
ED ^b	Exposure duration	(years)	1	2	15	40	
ET	Exposure time	(h)	Inside: 7.25 Outside: 0.45	Inside: 6.25 Outside: 1.75	Same as children	Same as children	
IR	Inhalation rate	(m ³ day ⁻¹)	8.5 ^c	10.1 ^c	15.9 ^d	14.9 ^d	
BW	Body weight	(kg)	14 ^e	21 ^e	77 ^f	77 ^f	
AT	Averaging time	(days)	^a THQ calculations: 365 × ED TR calculations: 25500 (365 × 70)				
RfD	Inhalation reference dose	(mg kg ⁻¹ day ⁻¹)	Values are indicated in Table 3.6				
RfC	Reference concentration	(mg m ⁻³)					
IUR	Chronic inhalation unit risk	(μg m ⁻³) ⁻¹					
C	Concentration of given element in air	(μg m ⁻³)	Values in Table 3.8				

Notes:

^aAccording to USEPA (2015);

^bThe lowest possible durations in order to not overestimate the risk values;

^cAdapted from USEPA (2008) based on children activity patterns;

^dAdapted from USEPA (2008);

^eDesignated for light physical activity, (USEPA 2011);

^fAdapted from USEPA (2011);

Table 3.6. Existent chronic inhalation unit risk (IUR), inhalation reference dose (RfD) and reference concentration (RfC) values of eight elements.

Metal	IUR ($\mu\text{g}/\text{m}^3)^{-1}$)	RfC (mg/m^3)	RfD ($\text{mg}/\text{kg}/\text{day}$)
Al	n.a.	5.00×10^{-3}	1.43×10^{-3}
Mn	n.a.	5.00×10^{-5}	1.43×10^{-5}
Ba	n.a.	5.00×10^{-4}	1.43×10^{-4}
Cd	n.a.	2.00×10^{-5}	5.71×10^{-6}
Cr ^(VI)	8.4×10^{-2}	1.00×10^{-4}	2.86×10^{-5}
Ni (refinery dust)	4.8×10^{-4}	5.00×10^{-5}	1.43×10^{-5}
As inorganic	4.3×10^{-3}	1.50×10^{-5}	4.29×10^{-6}
Pb (acetate)	1.2×10^{-5}	n.a.	n.a.

n.a. – not available;
 Note: RfD values can be also converted from existent USEPA RfC values (USEPA 2015a) according to USEPA methodology (USEPA 2015b) using IR (20 m³/day) and BW (70 kg) of an adult.

3.3 Results and Discussion

3.3.1 Indoor and outdoor concentrations of PM and gases

Over the sampling campaign, average PM_{2.5} indoor concentration was 17.6 $\mu\text{g m}^{-3}$ and 26.8 $\mu\text{g m}^{-3}$ for PM₁₀; the corresponding ranges are summarized in Table 3.7. PM_{2.5} and PM₁₀ concentrations were below the limits of 25 $\mu\text{g m}^{-3}$ and 50 $\mu\text{g m}^{-3}$, respectively, established by national regulations for air quality of public buildings in Portugal (Decreto Lei 118/2013; Portuguese Regulation 2013). The corresponding outdoor PM levels were slightly, but not significantly ($p > 0.05$) higher, with ranges of 11.3–36.3 $\mu\text{g m}^{-3}$ (mean concentration 19.3 $\mu\text{g m}^{-3}$) for PM_{2.5} and 27.3–36.9 $\mu\text{g m}^{-3}$ (30.9 $\mu\text{g m}^{-3}$) for PM₁₀; outdoor PM₁₀ fulfilled 24-h target (50 $\mu\text{g m}^{-3}$) settled in Directive 2008/50/EC for ambient air. Furthermore, PM₁₀ was mostly comprised of PM_{2.5}; fine fraction accounted, respectively, for 66% and 62% of indoor and outdoor PM₁₀. Overall, the PM in this work was lower than in other studies from Iberian Peninsula (Moreno et al., 2014; Rivas et al., 2014).

In BREATHE study that evaluated 39 schools, authors reported indoor PM_{2.5} levels 2 times higher, and 1.5 times higher for ambient air (Moreno et al., 2014; Rivas et al., 2014) which might be due to different sample duration (8 *versus* 24 h in this work, i.e. also when classrooms were empty, thus consequently leading to overall lower concentrations). In addition, this work was conducted in summer season when (compared to winter), PM levels also tend to be lower (Rivas et al., 2014).

Table 3.7. Pollutant levels and meteorological conditions at studied preschool.

Mean (Min – Max)	
Indoors	
Pollutants ^a	
($\mu\text{g m}^{-3}$)	PM ₁₀ : 26.8 (15.9 – 35.7) PM _{2.5} : 17.6 (11.3 – 26.6) Formaldehyde: 26.4 (n.d.– 61.4) O ₃ : 119 (20.0 – 230)
(mg m^{-3})	TVOCs: 1.98 (n.d. – 3.91) CO ₂ : 1252 (796 – 3605) CO: 0.77 (0.23 – 2.73)
Meteorological parameters ^b	T: 25.4 °C (21.8 – 32.0) RH: 42.7% (19.6 – 71.2)
Outdoors	
Pollutants ^a	
($\mu\text{g m}^{-3}$)	PM ₁₀ : 30.9 (27.3 – 36.9) PM _{2.5} : 19.3 (11.3 – 36.3) Formaldehyde: – (n.d.) O ₃ : 188 (20 – 401)
(mg m^{-3})	TVOCs: 1.37 (3.92×10^{-2} – 3.00) CO ₂ : 817 (688 – 1100) CO: 1.08 (n.d. – 4.10)
Meteorological parameters	T: 25.6 °C (17.3 – 36.1) RH: 73.0% (17.0 – 89.6) Precipitation: 18 days; 3.6 mm (0.0 – 53) Atmospheric pressure: 1017.0 hPa (1002.4 – 1024.9)

^aTotal volatile organic compounds (TVOCs); carbon dioxide (CO₂); carbon monoxide (CO); ozone (O₃).

^bTemperature (T); relative humidity (RH).

n.d. – not detected.

As expected, indoor levels of CO₂ and TVOCs (Table 3.7) were significantly higher than the outdoor ones ($p < 0.001$) (Table 3.7). The indoor means of CO₂ and formaldehyde fulfilled the limits (CO₂: 2250 mg m^{-3} ; formaldehyde: 100 $\mu\text{g m}^{-3}$) established for air quality of Portuguese public buildings (Decreto Lei 118/2013; Portuguese Regulation 2013). Formaldehyde probably resulted from used indoor materials (such as carpeting and paints; Jovanović et al., 2014) whereas indoor CO₂ levels were caused by the limited ventilation conducted at the preschool (Table 3.2) combined with room occupancy; indoor CO₂ levels were approximately 1.5 times higher than outdoor ones. For TVOCs the indoor concentrations highly exceeded (approximately 3.3 times) the limit value of 600 $\mu\text{g m}^{-3}$ (Decreto Lei 118/2013; Portuguese Regulation 2013), potentially due to the emissions from cleaning agents, materials for the room construction and surface coatings (Table 3.2) and from items (such as dyes, solvents, and adhesives) for children activities (Annesi-Maesano et al., 2013; Guo et al., 2004; Su et al., 2013). The indoor levels of CO also met the legislation standard (10 mg m^{-3} ; Decreto Lei 118/2013; Portuguese Regulation 2013)

but unlike PM, indoor levels of CO and O₃ were significantly lower ($p < 0.05$; 1.4 and 1.6 times for CO and O₃, respectively) than outdoor ones. Since there was no indoor source of CO such gas appliances or heaters at the sampled room (Table 3.2) and smoking was prohibited (for parents and school staff) at the premises of the preschool, the indoor CO probably resulted from transfer of outdoor air (Annesi-Maesano et al., 2013). O₃ (no limit value in indoor air exists according to Decreto Lei 118/2013; Portuguese Regulation 2013) which is formed during a complex photochemical reactions that includes solar radiation, anthropogenic and natural gaseous precursors (namely nitrogen monoxide (NO) and dioxide (NO₂) and volatile organic compounds. The main sources of O₃ in school environment include electronic equipment, namely old printers and photocopy machines, and air humidifiers, none of which were registered in the studied preschool (Table 3.2); its presence indoors was probably caused by infiltration of outdoor air. In agreement, Uchiyama et al. (2015) reported much lower levels of O₃ in indoor air of dwellings than outdoors; those authors suggested a quick decomposition of O₃ from outdoor emissions due chemical compounds present in indoor air.

3.3.2 PM elemental concentrations

Elemental characterization of B, Na, Mg, Al, P, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Rb, Cd, Sn, Sb, Ba, La, Ce, Nd, Pr, and Pb were performed in both indoor and outdoor PM_{2.5} and PM₁₀ fractions; the levels of Be, Se, Mo, and Cs were below the LODs in 100% of the analysed samples. The mean concentrations and the respective ranges of the elements in indoor and outdoor PM₁₀ and PM_{2.5} of studied preschool are shown in Table 3.8.

For indoor PM, the compositional profiles were similar for both fractions. Out of 26 elements, Ca, Na, Al, and K were the most abundant in both PM fractions, composing 82 and 84% of the total sum of elements (ΣE) in PM_{2.5} and PM₁₀, respectively. Specifically, Ca and Na were the most abundant in PM₁₀ (30% and 25% of ΣE , respectively); the abundances of K (18% of ΣE) and Al (11%) were lower; in PM_{2.5} Na and Ca accounted for 24% and 20% of ΣE , whereas Al and K contributed 21% and 17% of ΣE , respectively. According to Wang et al. (2006) these four elements can be considered as earth crust elements.

Table 3.8. Concentrations of elements in PM₁₀ and PM_{2.5} in indoor and outdoor air at the studied preschool (ng m⁻³).

Element	Indoors						Outdoors					
	PM ₁₀			PM _{2.5}			PM ₁₀			PM _{2.5}		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
B	12.7	3.92	18.7	7.26	1.90	12.4	8.80	3.80	18.1	7.58	1.96	13.4
Na	574	204	1016	236	13.9	536	756	313	1414	95.1	13.7	240
Mg	124	58.0	209	44.9	6.94	91.2	187	136	273	31.8	6.83	68.1
Al	252	130	733	207	6.94	480	302	107	483	73.2	15.2	149
P	50.5	33.0	103	38.3	32.3	56.3	69.5	34.1	167	61.9	33.6	112
K	404	233	697	164	13.9	345	357	78.7	581	65.7	12.7	302
Ca	676	229	1496	202	13.9	376	350	248	482	59.2	13.7	138
Cr	4.43	2.00	8.18	4.01	0.951	6.21	4.20	1.92	6.02	3.69	0.980	6.01
Mn	7.84	5.10	12.6	3.54	0.416	8.81	16.2	10.8	20.2	3.94	0.410	11.2
Co	0.523	0.132	1.95	0.209	0.0634	0.414	0.227	0.127	0.446	0.210	0.0653	0.393
Ni	6.39	3.07	11.0	2.59	0.634	5.88	2.86	1.28	7.07	2.46	0.654	4.46
Cu	13.1	10.2	16.4	6.14	0.986	12.5	27.3	24.2	29.6	3.94	1.37	8.62
Zn	123	40.5	314	48.1	6.94	150	191	72.1	392	76.3	18.2	123
As	6.28	2.61	10.9	5.19	1.27	8.82	5.97	2.56	10.9	4.96	1.31	8.93
Rb	1.32	0.847	2.22	0.702	0.259	1.24	1.26	0.712	1.68	0.755	0.370	1.34
Sr	2.85	1.08	5.73	1.21	0.416	2.31	2.15	1.60	3.54	0.978	0.410	1.80
Cd	0.293	0.231	0.388	0.257	0.0634	0.387	0.413	0.132	0.596	0.322	0.0653	0.464
Sn	4.01	2.61	8.28	3.70	1.27	7.75	4.36	2.56	8.03	4.01	1.32	7.85
Sb	1.28	0.653	2.00	0.790	0.197	1.64	2.32	1.25	3.15	0.895	0.379	1.37
Ba	7.25	3.99	9.79	2.65	0.416	4.23	13.7	9.67	18.8	2.67	0.633	7.01
La	0.539	0.151	2.79	0.378	0.0685	1.17	0.533	0.206	1.62	0.157	0.0671	0.370
Ce	5.44	0.396	30.9	2.80	0.259	11.6	5.44	0.384	36.0	4.00	0.381	30.9
Nd	0.215	0.0691	0.878	0.168	0.0317	0.409	0.271	0.0928	0.669	0.132	0.0635	0.232
Pr	0.168	0.0660	0.332	0.0829	0.0431	0.146	0.127	0.0633	0.333	0.0932	0.0631	0.223
Pb	11.9	4.20	26.2	9.79	1.24	25.9	15.5	3.76	42.7	7.65	1.16	24.4
ΣE _{calc}	29.3	21.7	39.0	21.8	4.98	47.3	28.9	20.8	42.7	19.1	7.74	30.5
ΣE	2.292	1.208	4.108	993	830	1634	2324	1738	2939	512	144	865

Note: When the concentration of a given element was below the LOD, the value of the respective LOD/√2 was used (Hornung and Reed 1990);

Statistical significance defined as $p < 0.05$ for non-parametric Mann-Whitney U analysis;

ΣE is total elemental concentration; ΣE_{calc} is total concentration of five carcinogenic elements (Cr, Ni, Cd, Ni, As and Pb);

In agreement, Begonha (2001) reported that geological layer of Oporto district is composed of granite (Al and K are the main species), which is due to its accessibility often used indoors (tiles, work surfaces). Thus, high abundance of those elements could be caused by indoor material abrasion or from infiltration of ambient emissions (through ventilations, or due to inferior building isolation, etc.; Rivas et al., 2014, 2015). In the context of BREATHE study air pollution was evaluated in Spanish schools. In the series of works (Amato et al., 2014; Moreno et al., 2014; Rivas et al., 2014, 2015; Viana et al., 2015) those authors reported similar findings to this work with high abundance of mineral components in indoor air, originating from soil re-suspension and entrainment of soil particles on children clothing, in shoes and etc. Thus, Na and K could be a result of sodium and potassium-rich sulphates that are often found indoors (due penetration of outdoor air). Ca can be attributed to blackboard chalk dust (Amato et al., 2014; Rivas et al., 2014); similarly to the findings of this work, in preschools in Stockholm. Molnár et al. (2007) also found higher levels of Ca indoors than outdoors. The abundances of the other trace metals in indoor PM were much lower: Zn (5% of ΣE in both PM), Pb (1% and 0.5% of ΣE in $PM_{2.5}$ and PM_{10} , respectively), and Cu (0.6% of ΣE in both PM). Finally, the remaining metals such Rb, Sn or Ba contributed less than 0.5% of ΣE .

The composition of PM collected in preschool yard (i.e. in ambient air) is also shown in Table 3.8. The obtained findings demonstrate that total elemental concentrations of outdoor PM_{10} was remarkably similar to the indoor one. In addition, compositional profiles of both PM fractions in outdoor air and indoors were rather similar. In ambient air, once again Ca, Na, Al and K were the most dominant elements in both PM (58% of analysed ΣE in $PM_{2.5}$, 76% of ΣE in PM_{10}) but the abundances were slightly different: Na ($PM_{2.5}$: 19% of analysed ΣE ; PM_{10} : 33% of analysed ΣE), being followed by Al ($PM_{2.5}$: 14% of analysed ΣE ; PM_{10} : 13% of analysed ΣE), K (13% and 15%, respectively), and Ca (12-15% of analysed ΣE in both PM fractions). A specific occurrence was observed for Zn, which accounted 15% of ΣE in $PM_{2.5}$ but its content in PM_{10} was lower (8%). These percentages were somewhat high in comparison with anthropogenic tracers such as Cr, Cd, Ni, Cu, Pb As, Sn: Pb (0.7–1.9%), and Cu (0.8–1.2%); the rest of the elements accounted for less than 0.5% of analysed ΣE . No particular emission source of Zn was found; however, obtained outdoor levels were in the similar ranges to those reported in ambient air of urban sites in Oporto area (mean concentrations 104–172 $ng\ m^{-3}$ for PM_{10} and 84–134 for $PM_{2.5}$; Slezakova et al., 2007).

As far as it is known, the information regarding elemental composition of PM in preschools or kindergartens is limited. Furthermore, comparisons between various studies can be hampered by different study designs, namely in terms of different PM fractions and number of considered elements. Concerning preschool environments, Latif et al. (2014) recently analysed a number of metals (Pb, Zn, Cd, Fe, and Cr) in indoor dust (i.e. particles with aerodynamic diameter up to 100 μm) of preschools in Selangor, Malaysia. According to our knowledge only two studies related to PM elemental compositions in preschools were identified in literature: Molnár et al. (2007) and lately Mainka et al. (2015). The latter analyzed As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, and Zn in $\text{PM}_{2.5}$ collected in air of Polish preschools. For indoor air of preschools in urban zones, the authors reported total elemental concentrations of 838 ng m^{-3} (in winter) and 1 026 ng m^{-3} (spring), being rather similar to the overall levels in this work ($\text{PM}_{2.5}$ mean of 993 ng m^{-3}). Furthermore, in ambient air total means varied between 433 ng m^{-3} (winter) and 571 ng m^{-3} (spring), being also similar to this study. Molnár et al. (2007) also reported $\text{PM}_{2.5}$ elemental composition using eight elements equal as in this work (namely Ca, K, Cr, Mn, Ni, Cu, Zn, and Pb) with a mean of 139 ng m^{-3} in ambient air of ten Swedish preschools (opposed to 223 ng m^{-3} in this study); they though reported much lower indoor levels of these 8 elements (mean of 162 ng m^{-3}) compared to this study (441 ng m^{-3}). Certainly, geographical and cultural differences and consequently different indoor emission sources as well as different urbanizations of preschool areas, seasonal and meteorological differences could account for the significant differences between the existent data.

Concerning the indoor carcinogenic metals, the present work considered As, Cd, Cr and Ni (known human carcinogens) and Pb (based on animal studies as probable carcinogen). The average concentration of these five elements ($\Sigma\text{E}_{\text{carc}}$) indoors was 21.8 ng m^{-3} in $\text{PM}_{2.5}$ and 29.3 ng m^{-3} in PM_{10} , thus representing 2.2 and 1.3% of ΣE in $\text{PM}_{2.5}$ and PM_{10} . Carcinogenic elements were predominantly found in fine particles (83% for indoor As and Pb; 88–91% for Cd and Cr) except for Ni which 40% of indoor PM_{10} content was in $\text{PM}_{2.5}$. Apart from Pb, As was the most abundant carcinogen indoors, accounting for 0.5% of ΣE in $\text{PM}_{2.5}$ and 0.3% of ΣE in PM_{10} ; concentrations of other carcinogens in indoor air of the preschool were much lower (i.e. less than 0.5%). Concerning arsenic, no particular emission source of this metal was determined in the preschool. Arsenic is not a common pollutant. Its presence indoors can result from infiltrations of outdoor emissions (Moreno et al., 2014; Rivas et al., 2015). High indoor levels of As are associated with cigarette smoke (Slezakova et al., 2009) which is the

most-known source of this metal indoors. As tobacco was banned at all the premises of the preschool, a possible contribution might result from thirdhand tobacco smoke (i.e. residues transferred by clothes of individuals exposed to smoking in other microenvironments; Bahl et al., 2014; Sleiman et al., 2014). Evaluating penetration of particles from outdoor sources to indoor air within the BREATHE study (39 Spanish schools), Rivas et al. (2015) also found high indoor concentrations of As, namely in schools that had Al/PVC windows. The authors demonstrated that frame material affects PM mineral components, hindering the dispersion and leading to higher indoor concentration. In regard to As, they suggested higher indoor levels due to emissions from material-treatments or from sand from school playgrounds (that reached indoor by soil adhering to footwear). At this moment no legislation regulating levels of particulate metals for indoor air exists, but EU Directive 2004/107/EC defines annual mean target concentrations (in PM_{10}) for Ni, Cd and As in outdoor air. The average levels of As (5.97 ng m^{-3} in outdoor PM_{10} , 4.96 ng m^{-3} in outdoor $PM_{2.5}$) were below the given limit of 6 ng m^{-3} but the daily measurements surpassed it during 45% of the sampling period. The sample collection was though done only during 13% of calendar year (corresponds to 7 weeks). Henke (2009) summarised existent information on this carcinogenic metalloid in outdoor air and reported range between 0.008 ng m^{-3} in distant places up to 15 ng m^{-3} in strongly polluted areas (Henke 2009). However, studies from Iberian Peninsula reported lower ranges of As in ambient air than in this work: approximately 1 ng m^{-3} for Lisbon (Almeida et al., 2009), $0.46\text{--}0.5 \text{ ng m}^{-3}$ in Barcelona (Moreno et al., 2014; Rivas et al., 2014) and thus further research regarding the sources of this metalloid would be needed. Concerning Cd and Ni, both estimated means and range of concentrations in outdoor PM fractions were well below the target values. The total abundance of five carcinogenic elements in outdoor PM_{10} was rather similar to the indoor one (1.3% of outdoor ΣE), and for $PM_{2.5}$ outdoors being slightly higher (4.1% of ΣE) than indoors.

3.3.3 Indoor-to-outdoor ratios

Evaluation of indoor-to-outdoor ratios (I/O) of the elemental concentrations can provide further insight on impacts of indoor/outdoor sources on indoor environments and possible identification of the pollutants origin. The calculated I/O ratios for both PM fractions are presented in Figure 3.2. Average values of I/O varied from 0.62 (P) to 3.4 (Ca) in $PM_{2.5}$, and 0.48 (Cu, Mn) to 2.3 (Co) in PM_{10} . As shown in Figure 3.2, mean I/O

of both PM fractions can be considered in two main groups: I/O values below (or very close to) unity (P, Cr, Mn, Zn, As, Rb, Cd, Sn, Sb, Ba, Ce) suggesting absence of relevant indoor emission source and I/O > 1 (Na, Mg, Al, K, Ca, Cu, La, Nd, Sr, and Pb in fine fraction; Ca, Co, Ni, Sr, and Pr in PM₁₀) suggesting existence of an indoor source. The high I/O ratios of the latter group indicates that a considerable part of both PM was caused by indoor origin, i.e. from suspension of settled mineral dust (such as due to cleaning, i.e. sweeping) and due to children activities and movements (Chithra and Shiva Nagendra 2012; Diapouli et al., 2008). In agreement, resuspension of mineral particles by children's activities was the major source of indoor PM_{2.5} in BREATHE study (Moreno et al, 2014; Rivas et al., 2014, 2015); the respective dust originated mostly from outdoors from school playgrounds, especially when these were unpaved (Rivas et al., 2014, 2015). Human activity is known to contribute mostly to coarse PM re-suspension which was not observed in the present work. Nevertheless, for fine particles Na, Al, Mg, K and La exhibited the biggest values of I/O ratios; these are the elements typically indicative of natural sources (i.e. crustal origin: Al, Mg, La; K and Na from sea salt; Calvo et al., 2013; Slezakova et al., 2009, 2010). In general the scientific community agrees that crustal-derived compounds represent lesser risks for potential health outcomes than components produced by combustions. Still, PM_{2.5} from crustal emission sources can interfere with other kinds of particulates thus causing negative consequences to human health (Schlesinger et al., 2006).

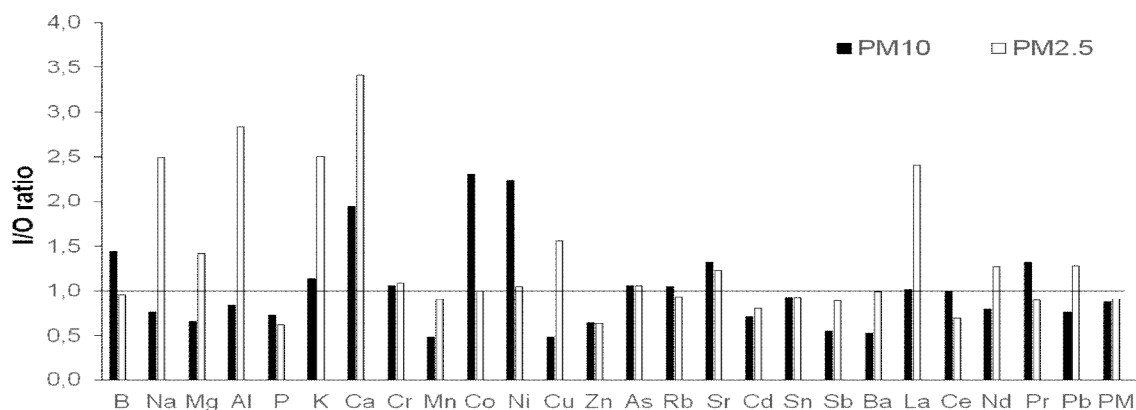


Figure 3.2. Average indoor to outdoor (I/O) elemental ratios of PM_{2.5} and PM₁₀ at the studied preschool.

3.3.4 Enrichment factors

The results for EF calculations for fine and coarse fractions (Figure 3.3) show that Na, Mg, K, lanthanides (La, Pr, Nd), and trace metals (namely Ba, Rb, Sr, Mn and Cr) exhibited mean $EF \leq 10$, which indicates a crustal origin. Their emissions sources were though man-made, results in previous section indicate that their presence indoors resulted from resuspension of mineral particles by children's activities. In majority of crustal materials the abundances of common lanthanides is typically as the following: $Ce > La > Nd > Pr > Sm$ (samarium) $> Yb$ (ytterbium) $> Lu$ (lutetium); Hsu et al. (2016). Whereas Sm, Yb, and Lu were not analyzed in this study, the data in Table 3.8 reflect this order of abundance and support the conclusion that the presence of lanthanides was mostly due to natural occurrence (i.e. from the crustal origin).

Trace metals with mean $EF \geq 10$, namely Ni, Cu, As, Zn, Cd, Sn, Sb, and Pb were predominantly from anthropogenic emissions (Hsu et al., 2010). The calculated results of EF of these elements decreased as the following: $Sb > As > Zn > Cd > Sn > Pb > Ni$. Specifically, Ni showed values close to 10, indicating minimal enrichment whereas Cu, Zn, Cd, Sn, Pb (EF values between 1×10^2 – 5×10^2), As and Sb (EF above 1×10^3) exhibited significant enrichment. It should be mentioned that EF values (and patterns) in $PM_{2.5}$ and PM_{10} were similar (Figure 3.3; aluminium used as reference for the calculations) which suggests that similar sources contributed to both PM; these results were unchanged if La was used as reference element (Figure 3.4). These elements may result from some specific indoor sources. Canha et al. (2014) reported presence of Zn in indoor air of primary schools due to contributions of wall paintings, protective coatings for steel materials, wood surfaces, walls, doors, and windows. The presence of trace metals can be also due to the equipment present in the monitoring places (Slezakova et al., 2010). Fabrics with Sb-containing flame retardant materials are the most typical indoor sources of Sb (de Perio et al., 2010; Mansson et al., 2009). Majestic et al. (2012) used ratios of Cu to Sb in order to distinguish between possible sources of Sb. These authors reported Cu:Sb ratio of 125 for upper continental crust, a range from 2.3 to 10.1 for the sources associated with vehicular traffic (mostly from brake wear), and Cu:Sb ratio ~ 20 for coal fly ash. In present work, $PM_{2.5}$ and PM_{10} in indoor air exhibited Cu:Sb ratios ranging from 7.8–10.2 which suggest traffic-related origin. Furthermore, EF patterns both indoors and outdoors are almost identical which confirms the findings that outdoor emissions were the main contributor to indoor PM.

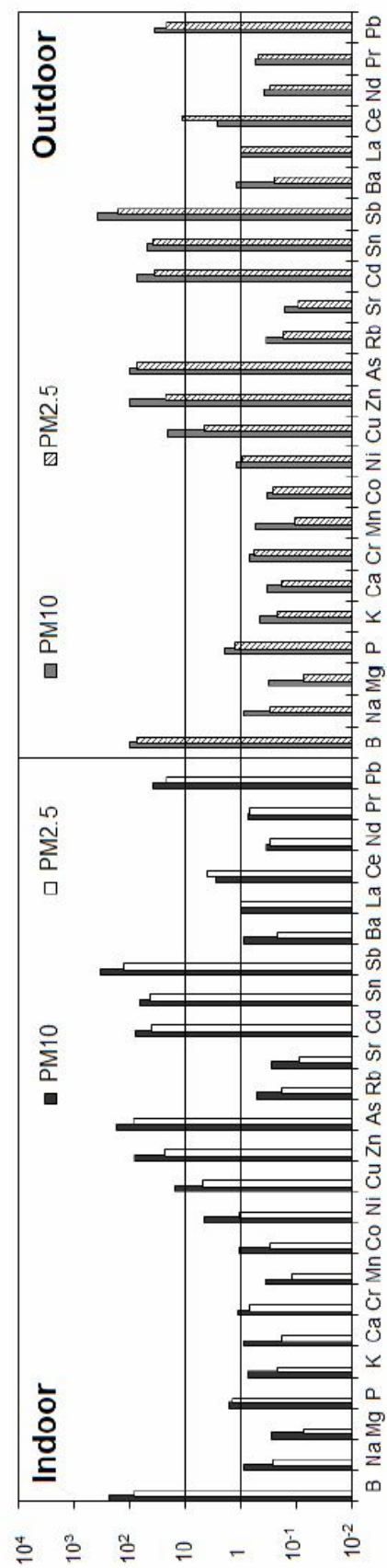


Figure 3.3. Enrichment factor (EF) analysis of PM_{2.5} and PM₁₀ collected in indoor and outdoor air of the studied preschool using aluminium as reference element

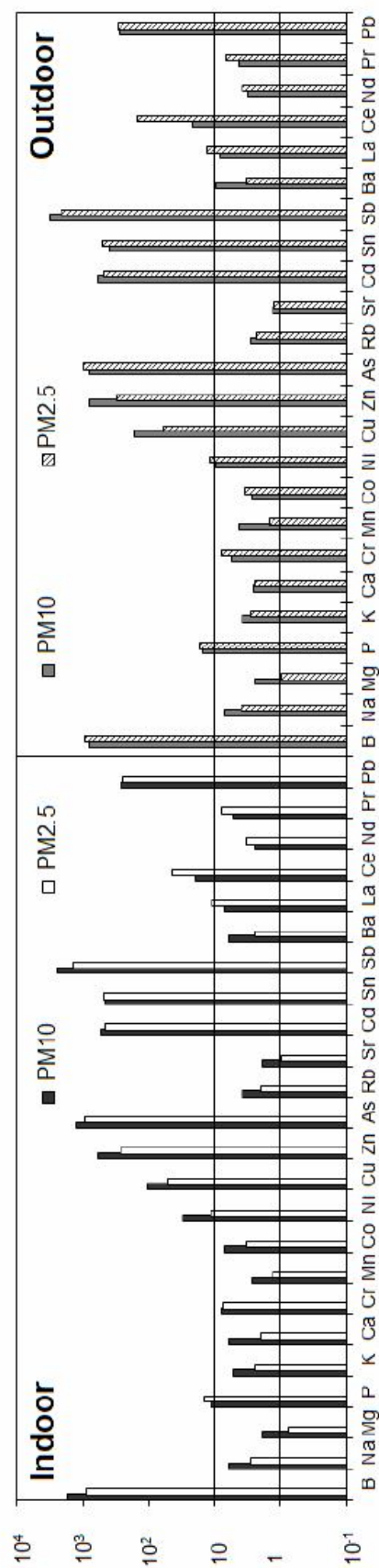


Figure 3.4. Enrichment factor (EF) analysis of PM_{2.5} and PM₁₀ collected in indoor and outdoor air of the studied preschool using lanthanum as reference element.

High abundance of Cu, Cd, and Sb has been associated with traffic-related dusts (Cheng et al., 2010; Christian et al., 2010; Dall'Osto et al., 2013; Zhai et al., 2014). Also, high levels of As, Sb and Pb have demonstrated coal combustion origin (Mokhtara et al., 2014; Tian et al., 2010; Zhang et al., 2013a, 2013b) with As and Sb usually identified as tracers for coal burning (this emission source is though not in the studied area), whereas Zn and Sb have been linked with combustions, waste incineration, and vehicular emissions (mainly tires and brake wear; Slezakova et al., 2007); Ni is typically known as an element coming from oil combustion. Thus it is possible to conclude that in the studied preschools outdoor anthropogenic sources (probably from traffic) were the major contributors to indoor particles.

3.3.5 Risk estimation

THQ risks estimated for preschools pupils and staff are presented in Table 3.9 considering for different PM fractions both in indoor and outdoor air. Concerning the indoor air, estimated values of THQ for elements in coarse fraction were between 8.35×10^{-5} for Cr (school staff 55–64 years old) and 3.77×10^{-2} for Mn (3 years old children). This range demonstrates that for coarse fractions all THQ values were <1 (Table 3.9). Furthermore, total THQ values (sum of THQ of all elements) were also lower than 1 (Table 3.9). Therefore, non-carcinogenic risks of $PM_{2.5-10}$ -bound components in indoor air of the preschool were acceptable to both preschool children and staff. For indoor $PM_{2.5}$ non-carcinogenic risks were significantly higher ($p < 0.05$) ranging between 8.56×10^{-4} for Cr (school staff 25–54 years old) and 1.51×10^{-1} for As (3 years old children). Due to its unusually high levels, As was the major component ($\approx 65\%$) of indoor $PM_{2.5}$ THQ, whereas the remaining elements demonstrated significantly lower non-cancer risks with the following contributions (in decreasing order): $Mn > Ni > Al > Cd > Cr > Ba$. However, it needs to be remarked that the values might be underestimated once calculations were done using concentrations estimated over 24 h period, i.e. included periods when room was not occupied (after classes, nights).

Similarly for the outdoor air of the respective preschool, ΣE_{THQ} (Table 3.9) did not exceed the safe level for all PM fractions (and all four age groups). The highest values (that are the greatest concerns) were found for groups with lower age.

Table 3.9. Risk assessment by target hazard quotients (THQ) in indoor and outdoor air of the studied preschool, as well as due to overall school exposure.

Indoor air	THQ							
	PM ₁₀							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	2.21×10^{-2}	6.86×10^{-2}	1.32×10^{-2}	2.77×10^{-3}	5.59×10^{-2}	1.83×10^{-1}	6.42×10^{-3}	3.52×10^{-1}
Children 4–5 years old	1.51×10^{-2}	4.69×10^{-2}	9.02×10^{-3}	1.89×10^{-3}	3.82×10^{-2}	1.25×10^{-1}	4.38×10^{-3}	2.40×10^{-1}
Adults 25–54 years old	7.54×10^{-3}	2.34×10^{-2}	4.51×10^{-3}	9.45×10^{-4}	1.91×10^{-2}	6.25×10^{-2}	2.19×10^{-3}	1.20×10^{-1}
Adults 55–64 years old	7.07×10^{-3}	2.20×10^{-2}	4.23×10^{-3}	8.86×10^{-4}	1.79×10^{-2}	5.86×10^{-2}	2.05×10^{-3}	1.13×10^{-1}
	PM _{2.5}							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	1.82×10^{-2}	3.10×10^{-2}	2.32×10^{-3}	2.51×10^{-3}	2.26×10^{-2}	1.51×10^{-1}	5.63×10^{-3}	2.33×10^{-1}
Children 4–5 years old	1.24×10^{-2}	2.11×10^{-2}	1.58×10^{-3}	1.71×10^{-3}	1.55×10^{-2}	1.03×10^{-1}	3.84×10^{-3}	1.59×10^{-1}
Adults 25–54 years old	6.20×10^{-3}	1.06×10^{-2}	7.92×10^{-4}	8.56×10^{-4}	7.73×10^{-3}	5.17×10^{-2}	1.92×10^{-3}	7.97×10^{-2}
Adults 55–64 years old	5.81×10^{-3}	9.91×10^{-3}	7.43×10^{-4}	8.02×10^{-4}	7.24×10^{-3}	4.84×10^{-2}	1.80×10^{-3}	7.47×10^{-2}
	PM _{2.5-10}							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	3.93×10^{-3}	3.77×10^{-2}	1.09×10^{-2}	2.61×10^{-4}	3.33×10^{-2}	3.18×10^{-2}	7.88×10^{-4}	1.19×10^{-1}
Children 4–5 years old	2.68×10^{-3}	2.57×10^{-2}	7.44×10^{-3}	1.78×10^{-4}	2.27×10^{-2}	2.17×10^{-2}	5.38×10^{-4}	8.10×10^{-2}
Adults 25–54 years old	1.34×10^{-3}	1.29×10^{-2}	3.72×10^{-3}	8.91×10^{-5}	1.14×10^{-2}	1.09×10^{-2}	2.69×10^{-4}	4.05×10^{-2}
Adults 55–64 years old	1.26×10^{-3}	1.21×10^{-2}	3.49×10^{-3}	8.35×10^{-5}	1.07×10^{-2}	1.02×10^{-2}	2.52×10^{-4}	3.80×10^{-2}

Table 3.9. Risk assessment by target hazard quotients (THQ) in indoor and outdoor air of the studied preschool, as well as due to overall school exposure (continued).

Outdoor air		THQ							
		PM ₁₀							
		Al	Mn	Ba	Cr	Ni	As	Cd	ΣE _{THQ}
Children 3 years old		2.73×10 ⁻³	1.47×10 ⁻²	1.24×10 ⁻³	2.71×10 ⁻⁴	2.58×10 ⁻³	1.80×10 ⁻²	9.35×10 ⁻⁴	4.05×10 ⁻²
Children 4–5 years old		5.05×10 ⁻³	2.71×10 ⁻²	2.30×10 ⁻³	5.01×10 ⁻⁴	4.78×10 ⁻³	3.33×10 ⁻²	1.73×10 ⁻³	7.48×10 ⁻²
Adults 25–54 years old		9.34×10 ⁻⁴	5.01×10 ⁻³	4.24×10 ⁻⁴	9.26×10 ⁻⁵	8.83×10 ⁻⁴	3.19×10 ⁻⁴	3.19×10 ⁻⁴	1.38×10 ⁻²
Adults 55–64 years old		8.75×10 ⁻⁴	4.69×10 ⁻³	3.98×10 ⁻⁴	8.68×10 ⁻⁵	8.27×10 ⁻⁴	2.99×10 ⁻⁴	2.99×10 ⁻⁴	1.30×10 ⁻²
		PM _{2.5}							
		Al	Mn	Ba	Cr	Ni	As	Cd	ΣE _{THQ}
Children 3 years old		6.62×10 ⁻⁴	3.57×10 ⁻³	2.42×10 ⁻⁴	2.59×10 ⁻⁴	2.34×10 ⁻³	1.49×10 ⁻²	7.28×10 ⁻³	2.27×10 ⁻²
Children 4–5 years old		1.22×10 ⁻³	6.60×10 ⁻³	4.47×10 ⁻⁴	4.79×10 ⁻⁴	4.33×10 ⁻³	2.75×10 ⁻²	1.35×10 ⁻³	4.19×10 ⁻²
Adults 25–54 years old		2.26×10 ⁻⁴	1.22×10 ⁻³	8.26×10 ⁻⁵	8.85×10 ⁻⁵	8.00×10 ⁻⁴	5.08×10 ⁻³	2.49×10 ⁻⁴	7.74×10 ⁻³
Adults 55–64 years old		2.12×10 ⁻⁴	1.14×10 ⁻³	7.74×10 ⁻⁵	8.30×10 ⁻⁵	7.49×10 ⁻⁴	4.76×10 ⁻³	2.33×10 ⁻⁴	7.26×10 ⁻³
		PM _{2.5-10}							
		Al	Mn	Ba	Cr	Ni	As	Cd	ΣE _{THQ}
Children 3 years old		2.07×10 ⁻³	1.11×10 ⁻²	1.00×10 ⁻³	1.20×10 ⁻⁵	2.43×10 ⁻⁴	3.16×10 ⁻³	2.07×10 ⁻⁴	1.78×10 ⁻²
Children 4–5 years old		3.83×10 ⁻³	2.05×10 ⁻²	1.85×10 ⁻³	2.22×10 ⁻⁵	4.49×10 ⁻⁴	5.84×10 ⁻³	3.82×10 ⁻⁴	3.29×10 ⁻²
Adults 25–54 years old		7.07×10 ⁻⁴	3.79×10 ⁻³	3.42×10 ⁻⁴	4.11×10 ⁻⁶	8.30×10 ⁻⁵	1.08×10 ⁻³	7.06×10 ⁻⁵	6.07×10 ⁻³
Adults 55–64 years old		6.63×10 ⁻⁴	3.55×10 ⁻³	3.20×10 ⁻⁴	3.85×10 ⁻⁶	7.78×10 ⁻⁵	1.01×10 ⁻³	6.62×10 ⁻⁵	5.69×10 ⁻³

Table 3.9. Risk assessment by target hazard quotients (THQ) in indoor and outdoor air of the studied preschool, as well as due to overall school exposure (continued).

Overall air school exposure	THQ							
	PM₁₀							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	2.48×10^{-2}	8.33×10^{-2}	1.45×10^{-2}	3.04×10^{-3}	5.85×10^{-2}	2.01×10^{-1}	7.35×10^{-3}	3.93×10^{-1}
Children 4–5 years old	2.01×10^{-2}	7.40×10^{-2}	1.13×10^{-2}	2.39×10^{-3}	4.30×10^{-2}	1.58×10^{-1}	6.11×10^{-3}	2.15×10^{-1}
Adults 25–54 years old	8.48×10^{-3}	2.84×10^{-2}	4.94×10^{-3}	1.04×10^{-3}	2.00×10^{-2}	6.87×10^{-2}	2.51×10^{-3}	1.34×10^{-1}
Adults 55–64 years old	7.94×10^{-3}	2.67×10^{-2}	4.63×10^{-3}	9.72×10^{-4}	1.87×10^{-2}	6.44×10^{-2}	2.35×10^{-3}	1.26×10^{-1}
	PM_{2.5}							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	1.88×10^{-2}	3.45×10^{-2}	2.56×10^{-3}	2.76×10^{-3}	2.50×10^{-2}	1.66×10^{-1}	6.36×10^{-3}	2.56×10^{-1}
Children 4–5 years old	1.36×10^{-2}	2.77×10^{-2}	2.03×10^{-3}	2.19×10^{-3}	1.98×10^{-2}	1.31×10^{-1}	5.19×10^{-3}	2.01×10^{-1}
Adults 25–54 years old	6.43×10^{-3}	1.18×10^{-2}	8.75×10^{-4}	9.44×10^{-4}	8.53×10^{-3}	5.67×10^{-2}	2.17×10^{-3}	8.75×10^{-2}
Adults 55–64 years old	6.02×10^{-3}	1.11×10^{-2}	8.20×10^{-4}	8.85×10^{-4}	7.99×10^{-3}	5.32×10^{-2}	2.03×10^{-3}	8.20×10^{-2}
	PM_{2.5-10}							
	Al	Mn	Ba	Cr	Ni	As	Cd	ΣE_{THQ}
Children 3 years old	6.00×10^{-3}	4.88×10^{-2}	1.19×10^{-2}	2.73×10^{-4}	3.35×10^{-2}	3.50×10^{-2}	9.94×10^{-4}	1.36×10^{-1}
Children 4–5 years old	6.57×10^{-3}	4.62×10^{-2}	9.29×10^{-3}	2.00×10^{-4}	2.32×10^{-2}	2.76×10^{-2}	9.20×10^{-4}	1.14×10^{-1}
Adults 25–54 years old	2.05×10^{-3}	1.67×10^{-2}	4.06×10^{-3}	9.32×10^{-5}	1.15×10^{-2}	1.19×10^{-2}	3.40×10^{-4}	4.66×10^{-2}
Adults 55–64 years old	1.92×10^{-3}	1.56×10^{-2}	3.81×10^{-3}	8.73×10^{-5}	1.07×10^{-2}	1.12×10^{-2}	3.18×10^{-4}	4.37×10^{-2}

In that regard, children 4–5 years exhibited THQ approximately twice higher than 3 years old ones, mostly due to their longer periods spent outdoors (1.75h *versus* 0.75h, respectively). Finally, it is possible to conclude that non-carcinogenic risks from overall school exposure (i.e. sum of indoor and outdoor) to PM-bound elements were acceptable to all age categories of children and adults.

Table 3.10 shows the TR values for the four groups of children and adults. USEPA defined a threshold of 10^{-6} (USEPA 1989), but the accumulative carcinogenic risks should not surpass the more stringent 10^{-4} threshold. For the considered elements (both indoors and outdoors) individual and total TR (based on Σ_E) were lower than 10^{-6} ; TR of overall exposure (sum of indoor and outdoor risks) were equally insignificant. Higher risks were obtained for metals bound to PM_{2.5} then to PM_{2.5–10}, possibly due to the overall higher levels. For both age groups of children, total indoor risks were 10 and 4 times higher than outdoor ones for younger (3–years) and older (4–5 years) preschool children, respectively. These differences were most likely caused by longer period which children were indoors (3–years old: 7.25 h in *versus* 45 min out; 4–5–years old: 6.25 h inside and 105 min outdoors).

Furthermore, risks values of overall school exposure were about 2 times greater (yet negligible) for older kids than for younger ones probably due to different exposure time and secondly, due to different daily schedules. Finally, the indoor risks contributed 91% of overall (sum of indoor and outdoor) school risks for younger children whereas it was 79% for older ones.

To better understand the level of risks at studied preschool, TR values were also estimated for school staff. The obtained values (Table 3.10) show that total carcinogenic risks due to overall school exposure (considering indoor and outdoor) exceeded the recommended USEPA level for both groups of staff (4–11 times) being 8–40 times higher for adults than for 3–5 years old children. However, if other parameters (medical history, eating habits, smoking, and etc.) would be considered, these concerns might be even greater. It is also necessary to point out that for adults, total risks due to indoor exposure also exceeded the recommended guideline of 10^{-6} for PM_{2.5} (3–10 times). The cancer risks decreased as the following: Cr>As>Ni>Cd>Pb. The definition of inhalation unit risk for Cr^{VI} assumes 1:6 ratio of Cr^{III}:Cr^{VI} (USEPA, 2015b). As the content of this element in present work was determined as total Cr, only $\frac{1}{7}$ of the respective concentrations (Table 3.8) were used to calculate the risk values. Cr was the element with the highest risks, probably due to its high IUR; the risks of this element accounted 43–67% in indoor and 55–66% of outdoor PM.

Table 3.10. Target carcinogenic risks (TR) in indoor and outdoor air of the studied preschool: estimation for different age groups of children and staff.

Children 3 years old	TR _{indoor}		ΣTR _{indoor}		TR _{outdoor}		ΣTR _{outdoor}		ΣTR _{school}	
	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5-10}	PM _{2.5}	PM ₁₀
Cr	1.42×10^{-7}	1.48×10^{-8}	1.57×10^{-7}	1.57×10^{-7}	1.36×10^{-8}	1.83×10^{-9}	1.54×10^{-8}	1.66×10^{-8}	1.56×10^{-7}	1.72×10^{-7}
Ni	3.67×10^{-9}	5.40×10^{-9}	9.07×10^{-9}	9.07×10^{-9}	3.62×10^{-10}	5.74×10^{-11}	4.19×10^{-10}	5.46×10^{-9}	4.03×10^{-9}	9.49×10^{-9}
As	6.59×10^{-8}	1.39×10^{-8}	7.98×10^{-8}	7.98×10^{-8}	6.48×10^{-9}	1.38×10^{-9}	7.86×10^{-9}	1.52×10^{-8}	7.24×10^{-8}	8.76×10^{-8}
Cd	1.37×10^{-9}	1.92×10^{-10}	1.56×10^{-9}	1.56×10^{-9}	1.77×10^{-10}	5.03×10^{-11}	2.27×10^{-10}	2.42×10^{-10}	1.55×10^{-9}	1.79×10^{-9}
Pb	3.47×10^{-10}	7.29×10^{-11}	4.20×10^{-10}	4.20×10^{-10}	2.81×10^{-11}	2.88×10^{-11}	5.69×10^{-11}	1.02×10^{-10}	3.75×10^{-10}	4.77×10^{-10}
Σ _E	2.14×10^{-7}	3.43×10^{-8}	2.48×10^{-7}	2.48×10^{-7}	2.06×10^{-8}	3.35×10^{-9}	2.40×10^{-8}	3.77×10^{-8}	2.34×10^{-7}	2.72×10^{-7}

Children 4-5 years old	TR _{indoor}		ΣTR _{indoor}		TR _{outdoor}		ΣTR _{outdoor}		ΣTR _{school}	
	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5-10}	PM _{2.5}	PM ₁₀
Cr	2.45×10^{-7}	2.55×10^{-8}	2.71×10^{-7}	2.71×10^{-7}	6.33×10^{-8}	8.55×10^{-9}	7.18×10^{-8}	3.41×10^{-8}	3.08×10^{-7}	3.43×10^{-7}
Ni	6.33×10^{-9}	9.31×10^{-9}	1.56×10^{-8}	1.56×10^{-8}	1.69×10^{-9}	2.68×10^{-10}	1.96×10^{-9}	9.58×10^{-9}	8.01×10^{-9}	1.76×10^{-8}
As	1.14×10^{-7}	2.39×10^{-8}	1.38×10^{-7}	1.38×10^{-7}	3.02×10^{-8}	6.42×10^{-9}	3.67×10^{-8}	3.03×10^{-8}	1.44×10^{-7}	1.74×10^{-7}
Cd	2.36×10^{-9}	3.30×10^{-10}	2.69×10^{-9}	2.69×10^{-9}	8.27×10^{-10}	2.35×10^{-10}	1.06×10^{-9}	5.65×10^{-10}	3.19×10^{-8}	3.75×10^{-9}
Pb	5.99×10^{-10}	1.26×10^{-10}	7.25×10^{-10}	7.25×10^{-10}	1.31×10^{-10}	1.34×10^{-10}	2.65×10^{-10}	2.60×10^{-10}	7.30×10^{-10}	9.90×10^{-10}
Σ _E	3.68×10^{-7}	5.92×10^{-8}	4.27×10^{-7}	4.27×10^{-7}	9.62×10^{-8}	1.56×10^{-8}	1.12×10^{-7}	7.48×10^{-8}	4.64×10^{-7}	5.39×10^{-7}

Table 3.10. Target carcinogenic risks (TR) in indoor and outdoor air of the studied preschool: estimation for different age groups of children and staff (continued).

Adults 25–54 years old	TR _{indoor}		ΣTR _{indoor}		TR _{outdoor}		ΣTR _{outdoor}		ΣTR _{school}	
	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM ₁₀
Cr	2.13×10 ⁻⁶	2.22×10 ⁻⁷	2.36×10 ⁻⁶	2.03×10 ⁻⁷	2.75×10 ⁻⁸	2.31×10 ⁻⁷	2.34×10 ⁻⁸	2.50×10 ⁻⁷	2.59×10 ⁻⁶	2.59×10 ⁻⁶
Ni	5.50×10 ⁻⁸	8.10×10 ⁻⁸	1.36×10 ⁻⁷	5.42×10 ⁻⁹	8.61×10 ⁻¹⁰	6.29×10 ⁻⁹	6.05×10 ⁻⁸	8.19×10 ⁻⁸	1.42×10 ⁻⁷	1.42×10 ⁻⁷
As	9.89×10 ⁻⁷	2.08×10 ⁻⁷	1.20×10 ⁻⁶	9.72×10 ⁻⁸	2.06×10 ⁻⁸	1.18×10 ⁻⁷	1.09×10 ⁻⁶	2.29×10 ⁻⁷	1.31×10 ⁻⁶	1.31×10 ⁻⁶
Cd	2.05×10 ⁻⁸	2.87×10 ⁻⁹	2.34×10 ⁻⁸	2.66×10 ⁻⁹	7.54×10 ⁻¹⁰	3.41×10 ⁻⁹	2.32×10 ⁻⁸	3.63×10 ⁻⁹	2.68×10 ⁻⁸	2.68×10 ⁻⁸
Pb	5.21×10 ⁻⁹	1.09×10 ⁻⁹	6.30×10 ⁻⁸	4.21×10 ⁻¹⁰	4.32×10 ⁻¹⁰	8.53×10 ⁻¹⁰	5.63×10 ⁻⁹	1.52×10 ⁻⁹	7.16×10 ⁻⁹	7.16×10 ⁻⁹
Σ _E	3.20×10 ⁻⁶	5.15×10 ⁻⁷	3.72×10 ⁻⁶	3.09×10 ⁻⁷	5.02×10 ⁻⁸	3.59×10 ⁻⁷	3.51×10 ⁻⁶	5.65×10 ⁻⁷	4.08×10 ⁻⁶	4.08×10 ⁻⁶

Adults 55–64 years old	TR _{indoor}		ΣTR _{indoor}		TR _{outdoor}		ΣTR _{outdoor}		ΣTR _{school}	
	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM _{2.5}	PM _{2.5-10}	PM ₁₀	PM ₁₀
Cr	5.69×10 ⁻⁶	5.92×10 ⁻⁷	6.28×10 ⁻⁶	5.42×10 ⁻⁷	7.33×10 ⁻⁸	6.16×10 ⁻⁷	6.23×10 ⁻⁶	6.65×10 ⁻⁷	6.90×10 ⁻⁶	6.90×10 ⁻⁶
Ni	1.47×10 ⁻⁷	2.16×10 ⁻⁷	3.63×10 ⁻⁷	1.45×10 ⁻⁸	2.30×10 ⁻⁹	1.68×10 ⁻⁸	1.61×10 ⁻⁷	2.18×10 ⁻⁷	3.80×10 ⁻⁷	3.80×10 ⁻⁷
As	2.64×10 ⁻⁶	5.55×10 ⁻⁷	3.19×10 ⁻⁶	2.59×10 ⁻⁷	5.50×10 ⁻⁸	3.14×10 ⁻⁷	2.90×10 ⁻⁶	6.10×10 ⁻⁷	3.51×10 ⁻⁶	3.51×10 ⁻⁶
Cd	5.48×10 ⁻⁸	7.66×10 ⁻⁹	6.24×10 ⁻⁸	7.09×10 ⁻⁹	2.01×10 ⁻⁹	9.10×10 ⁻⁹	6.18×10 ⁻⁸	9.68×10 ⁻⁹	7.15×10 ⁻⁸	7.15×10 ⁻⁸
Pb	1.39×10 ⁻⁸	2.91×10 ⁻⁹	1.68×10 ⁻⁸	1.12×10 ⁻⁹	1.15×10 ⁻⁹	2.28×10 ⁻⁹	1.50×10 ⁻⁸	4.07×10 ⁻⁹	1.91×10 ⁻⁸	1.91×10 ⁻⁸
Σ _E	8.54×10 ⁻⁶	1.37×10 ⁻⁶	9.91×10 ⁻⁶	8.24×10 ⁻⁷	1.34×10 ⁻⁷	9.58×10 ⁻⁷	9.36×10 ⁻⁶	1.51×10 ⁻⁶	1.09×10 ⁻⁵	1.09×10 ⁻⁵

Furthermore, TR of Cr and As in fine and coarse fractions (i.e. sum), estimated for indoor air, and due to combined school exposure (both indoor and outdoor), exceeded (3–7 and 1–4 times for Cr and As, respectively) the USEPA recommended guideline of 10^{-6} . Considering the elevated risks of As and Cr, mainly due to contribution of indoor exposure, preventive measures (such as use of filtered ventilation systems, more frequent cleaning, and change of shoes when being indoors) should be adapted aiming to decrease levels of atmospheric particles and to improve overall quality of indoor air in the studied preschool. In addition, characteristics of school buildings (level of deterioration, building isolation materials, type of windows, etc.) may help to reduce the infiltration of outdoor emissions and consequently influence the levels of pollutant indoors (Rivas et al., 2015).

3.4 Conclusions

The present study reports data on air pollution in preschool environments focusing on elemental composition of $PM_{2.5}$ and PM_{10} . Obtained concentrations of fine and coarse fractions of PM, CO_2 , formaldehyde, and CO fulfilled legislative limits designated for IAQ in Portuguese public facilities (Decreto Lei 118/2013; Portuguese Regulation 2013); TVOCs were the only pollutant which indoor levels highly (3.3 times) exceeded the existent limit.

The sum of 26 elements in indoor air of the preschool ranged between 1208–4108 $ng\ m^{-3}$ in PM_{10} and 830–1634 $ng\ m^{-3}$ in $PM_{2.5}$. Compositional profiles of both fractions were rather similar with Ca, Na, Al, and K being the most abundant elements in indoor particles (82% of ΣE in $PM_{2.5}$ and 84% in PM_{10}). Health hazardous elements, namely As, Cr, Ni, Cd, and Pb were mainly found in $PM_{2.5}$ (83–91%) and accounted for 2 and 1% of $PM_{2.5}$ and PM_{10} , respectively.

Values of I/O ratios showed that a considerable part of $PM_{2.5}$ and $PM_{2.5-10}$ was originated indoors (i.e., re-suspension of settled mineral dust and children activities). I/O ratios of trace metals (Cr, Mn, Zn, As, Rb, Cd, Sn, Sb and Ba) indicated a contribution from outdoor emissions sources. In addition, Na, Mg, K and Ca (with $EF < 10$) were mostly of crustal origin whereas trace metals (Ni, Cu, As, Zn, Cd, Sn, Sb, and Pb) were significantly enriched and associated with anthropogenic sources (namely from traffic).

Non-cancer risks from preschools total (i.e. both indoors and outdoors) exposure were acceptable both for children and adult staff. Carcinogenic risks due to overall

preschool exposure were negligible for children; risks due to exposure indoors were 10 and 4 times higher than for outdoors, respectively, for younger (3–years) and older (4–5 years) pupils. Finally for adults, carcinogenic risks due to overall school exposure exceeded (4–11 times) the recommended USEPA level of 10^{-6} being 8–40 times higher than for children. However, it is necessary to point out that these risks may be underestimated once PM concentrations were sampled over 24 h.

3.5 Ethical statement

Children at preschool age and school staff did not have an active participation in the study. Findings obtained in this work were disseminated to school personnel using the respective space as well as to the parents and/or students' representatives and to boards of directors of the preschool.

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**CHAPTER 4 – ASSESSMENT OF PARTICULATE MATTER AND
POLYCYCLIC AROMATIC HYDROCARBONS IN (PRE)SCHOOL
ENVIRONMENTS: LEVELS AND POTENTIAL HEALTH RISKS**

Chapter 4. Assessment of particulate matter and polycyclic aromatic hydrocarbons in (pre)school environments: levels and potential health risks

This work aims to characterize PM and PAH levels and phase distribution of PAHs in two pre- and ten primary schools and also to assess the respective risks for 3–5 and 8–10 years old children (in comparison with adults). PM mean concentrations were below the available recommendations. Particulate and gaseous PAHs (16 USEPA priority pollutants, dibenzo[a,l]pyrene and benzo[j]fluoranthene) were concurrently (indoors and outdoors) collected at classrooms and (pre)schools' yards in north of Portugal. The total (i.e. gaseous and particulate) concentrations of the analysed PAHs were higher indoors than outdoors. Gaseous PAHs accounted with more than 85% (indoors) and 77-91% (outdoors) of total air Σ PAHs. Particulate PAHs were predominantly associated with PM₁ (54-76% particulate Σ PAHs) with 5-ring PAHs being the most abundant. Mean PM_{2.5}-benzo[a]pyrene concentrations at preschools were always below the guideline of 1 ng/m³ define for PM₁₀ content (Directive 2004/107/EC), however at some primary schools that guideline was exceeded. Indoor/outdoor ratios of lighter PAHs (2–3 rings) indicated a contribution from indoor sources whereas compounds with 4–6 rings resulted mainly from penetration of outdoor emissions; vehicular traffic (both from diesel and gasoline combustions) was the main source of indoor PAHs. Total carcinogenic risks due to exposure to PAHs in the indoor air of pre- and primary schools were higher than in outdoor air, which was attributed to the longer periods that children spend indoors. Overall cancer risks of children and staff attending at preschools and primary schools exceeded from 5 to 37 and 7 to 87 times the WHO health-based guideline of 10⁻⁵.

4.1 Introduction

Because of PAHs potential health impacts, data concerning levels in educational environments have been slowly emerging but information is far from comprehensive. According to the study performed by Wilson et al. (2000) in preschool children attending two day care centers in North Carolina (USA), inhalation is clearly the predominant exposure pathway for PAHs (76.9%), which includes both relatively volatile and semi-volatile PAHs, followed by dietary ingestion (21.8%) and non-dietary ingestion (1.3%). Table 4.1 reviews the 17 studies regarding the PAH levels in school and day-care centers indoor and outdoor areas available in the literature during 2000-2014, being 47% conducted in European countries, 29% in Asia (in Thai and India) and 24% in USA. About 70% of the studies reported PAH levels simultaneously in indoor and outdoor air of schools. In contrast, only 35% of the studies assessed the PAH levels in the gas phase of school environments (Cirillo et al., 2006; Eiguren-Fernandez et al., 2007; Fiala et al., 2001; Krugly et al., 2014; Wilson et al., 2000, 2003). In addition, only four works focused on preschool children (Fiala et al., 2001; Vyskocil et al., 2000; Wilson et al., 2000, 2003) and none from Portugal. Overall, PAH levels ranged from 0.21 (Rome, Italy during the summer season) to 1301 ng/m³ (North Carolina, USA) for indoor air and from 0.28 (Chonburi, Thailand) to 217.3 ng/m³ (Delhi, India) for outdoors (Table 4.1). Outdoor air is usually the dominant contributor to indoor PAH levels at urban schools. High PAH levels were reported in Asian countries and in North America (Southern Carolina; Wilson et al., 2000, 2003). Many countries in Southeast Asia have undergone a rapid economic development and industrialization (Bae et al., 2010). It is also known that Asian countries share many common environmental problems, particularly high air pollution in the major cities with a large population and high levels of traffic congestion. In that regard, Ruchirawat et al. (2005, 2006, 2007) and Tuntawiroon et al. (2007) (Thailand, Asia) concluded that schoolchildren from Bangkok were potentially exposed to much higher levels of PAHs than schoolchildren from the rural city. However, several studies (Alves et al., 2014; Cirillo et al., 2006; Krugly et al., 2014; Wilson et al., 2003) reported higher PAH levels in school indoors comparatively to the outdoor areas.

Table 4.1. PAH (mean $\pm$ SD or range, expressed as ng/m³) concentrations reported in indoor and outdoor air of school environments.

Continent	Country, city	Note	Children n	age	Analysed phase	No. of PAHs	ΣPAHs indoor	ΣPAHs outdoor	Reference
Europe	Austria, Linz	Elementary school	164	7-10	Air			1.5 - 174.7	Moshammer and Neuberger (2003)
	Czech Republic, Hradec Králové	Kindergartens	25	3-6	Total air (PM + gas)	12			Fiala et al. (2001)
		Polluted area							
		Summer					18.17 ± 1.96	4.70 ± 0.36	
		Winter					5.20 ± 0.56	12.70 ± 3.70	
		Non polluted area							
		Summer					2.73 ± 0.45	1.40 ± 0.26	
		Winter					2.07 ± 0.45	4.40 ± 1.35	
	Italy, Campania Ercolano Pollica	Urban	30	7-9	Total air (PM + gas)	16			Cirillo et al. (2006)
		Rural					0.30 - 6.30	0.3 - 3.4	
						1.70 - 7.90	0.6 - 1.4		
Italy, Rome	Elementary school	5	8-11	PM _{2.5}	8			Gatto et al. (2014)	
	Summer					0.21 - 1.32	0.36 - 2.56		
	Winter					1.6 - 16	4.2 - 18.5		
Italy, Rome	Winter				PM _{2.5}	8	1.8 - 8.3	7.0 - 9.54	Romagnoli et al. (2014)
							B[a]P: 0.30 - 1.58	B[a]P: 1.36 - 1.69	
							0.3 - 1.02	0.42 - 1.21	
	Spring						B[a]P: 0.07 - 0.16	B[a]P: 0.07 - 0.21	
Lithuania, Kaunas	Primary schools				PM _{2.5}	15	20.1 - 131.1	40.7 - 121.1	Krugly et al. (2014)
	Urban, winter				Gas	15	35.6 - 80.6	B[a]P: 1.0 - 3.2	
								n.f.	
Portugal, Aveiro	Primary school, city center				PM ₁₀		0.89 ± 0.65	0.83 ± 0.42	Alves et al. (2014)
Serbia	Primary school, downtown				PM ₁₀	16	10.2 - 198.7		Jovanović et al. (2014)
India, Delhi	School located in proximity of industrial and heavy traffic roads				PM ₁₀	10		38.1- 217.3 B[a]P: 4.45 - 24.58	Jyethi et al. (2014)

Table 4.1. PAH (mean $\pm$ SD or range, expressed as ng/m³) concentrations reported in indoor and outdoor air of school environments (continued).

Continent	Country, city	Note	Children n	age	Analysed phase	No. of PAHs	Σ PAHs indoor	Σ PAHs outdoor	Reference
Asia	Thailand, Bangkok	Urban	44	10-12	PM		6.70 $\pm$ 0.47	7.10 - 83.04	Ruchirawat et al. (2005)
		Rural					1.25 $\pm$ 0.24		
	Thailand, Bangkok	Urban		10-12	PM	10			Ruchirawat et al. (2006) Ruchirawat et al. (2007)
		Playground Classroom	180	9-13	PM-air particulates		2.64 - 25.54 5.06 - 9.39	12.01 - 99.95	
	Chonburi	Rural			Playground Classroom		0.28 - 1.92 0.28 - 2.31	0.31 - 2.91	
North America	Thailand, Bangkok Chonburi	Urban Rural	184	8-13	PM	10		2.10 - 25.54 0.28 - 2.31	Tuntawiroon et al. (2007)
	Canada, Montreal	Kindergartens				12			Vyskocil et al. (2000)
		Polluted area					2.8; 2.6; 3.6 1.0; 2.8; 2.5	36.1; 17.9; 14.8 1.6; 2.2; 1.9	
		Non polluted area							
	USA, North Carolina	Day care centers	9	2-5	Total air (PM + gas)	20	619.05 B[a]P: 0.061 - 0.082	70.26 B[a]P: 0.060 - 0.100	Wilson et al. (2003)
	USA, North Carolina	Day care centers	9	2-5	Total air (PM + gas)	20	205 - 1301	34 - 176	Wilson et al. (2000)
	USA, Southern California	School communities			Gas	15		94.1 - 151	Eiguren-Fernandez et al. (2007)
					PM _{2.5}			B[a]P: n.d. 0.42 - 1.78	
								B[a]P: 0.0198 - 0.111	
					Total air (PM + gas)			94.5 - 152	

n.r - not reported
n.d. - not detected
B[a]P - benzo[a]pyrene

The re-emission and sorption of gaseous PAHs from indoor surfaces, infiltration of outdoor emissions (both gaseous and particulate), re-suspension of indoor dust, and emissions from cooking activities and children activities could account for the higher indoor PAH levels in the school environments (Alves et al., 2014; Gatto et al., 2014; Krugly et al., 2014). Furthermore, indoor environment is protected from PAHs atmospheric degradation and the particulate-bound PAHs may persist for longer periods (Kang et al., 2010).

Thus the main aims of this study were i) to investigate levels of particulate and gaseous PAHs (16 considered by USEPA as priority pollutants, and dibenzo[a,l]pyrene and benzo[j]fluoranthene; the latter recommended by EU Directive 2004/107/EC) at ten primary schools representative of different urban environments (city center, residential, industrial) and two preschools; ii) to characterize the phase distribution of indoor PAHs; iii) to assess the impacts of outdoor PAH emissions to indoor environments; iv) to identify possible emission sources of indoor PAHs; v) to estimate the potential health risks by TEF and according to the methodology recommended by USEPA (USEPA 2014) for 3–5 and 8–10 years old children (and compared with those of the adult staff).

4.2 Materials and Methods

4.2.1 Characterization of sampling sites

PM_{2.5} levels and sixteen particulate-bound PAHs considered by USEPA as priority pollutants, dibenzo[a,l]pyrene and benzo[j]fluoranthene were sampled during ten consecutive weeks of January–April 2014 at ten primary public schools (S1–S10) situated in Oporto Metropolitan Area (north of Portugal). The schools (Figure 4.1) were selected in order to obtain the representative data from various urban environments (city center, residential, industrial). The information regarding building (year of construction, age and size, number of floors), number of occupants, ventilation and heating systems, past occurrences, or visible problems, building usage, and potential indoor sources, as well as outdoor environment are shown in Table 4.2.

This work also evaluated the concentrations of PM and PAHs at two Portuguese preschools. Preschool PS1 was situated in Oporto Metropolitan Area (previously described in detail in section 3.2.1). Preschool PS2 was situated in Chaves (approximately 150 km northeast of Oporto) that represents the second most populous municipality in district of Vila Real (INE 2014).

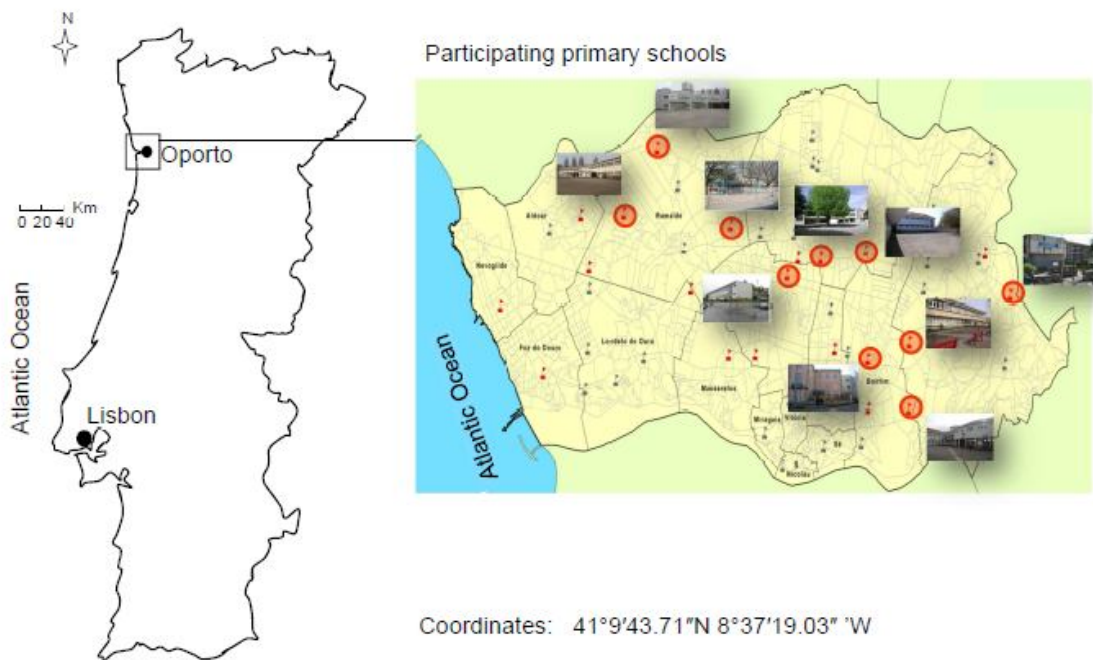


Figure 4.1. Geographic locations of the characterized ten primary schools.

This preschool was situated next to the road, which is the main traffic connection to city centre. A petrol station and main city shopping centre are situated directly next to the school complex, which leads to relatively consistent traffic density throughout the day. Information related with general description, location, traffic density and ventilation at each preschool is presented in Table 4.3. During two consecutive days, the number of road vehicles, namely passenger cars, trucks/buses, motorbikes, and others was manually counted between 5 a.m. and 12 p.m. during 10 min of each hour. The traffic densities were estimated for the streets surrounding each preschool (Table 4.3). These data were used to better describe the surroundings of the selected preschools. The total number of enrolled students (3–5 years old) was 173 and 44, respectively at PS1 and PS2.

Location	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
	Residential area with industrial influence	City centre	City centre	City centre	Residential area, industrial influence	City centre	Residential area surrounded with greens	City centre	Residential area	City centre
Site characterization	Urban-industrial	Urban-traffic	Urban-traffic	Urban-traffic	Urban-industrial	Urban-traffic	Urban-background	Urban-traffic	Urban-traffic	Urban-traffic
Year of construction	1968	1938	1959	1959	1980	1958	1963	1957	1982	1950
Year of refurbishment	2012	2011	2007	2010	2008	2004	2005	2004	2008	2005
Outdoor emissions sources	Local traffic; Car park; Industrial site within 10 km	Local traffic; Car park; Gasoline station	Local traffic	Local traffic; Car park; Gasoline station	Local traffic; Car park; Industrial site within 10 km	Local traffic; Car park	Local traffic; Car park; Gasoline station	Local traffic; Car park	Local traffic; Car park	Local traffic
Number of students (6–11 years old)	256	446	452	442	386	394	370	288	264	420
Classroom area (m ²)	48.9±0.0	50.3±5.4	46.2±0.0	46.8±0.0	56.6±0.2	59.2±23.5	50.1±0.9	50.4±0.1	56.16±0.08	50.6±5.1
Classroom height (m ²)	3.50±0.0	3.2±0.2	3.4±0.0	3.4±0.0	3.3±0.3	3.3±0.0	3.4±0.0	3.0±0.1	3.3±0.0	3.6±0.0
Classroom occupancy density (m ² per occupant)	2.7±0.2	2.6±0.3	2.0±0.3	2.1±0.2	2.9±0.4	2.96±0.9	3.2±0.6	2.0±0.1	2.4±0.2	2.3±0.5
Furniture	Plywood furniture	Plywood furniture	Wood furniture	Wood furniture	Wood furniture	Wood furniture	Wood and metal furniture	Wood and metal furniture	Wood furniture	Wood furniture
Windows	Al frame, double glazed	Metal frame, single glazed	Al frame, single glazed	Al frame, single glazed	Al frame, double glazed	Al frame, double glazed	Al frame, single glazed	Al frame, single glazed	Al frame, single glazed	Al frame, double glazed
Heating units	Hot water radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors	Electrical radiators / convectors
Ventilation	no	yes	no	no	no	no	no	no	no	no
beginning of day during classes	no	no	no	yes	no	no	no	yes	no	yes
during brakes	no	yes	yes	yes	no	yes	no	yes	no	yes
after classes	no	no	no	no	no	no	no	no	no	no
during cleaning	no	yes	yes	yes	yes	yes	yes	yes	yes	yes
Cleaning schedule per day	beginning/ end of day	beginning/end of day	beginning/ end of day	beginning of day	beginning/ end of day	beginning/end of day	beginning of day	beginning/ end of day	beginning of day	beginning/ end of day
Number of copy machine	1	1	1	3	1	2	1	1	1	2
Other					Meals cooked at school					

4.2.2 Sample collection

4.2.2.1 Primary schools

At each school particulate samples were collected during three consecutive week days (Tuesday–Thursday), avoiding Mondays and Fridays (children daily schedules were often irregular). The sample collection was conducted in classrooms because children spent majority of their school time in those microenvironments. Two rooms (8–10 years old children) per each school were simultaneously assessed, resulting in a total of 20 classrooms. Classroom characteristics (room area, height), used materials (windows, furniture, and etc.), information about maintenance and cleaning procedures were registered, as well as present equipment (copiers, chalkboards, plants), and use of environmental modifiers (i.e., air fresheners and insecticides). The main characteristics of studied rooms are shown in Table 4.2.

The indoor $PM_{2.5}$ was sampled according to USEPA IP-10A method (USEPA 1990). Samples were collected daily during 24 h using a personal environmental monitor (PEM™; SKC Ltd., United Kingdom), i.e. single stage impactor ($PM_{2.5}$) that was combined with personal air sampling pump (AirChek® 2000; SKC Ltd., United Kingdom); an air flow rate of 2.0 L min^{-1} was used. The impactors were positioned 1.0 m above the floor and minimally 1 m from the walls, without obstructing the normal usage of the rooms. The samplers were located as far as possible from windows or doors in order to minimize direct influence of any source. All requirements to maintain children safety were fulfilled.

In order to better understand the impacts of outdoor PAH emissions to indoor school environment, the levels of particulate-bound PAHs were concurrently measured in ambient air (i.e. outdoors) except in S1, S3 and S4 because of the school outdoor dispositions and/or safety reasons. Outdoor $PM_{2.5}$ -bound PAHs were measured in school yards; the samplers were positioned in open area avoiding any obstacles such as walls, fences and vegetation. Outdoor sampling was conducted with constant flow samplers (model Bravo H2; TCR TECORA, Italy) that were combined with PM EN LVS sampling heads for $PM_{2.5}$ (in compliance with norm EN14907); an air flow rate of $2.3 \text{ m}^3 \text{ h}^{-1}$ was used.

Table 4.3. Characterization of two studied preschools (PS1 and PS2).

	PS1	PS2
Preschool description	Private institution 173 students 3–5 years old	Public institution 44 students 3–5 years old
Location	Urban – traffic Situated on moderately trafficked streets	Urban – traffic Situated next to the main road connecting city centre
Traffic density	Mean: 16 cars min ⁻¹ Peak hours: 8:30h (27 cars min ⁻¹) 18:30h (25 cars min ⁻¹)	Mean: 5 cars min ⁻¹ Peak hours: 8:30h (7 cars min ⁻¹) 18:30h (10 cars min ⁻¹)
Layout	Constructed in 1940 Two-floors building, built in 1940 Fence (4 m), brick wall (≈3 m), and combination of both (brick-wall base with fence above)	Constructed before 1950 Two-floors building, built before 1950 3 m high fence
Ventilation	Morning (8.00 a.m.) ≈10 min After classes (6.00 p.m.) 15–20 min Throughout day as necessary Doors maintained always closed during the educational activities (classes) and physical activities	Morning (before classes) approximately 5 min After classes (≈6.00 p.m.) approximately 5–10 min During day as necessary Room always opened during recesses and recreational activities
Cleaning	Once per day (end of day)	Twice per day (beginning and end of day)
Other	Meals cooked in school canteen	Meals delivered from out-of-school supplier

Indoor places		
Floor surface (m ²)	101	69
Room height (m)	2.90	2.90
Volume (m ³)	292	200
Heating systems	None	None
Main materials	Concrete, plastic and wood	Concrete, plastic and wood
Floor coating	Plastic (PVC)	Parquet
Wall coating	White paint	White paint
Ceiling coating	White paint	White paint
Door area (m ²)	4.1	2.1
Type of door	Double, wood and glass	Single, wood
Doors number	2	1
Window areas (m ²)	5.7	3.8
Type of window	Single glass layer, wood	Single glass layer, wood
Windows number	10	6

4.2.2.2 Preschools

Both gaseous and particulate samples were collected daily for a period of 24 h. During the sampling periods, daily samples of particulate and gaseous phases were collected. At PS1 indoor sampling was conducted in a common room that was used throughout day both for educational and entertaining activities as well as for physical exercising. At PS2, samples were collected in a classroom (also used for educational physical activities). The main characteristics and images of the studied rooms are shown in Table 4.3 and Figure 4.2, respectively.

Sampling was done by constant flow samplers (model Bravo H2; TCR TECORA, Italy) that were combined with PM EN LVS sampling heads for gaseous and particulate samples (in compliance with norm EN14907 for PM_{2.5}, and PM₁); an air flow rate of 2.3 m³ h⁻¹ was used. The inlets were positioned at 1.5 m above floor and minimally 1 m from walls, without obstructing the normal usage of rooms. Samplers were located as far as possible from windows or doors in order to minimize direct influence of any source. Outdoor (i.e. in ambient air) particulate and gaseous PAHs were measured in preschools yards.

At both primary and preschools, PM fractions were collected on PTFE membrane filters with polymethylpentene support ring (2 µm porosity, Ø 37 and 47 mm for indoor and outdoor air, respectively; SKC Ltd., United Kingdom). Total suspended particles (TSP), PM₁₀, PM_{2.5} and PM₁ masses were determined gravimetrically; description in detail at section 3.2.3. Gaseous samples were collected on polyurethane foam (PUF) plugs (75 mm, SKC Ltd., United Kingdom) that were pre-cleaned using the procedure according to Castro et al. (2011). After sampling both filters and PUF plugs were stored in a freezer (-20 °C) before consequent chemical analysis.

Physical parameters, namely T and RH were measured by multi-gas sensor probe (model TG 502; GrayWolf Sensing Solutions, Shelton, USA).

Information regarding room occupancy, ventilation systems (door and window positions), and potential source activities was registered every day. In addition, teachers and staff were daily inquired regarding the occurrence of an additional source and/or activities.



Figure 4.2. Characterization of the indoor environments at the sampled preschools: (a) at PS1, and (b) at PS2.

4.2.3 Extraction and chromatographic analysis of PAHs

The extractions of PAHs from particles (TSP, $PM_{2.5}$ and PM_{10}) and PUF plugs were performed by previously validated analytical procedure from this research team (Castro et al., 2009, 2011). Briefly, filters and PUF plugs were microwave extracted with 30 and 45 ml of acetonitrile, respectively, for 20 min at 110 °C. After extraction, the vessels were allowed to cool at room temperature; extracts were then reduced to a small volume using a rotary evaporator (Rotavapor, R-200; BUCHI, Flawil, Switzerland) at 20 °C and carefully filtered through a PTFE membrane filter (0.45 μ m). A gentle stream of nitrogen was used to dry the extracts under low temperature; the residue was then dissolved in 1000 μ L of acetonitrile immediately before analysis.

To quantify PAHs, extracts were analyzed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a LC-20AD pump, DGU-20AS

degasser and photodiode array SPD–M20A (PAD) and fluorescence RF–10AXL (FLD) detectors. Separation of the compounds was performed in a C18 column (CC 150/4 Nucleosil 100–5 C18 PAH, 150 9 4.0 mm; 5 µm particle size; Macherey–Nagel, Duren, Germany) maintained at room temperature (20 ± 1 °C). The injected volume was 15.0 µl. A mixture of water and acetonitrile was used as the mobile phase. The initial composition of the mobile phase was 50% of acetonitrile and 50% ultrapure water, and a linear gradient to 100% of acetonitrile was programmed in 15 min, with a final hold of 13 min. Initial conditions were reached in 1 min and maintained for 6 min before next run. The total run time was 40 min with a flow rate of 0.8 ml/min. Fluorescence wavelength programming was used to achieve better sensitivity and minimal interference. Each compound was detected at its optimum excitation/emission wavelength pair: 260/315 nm (naphthalene, acenaphthene and fluorene), 260/366 nm (phenanthrene), 260/430 nm (anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and dibenzo[a,l]pyrene), and 290/505 nm (indeno[1,2,3–cd]pyrene). Acenaphthylene, which shows limited fluorescence, was analyzed at 254 nm in the PAD. Each analysis was performed at least in triplicate.

4.2.4 Quality control

The overall MAE-LC procedure for analysis of PAHs in atmospheric particulate samples was previously validated by systematic recovery experiments and analyzing the certified reference material SRM 1650b 'Diesel particulate matter' (Castro et al., 2009). PAHs were extracted from particles with recoveries ranging from $81.4 \pm 8.8\%$ to $112.0 \pm 1.1\%$ for all the compounds except for naphthalene ($62.3 \pm 18.0\%$) and anthracene ($67.3 \pm 5.7\%$). The validation of MAE procedure for extracting PAHs from PUFs was performed according to Castro et al. (2011). The extraction efficiency was consistent over the entire range of concentrations and the results ranged from $50.2 \pm 3.5\%$ (acenaphthylene) to $107.9 \pm 1.5\%$ (fluoranthene) for all PAHs. External calibrations with PAHs mixed standards, using at least six calibration points, were performed. Calibrations curves were linearly fitted with correlation coefficients always higher than 0.9997 for all PAHs. LODs between 1.0 pg/m^3 (for anthracene, benzo[k]fluoranthene, chrysene, benz[a]anthracene, phenanthrene and indeno[1,2,3–cd]pyrene) and 148 pg/m^3 (for acenaphthylene) were obtained, with corresponding limits of quantification (LOQs) in the range $3.4 - 492 \text{ pg/m}^3$ (calculated as the minimum

detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, respectively, (Miller and Miller 2000). During each set of MAE extractions, a filter blank or a PUF plug blank was included. The repeatability was evaluated by RSD of triplicate samples. RSD values ranged from 1.8% (dibenzo[a,l]pyrene) to 9.1% (naphthalene) and from 0.9% (chrysene) to 9.8% (naphthalene) for PAHs extraction from filters and PUFs, respectively. Standards used for calibration were analyzed daily and regularly, as well as blank MAE extracts (from filter blank or PUF plug blank), between samples to check instrument performance during PAHs analysis. Each analysis was run at least in triplicate.

4.2.5 Exposure assessment

The dose rates from inhalation exposure to PAHs in preschool environment were calculated using Equation 1 (Kalaierasan et al., 2009):

$$\text{Dose rate (D)} = (\text{BR}_{\text{WA}}/\text{BW}) \times C_{\text{WA}} \times \text{OF} \times \text{N} \quad (4.1)$$

where D is the age-specific dose rate ($\text{ng kg}^{-1} \text{ day}^{-1}$); BR_{WA} is the age-specific weighted average breathing rate (L min^{-1}); BW is age-specific body weight (kg); C_{WA} is the time-weighted average concentration (ng L^{-1}); OF is the occupancy factor (considered 1, as children kept their schedules and associated locations tightly); N is the total time per day spent by age-specific children in the preschool (min day^{-1}). The dose rates were estimated for 3–5 years old children.

The daily activity patterns of these children were analysed throughout each day. During the sampling period children spent approximately 8 hours at the preschool during each day. The daily residence time of children indoors and outdoors and the types of activities performed were registered. Each activity was characterized in terms of intensity level in order to assess the corresponding BR. An example of children timetable and activity patterns is shown in Table 4.4. As the information concerning the Portuguese population is not available, the age-specific factors were retrieved from USEPA data (USEPA 2011) considering the mixed population (both male and females).

Table 4.4. Timetable and child activity patterns: an example for 3–years old children.

Time		Environment	Observed activities	Activity intensity
8:30-9:00	Arrival to school	Indoor	Playing (calm, seated, TV)	Sedentary
9:03-10:29	Classes/education	Indoor	Seated only (talking)	Sedentary
10:30-11:15	Recess	Playground	Running, jumping, swings	High intensity
11:17-11:40	Classes/education	Indoor	Sedentary and other (painting, walking)	Sedentary
11:45–13:00	Lunch	Indoor	Seated (eating, drinking, talking)	Light
13:05-15:00	Rest	Indoor	Sleeping	Sleep
15:04-16:00	Classes/education	Indoor	Seated, and other	Sedentary
16:00–17:30	Leaving school	Indoor	Organized activities (singing dancing), running	High intensity

BW of 18.6 kg for 3–5 years old children was used. The values of BR were selected as the followings: 4.3 L min⁻¹ for rest or sleep; 4.5 L min⁻¹ for sedentary or passive activities; 11.0 L min⁻¹ for light intense activity, and 37.0 L min⁻¹ for highly intense activities (running, etc.). BR_{WA} was estimated then as weighted average, i.e. considering the intensity of each performed activities and the amount of time. The exposure doses were then estimated using the average indoor and outdoor concentrations (weighted by the real time that children spent in each place). Age-specific parameters BR_{WA} (11 L min⁻¹, i.e., light physical activity) and BW (77 kg) were used for school staff (USEPA 2011).

4.2.6 Health risk analysis

The risks associated with inhalation exposure to 18 particulate and gaseous PAHs were assessed by TEF using the values estimated by Nisbet and LaGoy (Boström et al., 2002). Consequently, the lifetime lung cancer risks were calculated according to the methodology provided by WHO (WHO 1987, 2000).

The carcinogenic risks of PAHs were assessed according to the methodology provided by USEPA Region III Risk-based Concentration Table (USEPA 2014). The risks were estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk; USEPA 1989). Acceptable risk levels for carcinogens range from 10⁻⁴ (risk of developing cancer over a human lifetime is 1 in 10

000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1 000 000). The carcinogenic risks were calculated using the following equation (4.2):

$$TR = [(Efr \times ED \times ET \times IUR \times C) / AT] \quad (4.2)$$

where TR is target carcinogenic risk (dimensionless); EFr is the exposure frequency (250 days year⁻¹); ED is the exposure duration (years); ET is indoor air exposure time (h day⁻¹); IUR is the chronic inhalation unit risk ($\mu\text{g m}^{-3}$)⁻¹ (USEPA 2014); C is the concentration of PAH ($\mu\text{g m}^{-3}$); and AT is the number of days over which the exposure is averaged (25 500 days, i.e. 70 years $\times$ 365 days year⁻¹; USEPA 2014). The carcinogenic risks were estimated only for PAHs for which IUR values are available (USEPA 2014), namely: naphthalene (IUR of 3.4×10^{-5} ($\mu\text{g m}^{-3}$)⁻¹); chrysene (1.1×10^{-5} ($\mu\text{g m}^{-3}$)⁻¹); benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene (IUR of 1.1×10^{-4} ($\mu\text{g m}^{-3}$)⁻¹); benzo[a]pyrene (IUR of 1.1×10^{-3} ($\mu\text{g m}^{-3}$)⁻¹); and dibenz[a,h]anthracene (1.2×10^{-3} ($\mu\text{g m}^{-3}$)⁻¹). The target risks for 3– and 4–5–years old children were estimated using the lowest possible ED (i.e. 1 and 2 years of exposure at the preschool, respectively) in order to not over–estimate the respective cancer risks. Over the sampling period children spent daily approximately 8 hours at a preschool. Residence time of children spent indoors and outdoors were registered in order to correctly assess total carcinogenic target risks. Per school day, 3–years old children spent 45 min outdoors whereas it was 1.75 h for older (4–5–years old) children. Table 4.5 and 4.6 shows an example of TR calculation respectively for 3–5 years old and 8–10 years old children. For comparison purposes, the health risks for two adult age categories of the school staff were also evaluated: 25–54–years and 55–64–years old (ED of 15 and 40 years of employment, respectively). Time schedules of preschool staff were considered the same as of children.

Table 4.5. Calculation of carcinogen risks (TR): example for benz[a]anthracene in indoor PM_{2.5} for 3–years old children.

Parameter ^a	TR
C _{Benz[a]anthracene} (µg m ⁻³)	1.19 × 10 ⁴
IUR _{Benz[a]anthracene} (µg m ⁻³) ⁻¹	1.1 × 10 ⁻⁴
EFr (days year ⁻¹)	250
ED (years)	1
ET (h day ⁻¹)	7.25 per 24 h, i.e. 0.302
AT (days)	25 500

^aTR is target carcinogenic risk (dimensionless); C is the concentration of PAH (µg m⁻³); IUR is the chronic inhalation unit risk (µg m⁻³)⁻¹; EFr is the exposure frequency (250 days year⁻¹); ED is the exposure duration (years); ET is indoor air exposure time (h day⁻¹); AT is the number of days over which the exposure is averaged (25 500 days, i.e. 70 years × 365 days year⁻¹).

Table 4.6. Calculation of carcinogenic risks (TR) for 8–10 years old children: example for dibenz[a,h]anthracene in indoor air at S1.

Parameter ^a	TR
C _{Dibenz[a,h]anthracene} (µg m ⁻³)	1.53 × 10 ⁻³
IUR _{Dibenz[a,h]anthracene} (µg m ⁻³) ⁻¹	1.20 × 10 ⁻³
EFr (days year ⁻¹)	250
ED (years)	2
ET (h day ⁻¹)	6.0 per 24 h, i.e. 0.250
AT (days)	25 500

^aTR is target carcinogenic risk (dimensionless); C is the concentration of PAH (µg m⁻³); IUR is the chronic inhalation unit risk (µg m⁻³)⁻¹; EFr is the exposure frequency (250 days year⁻¹); ED is the exposure duration (years); ET is indoor air exposure time (h day⁻¹); AT is the number of days over which the exposure is averaged (25 500 days, i.e. 70 years × 365 days year⁻¹).

4.2.7 Statistical analysis

SPSS (IBM SPSS Statistics 20) and Statistica software (v. 7, StatSoft Inc., USA) were used to conduct the statistical analysis. As normal distribution was not obtained by Shapiro–Wilk’s test, means were compared using the nonparametric Mann–Whitney U test. Statistical significance was defined as $p \leq 0.05$.

4.3 Results and Discussion

4.3.1 Primary school environments

4.3.1.1 PAH levels in indoor and outdoor air

The levels of indoor $PM_{2.5}$ -PAHs in ten studied primary schools are summarised in Table 4.7, which shows the indoor mean concentrations (as well as the ranges). In 100% of collected samples dibenzo[a,l]pyrene was below LOD and hence it was excluded from further assessment. Total levels of $\Sigma PAHs$ indoors ranged from 1.68–59.7 ng/m^3 whereas the school mean concentration ranged from 2.82 to 54.1 ng/m^3 . Among all the schools, the highest concentrations of $\Sigma PAHs$ were observed at S2 (Table 4.7) being approximately 2–5 times higher than in remaining places. No specific indoor emission source of PAHs or occupant activities was observed at this school. It must be though pointed out that the corresponding outdoor PAHs levels were similarly the highest at that school (Table 4.8). On the contrary, the lowest indoor levels were observed at S9 which was situated in a residential area.

In general the information regarding indoor PAHs in schools is limited. Furthermore, comparison between various studies is hampered by different study designs, namely in terms of considered particulate fractions (differing between PM_{10} , $PM_{2.5}$, PM_1 , TSP or even unspecified), considerations of compounds in gas phase, or even the number of individual PAHs compounds analysed. Analyzing 15 PAHs, Krugly et al. (2014) found much higher levels of $PM_{2.5}$ -bound PAHs (20.1–131 ng/m^3) in indoor air of schools in Lithuania. With exception to S2, the levels in schools of this study ($\Sigma PAHs$ 2.8–19.9 ng/m^3) were rather similar to those reported for indoor air of schools in Rome (1.6–16.0 ng/m^3 ; Gatto et al., 2014).

The compositional profiles between ten characterized schools of this study were relatively similar. In majority of the schools (S1–S6, S8) acenaphthylene was the most (or the second most abundant at S7 and S9) indoor particulate-bound PAH. On average this compound accounted for 40% of total particulate content ($\Sigma PAHs$), at each school its contribution ranged between 29% (at S2) up to 68% (S4) of $\Sigma PAHs$.

Table 4.7. Indoor levels of PM_{2.5}-bound PAHs (mean, range) at ten studied primary schools S1– S10 (ng/m³).

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Naph	2.13 (0.797–3.48)	1.16 (0.882–1.47)	0.387 (0.363–0.406)	0.214 (0.180–0.247)	0.110 (0.101– 0.123)	0.223 (0.182–0.269)	0.264 (0.195–0.328)	0.275 (0.142–0.431)	0.174 (8.56×10 ⁻² –0.258)	0.263 (0.192–0.335)
Acen	11.4 (11.0–11.8)	15.7 (9.97–21.4)	6.56 (5.82–7.33)	5.42 (4.45–6.42)	4.69 (3.66–5.59)	7.94 (6.45–9.37)	3.90 (1.82–5.95)	4.44 (3.17–5.65)	0.881 (n.d.–1.79)	0.424 (0.395–0.445)
Ace	1.50 (1.25–1.77)	2.33 (2.29–2.39)	0.908 (0.706–1.09)	1.20 (0.806–1.58)	0.914 (0.790–1.04)	1.21 (1.05–1.38)	1.26 (1.05–1.46)	1.07 (0.929–1.22)	1.01 (0.885–1.14)	1.15 (0.714–1.57)
Flu	6.73×10 ⁻² (5.04×10 ⁻² – 8.56×10 ⁻²)	8.34×10 ⁻² (7.25×10 ⁻² – 9.62×10 ⁻²)	1.83×10 ⁻² (1.70×10 ⁻² – 1.99×10 ⁻²)	1.16×10 ⁻² (1.03×10 ⁻² – 1.28×10 ⁻²)	1.60×10 ⁻² (1.32×10 ⁻² – 1.92×10 ⁻²)	4.09×10 ⁻² (2.58×10 ⁻² – 5.60×10 ⁻²)	3.14×10 ⁻² (2.71×10 ⁻² – 3.57×10 ⁻²)	2.14×10 ⁻² (2.03×10 ⁻² – 2.40×10 ⁻²)	5.91×10 ⁻³ (1.64×10 ⁻³ – 1.02×10 ⁻²)	1.80×10 ⁻² (1.57×10 ⁻² – 2.07×10 ⁻²)
Phen	0.374 (0.328–0.419)	0.870 (0.722–0.998)	0.171 (0.154–0.185)	0.101 (9.53×10 ⁻² – 0.111)	0.215 (0.183– 0.249)	0.244 (0.228–0.265)	0.240 (0.183–0.290)	0.253 (0.233–0.272)	8.63×10 ⁻² (7.85×10 ⁻² – 9.46×10 ⁻²)	0.264 (0.248–0.280)
Ant	7.54×10 ⁻² (7.15×10 ⁻² – 7.84×10 ⁻²)	1.68×10 ⁻² (7.39×10 ⁻² – 2.93×10 ⁻²)	1.54×10 ⁻² (5.31×10 ⁻² – 2.69×10 ⁻²)	5.40×10 ⁻³ (4.30×10 ⁻³ – 6.68×10 ⁻³)	8.11×10 ⁻² (5.59×10 ⁻³ – 1.04×10 ⁻²)	1.79×10 ⁻² (1.66×10 ⁻² – 1.97×10 ⁻²)	n.d.	1.08×10 ⁻² (6.73×10 ⁻³ – 1.52×10 ⁻²)	4.29×10 ⁻³ (3.86×10 ⁻³ – 4.68×10 ⁻³)	3.20×10 ⁻³ (2.15×10 ⁻³ – 6.49×10 ⁻³)
Flu	0.286 (2.76×10 ⁻² – 0.550)	6.13 (4.76–7.50)	0.105 (1.61×10 ⁻² – 0.216)	7.52×10 ⁻³ (3.11×10 ⁻³ – 2.34×10 ⁻²)	0.543 (0.106–1.092)	4.48×10 ⁻² (2.45×10 ⁻² – 6.78×10 ⁻²)	3.31×10 ⁻² (2.14×10 ⁻² – 4.67×10 ⁻²)	0.566 (4.95×10 ⁻² – 1.10)	1.92×10 ⁻² (1.35×10 ⁻² – 2.45×10 ⁻²)	3.02×10 ⁻² (2.03×10 ⁻² – 3.97×10 ⁻²)
Pyr	0.197 (0.178–0.211)	1.36 (1.08–1.70)	0.213 (0.169–0.260)	5.83×10 ⁻² (5.35×10 ⁻² – 6.36×10 ⁻²)	0.273 (0.242–0.294)	0.279 (0.247–0.306)	0.527 (0.267–0.770)	0.308 (0.231–0.388)	4.72×10 ⁻² (4.54×10 ⁻² – 4.91×10 ⁻²)	0.135 (9.92×10 ⁻² – 0.169)
B[a]A	4.64×10 ⁻² (4.14×10 ⁻² – 5.10×10 ⁻²)	0.668 (0.461–0.896)	9.29×10 ⁻² (8.02×10 ⁻² – 0.105)	1.24×10 ⁻² (1.00×10 ⁻² – 1.53×10 ⁻²)	7.39×10 ⁻² (6.14×10 ⁻² – 9.25×10 ⁻²)	7.38×10 ⁻² (6.76×10 ⁻² – 8.09×10 ⁻²)	0.160 (8.23×10 ⁻² – 0.239)	7.53×10 ⁻² (6.22×10 ⁻² – 0.877)	1.29×10 ⁻² (1.14×10 ⁻² – 1.55×10 ⁻²)	4.84×10 ⁻² (3.84×10 ⁻² – 5.75×10 ⁻²)
Chry	0.131 (0.111–0.148)	1.33 (0.889–1.79)	0.178 (0.167–0.189)	3.40×10 ⁻² (3.15×10 ⁻² – 3.79×10 ⁻²)	0.166 (0.129–0.201)	0.167 (0.151–0.181)	0.377 (0.189–0.607)	0.195 (0.180–0.209)	3.56×10 ⁻² (3.04×10 ⁻² – 4.10×10 ⁻²)	0.105 (7.46×10 ⁻² – 0.133)

Naph – naphthalene, Acen – acenaphthylene, Ace – acenaphthene, B[a]P – benzo[a]pyrene, DB[a]hA – dibenz[a,h]anthracene, B[a]A – benzo[a]anthracene, Chry – chrysene, B[b]F – benzo[b]fluoranthene, B[k]F – benzo[k]fluoranthene, B[a]P – benzo[a]pyrene, Flu – fluorene, Phen – phenanthrene, Ant – anthracene, Flt – fluoranthene, Pyr – pyrene, B[a]A – benzo[a]anthracene, Chry – chrysene, B[b]F – benzo[b]fluoranthene, B[k]F – benzo[k]fluoranthene, DB[a]hA – dibenz[a,h]anthracene, B[a]P – benzo[a]pyrene, Ind – indeno[1,2,3-cd]pyrene
n.d. – not detected

Table 4.7. Indoor levels of PM_{2.5}-bound PAHs (mean, range) at ten studied primary schools S1– S10 (ng/m³) (continued).

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
B[b+]/F	0.695 (0.585–0.826)	6.57 (5.23–8.06)	0.830 (0.803–0.868)	0.159 (0.154–0.162)	0.966 (0.846–1.07)	0.571 (0.545–0.599)	1.88 (0.933–2.833)	1.08 (0.852–1.32)	0.111 (9.44×10 ⁻² –0.130)	0.559 (0.547–0.572)
B[k]F	0.121 (0.101–0.145)	1.32 (1.04–1.59)	0.159 (0.152–0.164)	3.11×10 ⁻² (3.02×10 ⁻² – 3.28×10 ⁻²)	0.192 (0.178–0.205)	0.108 (9.73×10 ⁻² – 0.121)	0.341 (0.168–0.514)	0.194 (0.146–0.243)	1.96×10 ⁻² (1.64×10 ⁻² – 2.36×10 ⁻²)	8.87×10 ⁻² (7.11×10 ⁻² – 0.106)
B[a]P	0.265 (0.209–0.329)	3.53 (2.87–4.17)	0.389 (0.353–0.463)	5.95×10 ⁻² (5.57×10 ⁻² – 6.36×10 ⁻²)	0.565 (0.551–0.580)	0.224 (0.191–0.257)	0.769 (0.312–1.32)	0.399 (0.313–0.484)	3.73×10 ⁻² (3.06×10 ⁻² – 4.38×10 ⁻²)	0.204 (0.191–0.219)
DB[a,h]A	1.54 (1.27–1.82)	5.00 (n.d.–10.1)	1.44 (1.29–1.53)	0.373 (0.357–0.394)	2.25 (2.17–2.33)	1.06 (0.981–1.12)	4.11 (2.08–6.18)	1.94 (1.40–2.48)	0.157 (0.140–0.180)	1.24 (1.07–1.42)
B[ghi]P	0.608 (0.512–0.741)	4.05 (3.48–4.58)	0.611 (0.556–0.664)	0.235 (0.224–0.252)	0.855 (0.796–0.914)	0.468 (0.458–0.476)	1.29 (0.669–1.90)	0.782 (0.709–0.856)	0.153 (0.138–0.170)	0.461 (0.430–0.490)
Ind	0.442 (0.364–0.522)	4.00 (3.17–4.65)	0.521 (0.479–0.557)	0.115 (0.108–0.123)	0.749 (0.691–0.829)	0.263 (0.218–0.286)	1.27 (0.606–1.92)	0.600 (0.379–0.851)	5.66×10 ⁻² (4.97×10 ⁻² – 6.37×10 ⁻²)	0.326 (0.304–0.358)
ΣPAHs	19.9 (18.1–21.6)	54.1 (48.3–59.7)	12.6 (12.2–13.1)	8.02 (7.43–8.59)	12.6 (11.0–13.9)	12.9 (11.4–14.5)	16.4 (9.24–23.6)	12.2 (9.23–15.3)	2.82 (1.68–3.98)	5.31 (5.03–5.58)
ΣPAHscarc	5.38 (3.47–7.32)	23.6 (14.5–32.7)	3.99 (3.79–4.15)	0.999 (0.962–1.04)	5.07 (4.96–5.17)	2.69 (2.48–2.87)	9.17 (4.72–13.6)	4.76 (3.76–5.80)	0.605 (0.470–0.745)	2.83 (2.63–3.03)

Naph – naphthalene, Acen – acenaphthylene, Ace – acenaphthene, Flu – fluorene, Phen – phenanthrene, Ant – anthracene, Flt – fluoranthene, Pyr – pyrene, B[a]A – benz[a]anthracene, Chry – chrysene, B[b+]/F – benzo[b+]/fluoranthene, B[k]F – benzo[k]fluoranthene, B[a]P – benzo[a]pyrene, DB[a,h]A – dibenz[a,h]anthracene, B[ghi]P – benzo[ghi]perylene, Ind – indeno[1,2,3-cd]pyrene
n.d. – not detected

Table 4.8. Outdoor levels of PM_{2.5}-bound PAHs (mean, range) at studied primary schools (ng/m³).

	S2	S5	S6	S7	S8	S9	S10
Naph	0.343 (0.169–0.534)	9.80×10 ⁻² (6.27×10 ⁻² –0.128)	9.75×10 ⁻² (8.21×10 ⁻² –0.117)	0.159 (4.10×10 ⁻² –0.253)	0.1620 (0.152–0.170)	0.108 (2.61×10 ⁻² –0.206)	3.78×10 ⁻² (2.50×10 ⁻² –5.15×10 ⁻²)
Acen	3.88 (3.48–4.18)	2.23 (1.20–2.95)	2.23 (2.04–2.52)	3.58 (0.477–6.23)	0.962 (0.682–1.17)	2.15 (1.08–3.23)	0.785 (0.172–1.37)
Ace	0.969 (0.689–1.21)	0.378 (0.267–0.447)	0.363 (0.271–0.524)	1.15 (0.884–1.35)	0.352 (0.294–0.420)	0.487 (0.326–0.723)	0.393 (0.291–0.528)
Flu	9.66×10 ⁻² (8.51×10 ⁻² –0.114)	3.41×10 ⁻² (1.59×10 ⁻² –0.070)	1.84×10 ⁻² (1.70×10 ⁻² –1.97×10 ⁻²)	2.94×10 ⁻² (2.21×10 ⁻² –3.52×10 ⁻²)	3.00×10 ⁻² (9.62×10 ⁻³ –4.83×10 ⁻²)	2.33×10 ⁻² (1.35×10 ⁻² –3.33×10 ⁻²)	1.18×10 ⁻² (9.74×10 ⁻³ –1.59×10 ⁻²)
Phen	0.705 (0.468–0.971)	0.245 (0.146–0.431)	0.164 (0.151–0.175)	0.487 (0.455–0.528)	0.352 (0.146–0.638)	0.199 (0.107–0.387)	0.117 (4.00×10 ⁻² –0.208)
Ant	2.06×10 ⁻² (1.89×10 ⁻² –2.18×10 ⁻²)	5.85×10 ⁻³ (4.65×10 ⁻³ –7.30×10 ⁻³)	5.71×10 ⁻³ (3.77×10 ⁻³ –7.59×10 ⁻³)	3.66×10 ⁻² (2.23×10 ⁻² –6.43×10 ⁻²)	2.26×10 ⁻² (5.14×10 ⁻³ –6.46×10 ⁻²)	8.12×10 ⁻³ (6.94×10 ⁻³ –1.64×10 ⁻²)	2.21×10 ⁻³ (1.42×10 ⁻³ –2.88×10 ⁻³)
Fln	4.84 (0.290–9.43)	2.04×10 ⁻² (1.98×10 ⁻² –2.11×10 ⁻²)	2.09×10 ⁻² (1.70×10 ⁻² –2.46×10 ⁻²)	0.260 (0.159–0.339)	1.29 (2.36×10 ⁻² –3.33)	0.133 (1.74×10 ⁻² –0.379)	2.40×10 ⁻² (2.19×10 ⁻² –2.54×10 ⁻²)
Pyr	2.15 (1.90–2.35)	0.586 (0.184–1.38)	0.300 (0.198–0.426)	0.766 (0.707–0.824)	0.539 (0.179–0.878)	0.492 (0.165–1.04)	0.337 (0.274–0.339)
B[a]A	1.39 (0.951–1.86)	0.415 (3.69×10 ⁻² –1.18)	7.95×10 ⁻² (3.73×10 ⁻² –0.117)	0.184 (0.172–0.203)	0.321 (5.39×10 ⁻² –0.641)	0.239 (5.22×10 ⁻² –0.448)	0.118 (8.37×10 ⁻² –0.178)
Chry	2.73 (1.81–3.71)	0.809 (9.28×10 ⁻² –2.23)	0.223 (0.111–0.351)	0.423 (0.370–0.477)	0.756 (0.126–1.33)	0.566 (0.127–1.09)	0.265 (0.181–0.389)
B[b+]F	7.57 (5.25–10.0)	2.14 (0.306–5.50)	0.850 (0.391–1.46)	1.29 (1.04–1.71)	3.12 (0.632–5.63)	1.76 (0.395–3.33)	0.970 (0.789–1.29)
B[k]F	1.59 (1.06–2.10)	0.426 (5.92×10 ⁻² –1.10)	0.174 (7.63×10 ⁻² –0.310)	0.238 (0.193–0.321)	0.564 (0.108–1.03)	0.336 (7.56×10 ⁻² –0.673)	0.191 (0.159–0.251)
B[a]P	3.55 (2.29–4.79)	1.11 (9.43×10 ⁻² –3.29)	0.313 (0.152–0.525)	0.466 (0.432–0.519)	1.20 (0.171–2.21)	0.632 (9.05×10 ⁻² –1.52)	0.332 (0.301–0.383)
DB[a,h]A	11.2 (7.71–14.7)	3.25 (0.571–7.91)	1.39 (0.591–2.46)	2.30 (1.76–3.21)	4.15 (1.08–6.76)	2.40 (0.648–5.12)	1.71 (1.42–2.23)

Naph – naphthalene, Acen – acenaphthylene, Ace – acenaphthene, Flu – fluorene, Phen – phenanthrene, Ant – anthracene, Fln – fluoranthene, Pyr – pyrene, B[a]A – benz[a]anthracene, Chry – chrysene, B[b+]]F – benzo[b+]]fluoranthene, B[k]F – benzo[k]fluoranthene, B[a]P – benzo[a]pyrene, DB[a,h]A – dibenz[a,h]anthracene, B[ghi]P – benzo[ghi]perylene, Ind – indeno[1,2,3-cd]pyrene

The second most abundant compound was dibenz[a,h]anthracene which was in agreement with the previous studies in Oporto Metropolitan Area (Castro et al., 2011; Oliveira et al., 2015a, 2015b). This PAH approximately accounted for 13% Σ_{PAHs} (range 5–25% of Σ_{PAHs} at S4 and S7, respectively) and its high abundance suggests contribution of emissions from motor light-duty gasoline vehicles (Ravindra et al., 2008). Acenaphthene and benzo[b+j]fluoranthene were the other abundant PAHs, accounting approximately for 12% (4–22% at S2 and S10) and 7% (2–11% at S7 and S4) of Σ_{PAHs} ; the contributions of the remaining compounds were much lower (i.e. less than 4 %). The indoor variation of particulate-bound ΣPAHs was not statistically significant ($p > 0.05$) in all of the schools, except for S7, where one classroom exhibited significantly lower PAH levels. The variation of PAH concentrations in S7 was attributed to different position/distance of the classrooms from the main street.

Up to this date, there are no air quality standards for PAHs in indoor air except for a WHO recommendation for naphthalene (set as annual guideline value of $10 \mu\text{g}/\text{m}^3$; WHO 2010). The maximum levels of naphthalene ($2.13 \pm 1.45 \text{ ng}/\text{m}^3$) were observed at S1, being significantly higher than at other schools ($p < 0.05$). However, this mean (as well as other schools) was still below the recommended guideline. It is necessary to point out that naphthalene is a volatile PAH, predominantly found in gas phase (Krugly et al., 2014). Therefore, the assessment of PAHs vapour phase in school environments should be conducted in future in order to correctly evaluate levels of this compound.

Out of 18 analysed PAHs, naphthalene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]luoranthene, benzo[a]pyrene, dibenzo[a,l]pyrene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene were reported as carcinogenic ones (possible, probable) (IARC 2002, 2010). The indoor concentrations of ten carcinogenic PAHs (i.e. Σ_{PAHscarc}) at the ten studied schools are also presented in Table 4.7. On average Σ_{PAHscarc} accounted for 35% of Σ_{PAHs} (range between 12% at S4 to 56% at S7). Dibenz[a,h]anthracene was the most abundant carcinogenic PAH at all schools accounting for approximately 36% of Σ_{PAHscarc} (between 21% at S2 and 45% at S7). Considering the protection of public health, it is important to enhance that at all studied schools dibenz[a,h]anthracene was among the most abundant compounds when all 18 PAHs were considered. Other abundant carcinogen were: benzo[b+j]fluoranthene, that on average accounted for 23% of Σ_{PAHscarc} (range of 13–28% at S1 and S2, respectively), and benzo[a]pyrene (mean: 9% of Σ_{PAHscarc} ; range : 5–15% of Σ_{PAHscarc} at S1 and S2, respectively).

The levels of PM_{2.5}-bound PAHs in ambient air of schools are presented in Table 4.8. With exception to S2, levels of particulate-bound PAHs in outdoor air of Portuguese primary schools were similar to those in winter season in outdoor air of schools in Rome (4.2–18 ng/m³; 7.0–9.54 ng/m³ (Gatto et al., 2014; Romagnoli et al., 2014); for other seasons (0.42–1.74 ng/m³ in spring; 0.36–2.56 ng/m³ in summer) and in other European countries (Lithuania; 40.7–121 ng/m³) considerably different levels of particulate-bound PAHs were observed (Gatto et al., 2014; Krugly et al., 2014; Romagnoli et al., 2014).

The results in Tables 4.7-4.8 also show that total PAH levels in outdoor and indoor air of each schools were not significantly different ($p < 0.05$) with exception to S6 at which indoor Σ_{PAHs} was approximately twice higher than outdoors. School S6 was the only one equipped with kitchen. On contrary to the other schools, meals were directly cooked at the school premises which could result in overall higher indoor levels (in comparison to outdoors). In addition, emissions from some indoor sources could contribute to indoor levels of PAHs; the potential sources observed during the sampling included: use of heating systems, use of cleaning products, and use of artistic supplies (such as candles, wax melting, etc.). The compositional profiles of PAHs in outdoor air were rather similar between the schools and showed some similarities to indoors. Once again dibenz[a,h]anthracene (mean of 23% of Σ_{PAHs} ; 18–28% at S7 and S10), acenaphthylene (mean of 17% of Σ_{PAHs} ; 3–32% at S8 and S6) and benzo[b+j]fluoranthene (15% of Σ_{PAHs} ; ranging between 10% at S7 and 20% at S8) were among the most abundant compounds. Finally, carcinogenic content of PAHs in outdoor air accounted on average for 54% Σ_{PAHs} (range 45–74% at S7 and S8, respectively).

4.3.1.2 Indoor-to-outdoor ratios

Despite the common perception that building borders (walls and windows) can protect occupants from ambient air pollution, various indoor sources of PAHs exist. Evaluation of I/O ratio of PAH concentrations can provide further insight on impacts of indoor/outdoor PAHs on indoor environments and possible identification of the pollutants origin; ratios higher than unity (i.e. >1) suggest that predominantly indoor emissions contribute to indoor PAH levels. The calculated I/O ratios of PM_{2.5}-bound PAHs at studied primary schools are presented in Figure 4.3. At all schools, I/O ratios of light PAH congeners, namely naphthalene, acenaphthene, acenaphthylene highly

exceeded unity. These results indicated a potential contribution of these PAHs from an indoor source, which was consistent with other studies. Similarly, Krugly et al. (2014) reported higher I/O ratios for semivolatile PAHs in indoor air of the schools; authors suggested contribution from activities of the respective occupants and/or evaporation from building materials. The indoor sources that were registered during the sampling included (Morawska et al., 2013): use of artistic supplies such as candles, wax melting, use of cleaning products; during the time of study heating systems (electrical convertors) were used. In addition, on several occasions, birthday party took place in the classrooms (with candles on birthday cake) which could contribute to indoor PAH content (Derudi et al., 2013). Compounds with 3–4 rings, namely fluorene, phenanthrene, anthracene, and fluoranthene showed a mixed trend ($I/O > 1$ at S2, S6, S10; $I/O < 1$ at S7, S8, S9). The remaining PAHs (i.e. with 4–6 rings) exhibited I/O ratios lower 1 at all schools (with exception to S7), indicating an outdoor origin of these contaminants. Moreover, at S8 and S10 these ratios were even < 0.5 suggesting that outdoor sources of 4–6 ringed PAHs were prevailing. S7 was the only schools at which I/O of 5–6 ring PAHs were consistently > 1 . No specific source of PAHs that could justify this occurrence was identified, but this occurrence could be caused by combined effect of position/distance of the classrooms from the main street and the low frequency of ventilation in the respective rooms.

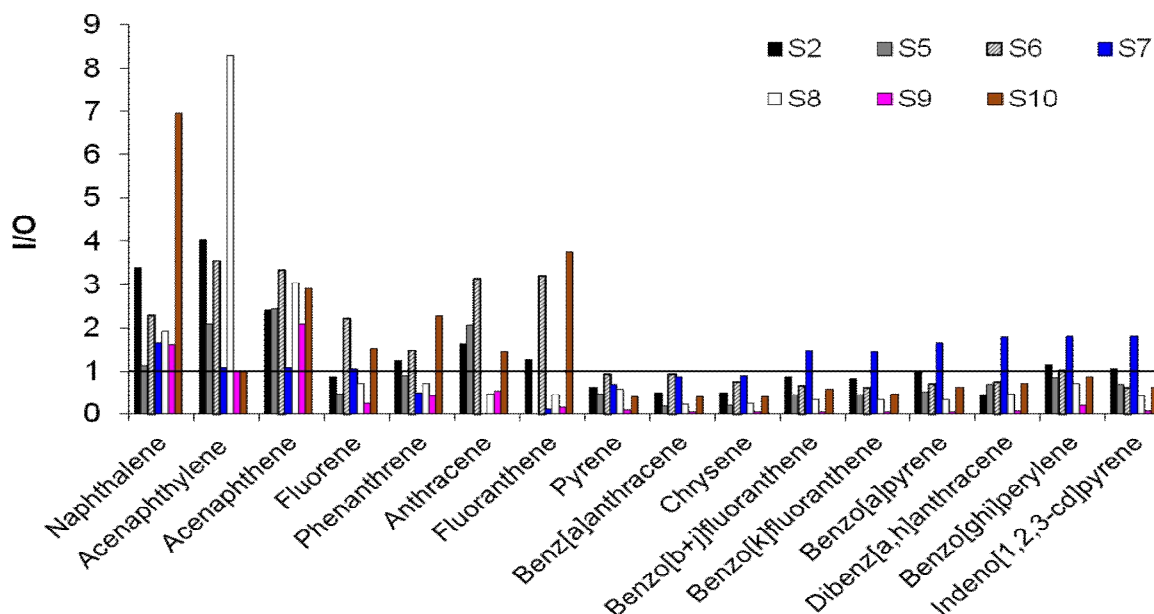


Figure 4.3. Indoor-to-outdoor (I/O) ratio $PM_{2.5}$ -bound PAH ratios at studied schools (S1 – S10).

4.3.1.3 Diagnostic concentration ratios

It is well known that composition of PAH mixtures depends on the nature of emission sources. Thus the ratio values of individual PAH concentrations can be used as a diagnostic tool to identify possible origin of PAHs. The diagnostic ratios calculated for indoor PAHs at ten primary schools are presented in Table 4.9. Ratios of the total concentration of 2 and 3-rings PAHs ($\Sigma\text{PAHs}_{\text{LMW}}$) to 4 and 6-ring PAHs ($\Sigma\text{PAHs}_{\text{HMW}}$) indicate if the origin of PAHs is pyrogenic or petrogenic. In case of ratio value >1 , a possible source of PAHs is petrogenic; otherwise the source may be considered as pyrogenic (Krugly et al., 2014; Zhang et al., 2008). At S2, S5, S7, S8, and S10 the values of this diagnostic ratio were lower than 1, indicating pyrogenic origin of PAHs. Traffic emissions were most likely the source of PAHs at these schools. S2, S8, and S10 were situated in a close vicinity to high-traffic streets (Table 4.2) whereas S7 was located in residential area; at these schools possible PAH sources included vehicular emissions and fuel burning. S5 was located in the residential area with traffic streets and potential impacts of an industrial site in the vicinity. In agreement with these findings, values of benz[a]anthracene/chrysene were higher than 0.35 at all schools and thus indicated vehicular emissions and/or fuel combustions of (Krugly et al., 2014); similarly the values of indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene + benzo[ghi]perylene) implied contributions of traffic emissions (specifically from diesel combustions; Table 4.9).

At S1, S3, S4, S6 and S9 the diagnostic ratios of $\Sigma\text{PAHs}_{\text{LMW}}$ to $\Sigma\text{PAHs}_{\text{HMW}}$ were greater than 1, suggesting petrogenic origin. These results were somewhat surprising considering that pyrogenic origin of PAHs was expected. It is though necessary point out that an oil refinery with a petrochemical complex is located approximately 15 km west from the respective area which could cause the unexpected findings. In addition, binary ratios are only crude indicators; it is often difficult to distinguish between different sources (Galarneau et al., 2008) so caution is always required when reviewing these values. Furthermore, values of the ratios can be altered due to the reactivity of some PAH congeners with other atmospheric species (such as O_3 and/or oxides of nitrogen; Ravindra et al. (2008)). Benz[a]anthracene/chrysene ratios at these schools were >0.35 which suggest an origin from fuel and/or vehicular emissions (Krugly et al., 2014); values of indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene + benzo[ghi]perylene) also pointed towards vehicular traffic emissions (i.e. contribution of diesel combustions and/or road dusts) (Kavouras et al., 2001; Pio et al., 2001; Rogge et al., 1993a).

Table 4.9. Diagnostic ratios for indoor PM_{2.5}-bound PAHs at the ten primary schools.

Ratio	This study										Value	Source	Reference
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10			
^a ΣPAHs _{LMW} /ΣPAH _{HMMW}	3.58	0.59	1.78	6.39	0.90	2.98	0.53	0.99	3.33	0.66	>1 <1	Petrogenic Pyrogenic	Zhang et al. (2008)
Benz[a]anthracene/Chrysene	0.36	0.50	0.52	0.37	0.45	0.44	0.42	0.39	0.36	0.46	>0.35	Fuel combustion	Krugly et al. (2014)
Indeno[1,2,3-cd]pyrene/(Indeno[1,2,3-cd]pyrene + Benzo[ghi]perylene)											0.21–0.22	Gasoline cars	Gogou et al. (1996); Grimmer et al. (1983);
											0.35–0.70	Diesel emissions	Kavouras et al. (2001); Khalli et al. (1995); Pio et al. (2001); Ravindra et al. (2008); Rogge et al. (1993a)
	0.42	0.50	0.46	0.33	0.47	0.36	0.50	0.43	0.27	0.41	0.56	Coal combustion	
											0.62	Wood combustion	
											0.36	Road dust	
Benzo[b]fluoranthene/Benzo[k]fluoranthene	5.75	4.98	5.23	5.12	5.03	5.27	5.51	5.58	5.67	6.31	>0.5	Diesel	Ravindra et al. (2008)
Benzo[ghi]perylene/Benzo[a]pyrene	2.29	1.15	1.57	3.95	1.51	2.09	1.67	1.96	4.11	2.16	1.2–2.2 2.5–3.3 0.86, 0.91	Diesel cars Gasoline cars Road dust	Oda et al. (2001); Rogge et al. (1993a, 1993b)
Benzo[a]pyrene/(Benzo[a]pyrene + Chrysene)	0.67	0.73	0.69	0.64	0.77	0.57	0.67	0.67	0.51	0.66	0.5 0.73	Diesel Gasoline	Ravindra et al. (2008)
Benz[a]anthracene/(Benz[a]anthracene+chrysene)	0.26	0.33	0.34	0.27	0.31	0.31	0.30	0.28	0.27	0.32	0.40, 0.38–0.64 0.76 0.43	Diesel Gasoline Wood combustion	Li and Kamens (1993); Manoli et al. (2004); Sicre et al. (1987)
Benzo[b+k]Fluoranthene/Benzo[ghi]perylene	0.89	6.90	1.09	0.29	1.19	0.80	2.15	1.33	0.24	0.75	1.60 0.33 2.18	Diesel cars Gasoline cars Wood combustion	Li and Kamens (1993)
Fluorene/(Fluorene + Pyrene)	0.25	0.06	0.08	0.17	0.06	0.13	0.06	0.07	0.11	0.12	>0.5 <0.5	Diesel Gasoline	Ravindra et al. (2008)
Phenanthrene/ (Phenanthrene+ Anthracene	0.83	0.98	0.92	0.95	0.96	0.93	1.00	0.96	0.95	0.99	>0.7	Lubricant oils and fossil fuels	Mirante et al. (2013)

^aTotal concentration of 2- and 3-rings PAHs (ΣPAHs_{LMW}), and 4- 6-rings PAHs (ΣPAHs_{HMMW});

Other ratios that were applied to the obtained indoor levels of PAHs at the ten primary schools were also indicative of vehicular influence. The comparison of results in Table 4.9 suggests that emissions from diesel combustions were a relevant source of PAHs. The values of benzo[b]fluoranthene/benzo[k]fluoranthene >0.5 (Ravindra et al., 2008) indicated a contribution of diesel emissions as well as the values of benzo[ghi]perylene/benzo[a]pyrene which mostly ranged between 1.2–2.2. These findings are in agreement with data from Portugal: diesel is the most used car fuel accounting for 78.3% of the total national car fuel consumption (INE 2013). Other diagnostic ratios can be also used to distinguish between diesel and gasoline emissions. Values of benzo[a]pyrene/(benzo[a]pyrene + chrysene), benz[a]anthracene/(benzo[a]anthracene + chrysene), and benzo[b+k]fluoranthene/benzo[ghi]perylene (Table 4.9) indicated a mixed contribution of diesel and gasoline emissions at the characterized schools. The fluorene/(fluorene + pyrene) can be also used to differentiate between diesel and gasoline exhaust origin. Whereas values > 0.5 suggest contribution of diesel emissions, ratio < 0.5 indicates gasoline emissions (Ravindra et al., 2008; Rogge et al., 1993a, 1993b); the present results show very low values thus suggesting some presence of gasoline emissions at schools (Li and Kamens 1993). Finally, at all ten schools, values of phenanthrene to phenanthrene and anthracene ratios were higher than 0.7, indicating emissions from lubricant oils and fossil fuels (Mirante et al., 2013).

4.3.1.4 Health risk assessment

As there is no identifiable threshold below which PAHs do not pose risk to humans (Directive 2004/107/EC), genotoxic and carcinogenic PAHs require the fullest possible risk assessment. Several approaches have been developed to evaluate the potencies of the components of a complex mixture of PAHs. Typically, most of the authors (and in this work) use TEF by Nisbeth and La Goy 1992 (Boström et al., 2002) who estimated values for all PAHs detected in this work. The results of TEF-adjusted concentrations for 18 PAHs in indoor and outdoor air of studied schools are presented in Table 4.10 and Table 4.11, respectively. Dibenz[a,h]anthracene was the second largest contributor (approximately between 5–25 %) to Σ_{PAHs} in indoor air, and due to its high TEF (5) it was the largest contributor to $\Sigma_{\text{TEF-PAHs}}$. This PAH accounted approximately for 93% of both indoor (range 84–95%) and outdoor (92–94%) $\Sigma_{\text{TEF-PAHs}}$ at the selected schools.

Table 4.10. Toxicity equivalency factor (TEF)-adjusted mean concentrations of PAHs in indoor air of studied primary schools (pg/m³).

Compound	TEF ^a	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Naphthalene	0.001	2.13	1.16	0.387	0.214	0.110	0.223	0.264	0.275	0.174	0.263
Acenaphthylene	0.001	11.4	15.7	6.56	5.42	4.69	7.94	3.90	4.44	0.881	0.424
Acenaphthene	0.001	1.50	2.33	0.908	1.10	0.914	1.21	1.26	1.07	1.01	1.15
Fluorene	0.001	6.73×10 ⁻²	8.34×10 ⁻²	1.83×10 ⁻²	1.16×10 ⁻²	1.60×10 ⁻²	4.09×10 ⁻²	3.14×10 ⁻²	2.14×10 ⁻²	5.91×10 ⁻³	1.80×10 ⁻²
Phenanthrene	0.001	0.374	0.870	0.171	0.101	0.215	0.244	0.240	0.253	8.63×10 ⁻²	0.264
Anthracene	0.01	0.754	0.168	0.154	5.40×10 ⁻²	8.11×10 ⁻²	0.179	0.0000	0.108	4.29×10 ⁻²	3.20×10 ⁻²
Fluoranthene	0.001	0.286	6.13	0.105	7.52×10 ⁻³	0.543	4.48×10 ⁻²	3.31×10 ⁻²	0.566	1.92×10 ⁻²	3.02×10 ⁻²
Pyrene	0.001	0.197	1.36	0.213	5.83×10 ⁻²	0.273	0.279	0.527	0.308	4.72×10 ⁻²	0.135
Benz[a]anthracene	0.1	4.64	66.8	9.30	1.24	7.39	7.38	15.9	7.53	1.29	4.84
Chrysene	0.01	1.31	13.3	1.78	0.340	1.66	1.67	3.77	1.95	0.360	1.05
Benzo[b+g]fluoranthene	0.1	69.5	657	83.0	15.9	96.6	57.1	188	108	11.1	56.0
Benzo[k]fluoranthene	0.1	12.1	132	15.9	3.11	19.2	10.8	34.1	19.4	1.96	8.87
Benzo[a]pyrene	1	265	3530	389	60	565	224	769	399	37.3	204
Dibenzo[a,h]anthracene	5	7720	24900	7190	1870	1130	5280	20600	9680	786	6180
Benzo[ghi]perylene	0.01	6.08	40.5	6.11	2.35	8.55	4.68	12.9	7.82	1.53	4.61
Indeno[1,2,3-cd]pyrene	0.1	44.1	400	52.1	11.5	74.9	26.3	127	60.0	5.66	32.6
Σ TEF-PAHs	–	8140	29850	7753	1966	12050	5625	21730	10300	848	6492

^aTEF estimated by Nisbeth and LaGoy 1992 (Boström et al., 2002).

Table 4.11. Toxicity equivalency factor (TEF)–adjusted mean concentrations of PAHs in outdoor air of studied primary schools (pg/m³).

Compound	TEF ^a	S2	S5	S6	S7	S8	S9	S10
Naphthalene	0.001	0.342	9.80×10 ⁻²	9.75×10 ⁻²	0.160	0.144	0.108	3.78×10 ⁻²
Acenaphthylene	0.001	3.88	2.23	2.23	3.58	0.534	2.15	0.785
Acenaphthene	0.001	0.970	0.378	0.363	1.15	0.352	0.487	0.393
Fluorene	0.001	0.0966	3.41×10 ⁻²	1.84×10 ⁻²	2.94×10 ⁻²	3.00×10 ⁻²	2.33×10 ⁻²	1.18×10 ⁻²
Phenanthrene	0.001	0.705	0.245	0.164	0.487	0.352	0.199	0.117
Anthracene	0.01	0.103	3.90×10 ⁻²	5.71×10 ⁻²	0.366	0.226	8.12×10 ⁻²	2.21×10 ⁻²
Fluoranthene	0.001	4.84	6.80×10 ⁻³	1.39×10 ⁻²	0.260	1.29	0.122	8.01×10 ⁻³
Pyrene	0.001	2.15	0.586	0.300	0.766	0.540	0.492	0.337
Benz[a]anthracene	0.1	139	41.5	7.95	18.4	32.0	23.9	11.8
Chrysene	0.01	27.3	8.09	2.23	4.23	7.60	5.66	2.65
Benzof[b+i]fluoranthene	0.1	757	214	85.0	129	312	176	97.0
Benzof[k]fluoranthene	0.1	159	42.6	17.4	23.8	56.4	33.6	19.1
Benzof[a]pyrene	1	3550	1113	313	466	1204	632	332
Dibenz[a,h]anthracene	5	55910	16240	6950	11510	20760	11980	8550
Benzofghi]perylene	0.01	35.1	10.1	4.53	7.13	11.0	7.72	5.35
Indeno[1,2,3-cd]pyrene	0.1	377	108	42.1	70.3	138	77.4	52.8
Σ TEF-PAHs	–	60960	17780	7421	12230	22530	12940	9075

^aTEF estimated by Nisbeth and LaGoy 1992 (Boström et al., 2002).

These results thus emphasize the importance of analysis and evaluation of this potent carcinogen that is being, among others, discussed as a possible surrogate for PAH mixtures from various environments (Okona-Mensah et al., 2005). Benzo[a]pyrene, the most known and studied carcinogenic PAH, was the second most abundant compound accounting for 3–11% of $\Sigma_{\text{TEF-PAHs}}$ at indoor, and 4–6% at outdoor air. The values of $\Sigma_{\text{TEF-PAHs}}$ were used to estimate the corresponding lifetime lung cancer risks for the exposed populations using the WHO unit risk of 8.7×10^{-5} per ng m^{-3} of benzo[a]pyrene for lifetime (70 years) exposure (WHO 2010). The lung cancer risks due to total school exposure (i.e. sum of both indoors and outdoors) ranged between 6.53×10^{-5} (at S9) and 8.70×10^{-4} (S2). Thus the values of lung cancer risks estimated for the characterized primary schools exceeded WHO health-based level of 10^{-5} (Boström et al., 2002), being approximately 7–87 times higher than the recommended guideline. It is though necessary to point out that the respective values might be even higher as risks were estimated considering 6 h of indoor exposure time (as spent in classrooms). Nevertheless, in the remaining school time (additional 1–1.5h) children move between other school indoor microenvironments (libraries, gymnasiums, canteens, schools public halls) and the exposures in these places could contribute to overall indoor exposure to PAHs (and consequently to the respective risks). Therefore, future assessment of PAHs levels and risks in these school microenvironments would be of a great importance.

The target carcinogenic risks associated with inhalation exposure to PAHs for children (8–10 years old) and adults were estimated by USEPA methodology and the means are presented in Table 4.12. USEPA sets a risk level of 10^{-6} for carcinogenic individual compounds and pathways with the understanding that it will generally cause negligible cancer risks. However, caution is recommended to ensure that cumulative cancer risks of all potential carcinogenic components do not have residual cancer risk exceeding this threshold. Target risks due to indoor exposure ($\Sigma \text{TR}_{\text{Indoor}}$) exceeded the recommended guideline at all schools with exception to S4 and S9 (due to much lower levels of the respective PAH indoors; Table 4.7), whereas risks due to outdoor exposure ($\Sigma \text{TR}_{\text{Outdoor}}$) were below 10^{-6} and thus negligible at all schools. Specifically, $\Sigma \text{TR}_{\text{Indoor}}$ were 2–10 times higher than $\Sigma \text{TR}_{\text{Outdoor}}$ probably due prolonged periods that children spent indoors (6 h indoors in classrooms versus 1–1.25 h outdoors). Overall, $\Sigma \text{TR}_{\text{Indoor}}$ contributed 70–90% of total school risks ($\Sigma \text{TR}_{\text{School}}$, i.e. sum of both indoors and outdoors). The especially high contributions of indoor risks emphasize the need for future studies on these types of environments.

Table 4.12. Target carcinogenic risks (TR) for 8–10 years old children at ten primary schools S1–S10.

Children 8–10 years old										
Indoor air	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Naphthalene	3.55×10^{-10}	1.93×10^{-10}	6.43×10^{-11}	3.55×10^{-11}	1.83×10^{-11}	3.70×10^{-11}	4.39×10^{-11}	4.57×10^{-11}	2.90×10^{-11}	4.37×10^{-11}
Benz[a]anthracene	2.50×10^{-11}	3.59×10^{-10}	5.00×10^{-11}	6.68×10^{-12}	3.97×10^{-11}	3.97×10^{-11}	8.58×10^{-11}	4.05×10^{-11}	6.94×10^{-12}	2.60×10^{-11}
Chrysene	7.02×10^{-12}	7.15×10^{-11}	9.58×10^{-12}	1.83×10^{-12}	8.92×10^{-12}	9.00×10^{-12}	2.03×10^{-11}	1.05×10^{-11}	1.91×10^{-12}	5.63×10^{-12}
Benzof[k]fluoranthene	3.74×10^{-10}	3.54×10^{-9}	4.47×10^{-10}	8.57×10^{-11}	5.20×10^{-10}	3.07×10^{-10}	1.01×10^{-9}	5.83×10^{-10}	5.99×10^{-11}	3.01×10^{-10}
Benzof[k]fluoranthene	6.50×10^{-11}	7.10×10^{-10}	8.54×10^{-11}	1.68×10^{-11}	1.03×10^{-10}	5.83×10^{-11}	1.84×10^{-10}	1.05×10^{-10}	1.06×10^{-11}	4.77×10^{-11}
Benzof[a]pyrene	1.43×10^{-6}	1.90×10^{-5}	2.09×10^{-6}	3.20×10^{-7}	3.04×10^{-6}	1.20×10^{-6}	4.14×10^{-6}	2.15×10^{-6}	2.01×10^{-7}	1.10×10^{-6}
Dibenz[a,h]anthracene	9.07×10^{-9}	2.93×10^{-8}	8.44×10^{-9}	2.19×10^{-9}	1.32×10^{-8}	6.20×10^{-9}	2.42×10^{-8}	1.14×10^{-8}	9.23×10^{-10}	7.25×10^{-9}
Indeno[1,2,3-cd]pyrene	2.38×10^{-10}	2.15×10^{-9}	2.81×10^{-10}	6.20×10^{-11}	4.03×10^{-10}	1.42×10^{-10}	6.82×10^{-10}	3.23×10^{-10}	3.05×10^{-11}	1.75×10^{-10}
ΣTR_{Indoor}	1.44×10^{-6}	1.90×10^{-5}	2.10×10^{-6}	3.23×10^{-7}	3.06×10^{-6}	1.21×10^{-6}	4.16×10^{-6}	2.16×10^{-6}	2.02×10^{-7}	1.11×10^{-6}
Outdoor air										
S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	
Naphthalene	9.50×10^{-12}			2.72×10^{-12}	2.70×10^{-12}	4.41×10^{-12}	3.99×10^{-12}	3.00×10^{-12}	3.00×10^{-12}	1.05×10^{-12}
Benz[a]anthracene	1.24×10^{-10}			3.72×10^{-11}	7.13×10^{-12}	1.65×10^{-11}	2.87×10^{-11}	2.14×10^{-11}	2.14×10^{-11}	1.06×10^{-11}
Chrysene	2.45×10^{-11}			7.25×10^{-12}	2.00×10^{-12}	3.79×10^{-12}	6.78×10^{-12}	5.08×10^{-12}	5.08×10^{-12}	2.37×10^{-12}
Benzof[k]fluoranthene	6.79×10^{-10}			1.92×10^{-10}	7.63×10^{-11}	1.15×10^{-10}	2.80×10^{-10}	1.58×10^{-10}	1.58×10^{-10}	8.70×10^{-11}
Benzof[k]fluoranthene	1.43×10^{-10}			3.82×10^{-11}	1.56×10^{-11}	2.14×10^{-11}	5.06×10^{-11}	3.01×10^{-11}	3.01×10^{-11}	1.72×10^{-11}
Benzof[a]pyrene	3.18×10^{-6}			9.98×10^{-7}	2.81×10^{-7}	4.18×10^{-7}	1.08×10^{-6}	5.67×10^{-7}	5.67×10^{-7}	2.98×10^{-7}
Dibenz[a,h]anthracene	1.09×10^{-8}			3.18×10^{-9}	1.36×10^{-9}	2.25×10^{-9}	4.06×10^{-9}	2.34×10^{-9}	2.34×10^{-9}	1.67×10^{-9}
Indeno[1,2,3-cd]pyrene	3.38×10^{-10}			9.65×10^{-11}	3.77×10^{-11}	6.30×10^{-11}	1.24×10^{-10}	6.95×10^{-11}	6.95×10^{-11}	4.74×10^{-11}
$\Sigma TR_{Outdoor}$	3.19×10^{-6}			1.00×10^{-6}	2.83×10^{-7}	4.21×10^{-7}	1.08×10^{-6}	5.70×10^{-7}	5.70×10^{-7}	3.00×10^{-7}

Table 4.12. Target carcinogenic risks (TR) for 8–10 years old children at ten primary schools S1–S10 (continued).

Children 8–10 years old										
Total air	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Naphthalene	3.55×10^{-10}	2.03×10^{-10}	6.43×10^{-11}	3.55×10^{-11}	2.11×10^{-11}	3.97×10^{-11}	4.83×10^{-11}	4.97×10^{-11}	3.20×10^{-11}	4.48×10^{-11}
Benz[a]anthracene	2.50×10^{-11}	4.84×10^{-10}	5.00×10^{-11}	6.68×10^{-12}	7.70×10^{-11}	4.69×10^{-11}	1.02×10^{-10}	6.92×10^{-11}	2.84×10^{-11}	3.66×10^{-11}
Chrysene	7.02×10^{-12}	9.60×10^{-11}	9.58×10^{-12}	1.83×10^{-12}	1.62×10^{-11}	1.10×10^{-11}	2.41×10^{-11}	1.73×10^{-11}	6.99×10^{-12}	8.00×10^{-12}
Benzo[b]fluoranthene	3.74×10^{-10}	4.22×10^{-9}	4.47×10^{-10}	8.57×10^{-11}	7.12×10^{-10}	3.83×10^{-10}	1.13×10^{-9}	8.64×10^{-10}	2.18×10^{-10}	3.88×10^{-10}
Benzo[k]fluoranthene	6.50×10^{-11}	8.53×10^{-10}	8.54×10^{-11}	1.68×10^{-11}	1.41×10^{-10}	7.39×10^{-11}	2.05×10^{-10}	1.55×10^{-10}	4.07×10^{-11}	6.49×10^{-11}
Benzo[a]pyrene	1.43×10^{-6}	2.22×10^{-5}	2.09×10^{-6}	3.20×10^{-7}	4.04×10^{-6}	1.49×10^{-6}	4.55×10^{-6}	3.23×10^{-6}	7.68×10^{-7}	1.40×10^{-6}
Dibenz[a,h]anthracene	9.07×10^{-9}	4.03×10^{-8}	8.44×10^{-9}	2.19×10^{-9}	1.64×10^{-8}	7.56×10^{-9}	2.64×10^{-8}	1.54×10^{-8}	3.27×10^{-9}	8.93×10^{-9}
Indeno[1,2,3-cd]pyrene	2.38×10^{-10}	2.49×10^{-9}	2.81×10^{-10}	6.20×10^{-11}	5.00×10^{-10}	1.80×10^{-10}	7.45×10^{-10}	4.47×10^{-10}	9.99×10^{-11}	2.23×10^{-10}
ΣTR_{School}	1.44×10^{-6}	2.22×10^{-5}	2.10×10^{-6}	3.23×10^{-7}	4.06×10^{-6}	1.49×10^{-6}	4.58×10^{-6}	3.24×10^{-6}	7.71×10^{-7}	1.41×10^{-6}

In addition, better understanding of the complexity of child school exposure and the respective health impacts will allow the development and implementation of protective guidelines of indoor air quality standards in these environments that are so vital for child healthy development.

The carcinogenic risks due to total (indoor and outdoor) school exposure were also estimated. Results in Figure 4.4a show that with exceptions to S4 (absence of outdoor PAH assessment) and S9 (significantly lower, i.e. the lowest indoor PAHs; Table 4.7), carcinogenic risks exceeded (up to 22 times) the recommended guideline at all schools. For adults, carcinogenic risks due to total exposure exceeded (2–166 times) the recommended level of 10^{-6} at all schools (Figure 4.4b), at S2 being even higher than the more stringent guideline of 10^{-4} . For adult populations of school personnel the risks of overall school exposure ($\Sigma TR_{\text{school}}$) were about 8 times greater than children, probably due to the longer exposure duration (15 *versus* 2 years).

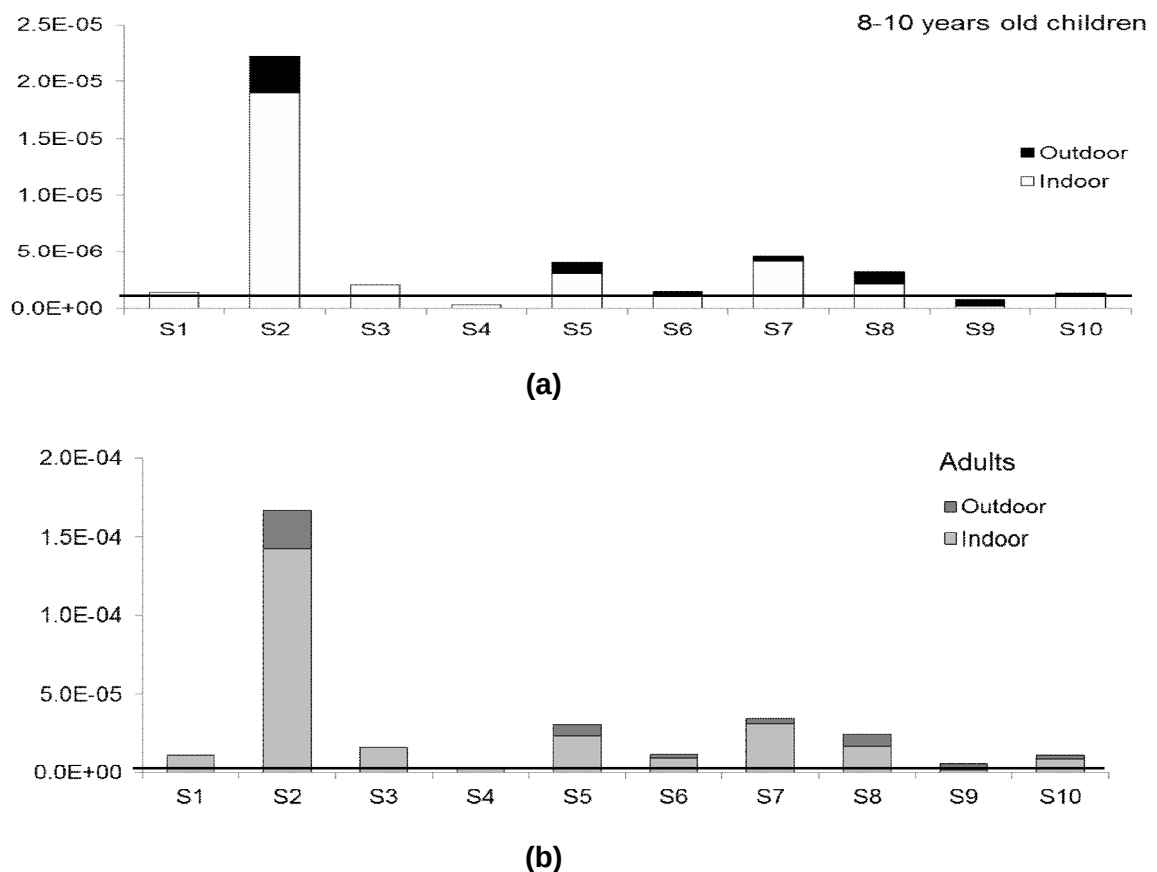


Figure 4.4. Total carcinogenic risks due to school (i.e. both indoor and outdoor) exposure: (a) for 8–10 years old children; (b) for adults. The black horizontal line identifies United States Environmental Protection Agency recommended guideline level of 10^{-6} .

4.3.2 Preliminary study at an Oporto preschool

4.3.2.1 Particulate matter levels

The indoor concentrations of $PM_{2.5}$ and PM_1 ranged, respectively, from 2.1 to $106 \mu g/m^3$ (mean of $27 \mu g/m^3$) and 1.1 to $82 \mu g/m^3$ (mean of $24 \mu g/m^3$). On average, a majority of the $PM_{2.5}$ was comprised of PM_1 ; these particles accounted for 91% of $PM_{2.5}$. Furthermore, the outdoor air exhibited similar levels of $PM_{2.5}$ as indoors, with outdoor concentrations ranging from 5.1 to $113 \mu g/m^3$ (mean of $29 \mu g/m^3$); the estimated indoor and outdoor means were not significantly different ($p < 0.05$). The concentrations of $PM_{2.5}$ in the indoor and outdoor air were slightly higher than $25 \mu g/m^3$ defined by Portuguese Regulation (2013).

4.3.2.2 PAH levels in indoor and outdoor air

The levels of indoor PAHs associated with each phase are summarised in Table 4.13, which shows the indoor mean concentrations (as well as median and interquartile range) of 18 PAHs in $PM_{2.5}$, PM_1 , and in the gas phase at the studied preschool. In agreement with the previous studies on IAQ in the Oporto Metropolitan Area (Castro et al., 2011; Slezakova et al., 2009) dibenz[a,h]anthracene was the most abundant indoor particle-bound PAH at the studied preschool. This compound reached mean concentrations of 1.7 and $1.4 ng/m^3$ in $PM_{2.5}$ and PM_1 , respectively, accounting in both PM size fractions for about 30% of Σ_{PAHs} . The dominance of this compound indicates influence from light-duty gasoline vehicle emissions (Ravindra et al., 2008). The other abundant PAHs in $PM_{2.5}$ and PM_1 were, in descending order, benzo[b+j]fluoranthene (approximately 15%), indeno[1,2,3-cd]pyrene (12–14% in $PM_{2.5}$ and PM_1 , respectively) and benzo[ghi]perylene (8% in $PM_{2.5}$, 10% in PM_1). Naphthalene was the least abundant particle-bound PAH (approximately 2%); but, in the gas phase, it was the second most abundant compound (26% of gaseous Σ_{PAHs}) reaching a mean of $7.7 ng m^{-3}$. Other abundant gaseous compounds were, in descending order, phenanthrene (32% of Σ_{PAHs}), fluorene (19%), and acenaphthylene (13%). These four PAHs accounted in total for 90% of indoor gaseous Σ_{PAHs} at the studied preschool whereas each of the 5–6 ringed compounds accounted approximately for less than 0.5% of gaseous Σ_{PAHs} . Regarding the levels of PAHs in air (i.e. sum of PAH levels in $PM_{2.5}$ and in gas phase; Table 4.13), the total concentrations of 18 PAHs (Σ_{PAHs}) ranged from 20 to $82 ng/m^3$.

with a mean of 35 ng/m³. Specifically, naphthalene reached a mean of 7.8 ng/m³ which is below the WHO recommend annual guideline value of 10 µg/m³ (WHO 2010). Krugly et al. (2014) observed naphthalene levels in similar ranges in five elementary schools in Lithuania (medians between 12 and 27 ng/m³). Wilson et al. (2003) reported higher concentrations of naphthalene in indoor air of a preschool (median of 546 ng/m³); still these levels were well below the WHO recommended guideline.

There is limited information on indoor PAHs in educational environments (i.e. in kindergartens, preschools and schools). The reported levels of all studies available in literature are summarized in Table 4.1. In general, the levels of PM_{2.5}-bound PAHs at PS1 were similar to those reported from elementary schools in Rome during winter season (Gatto et al., 2014) whereas no information was found on PM₁-bound PAHs in indoor air of schools and/or preschools. Concerning gaseous PAHs, the most similar range of concentrations was observed in primary schools in Lithuania (Krugly et al., 2014). Overall, there were very significant differences among the levels and ranges of PAHs reported in the literature for indoor air of educational environments, ranging from 0.21 ng/m³ in Rome, Italy (Gatto et al., 2014) to 619 ng/m³ in North Carolina, USA (Wilson et al., 2003). Certainly, geographical and seasonal influences, meteorological conditions, level of urbanization and development of the surrounding area could account for some of the differences. However, it is necessary to point out that study designs varied greatly between the mentioned works, namely in terms of considered indoor environments (preschool, elementary or primary school), particle size fractions (differing between PM₁, PM_{2.5}, PM₁₀, TSP, or unspecified), consideration of compounds present in the gas phase, and the number of individual PAHs compounds analysed, which can influence the significance of comparisons across studies.

Figure 4.5 shows the distribution of PAHs between particle and gas phases in the indoor air of the studied preschool. The distribution of PAHs between the two phases predominantly depends on the physical characteristics of the compounds as well as on the physical conditions of the studied environments such as temperature and relative humidity (Ravindra et al., 2008). Typically, PAHs with higher molecular weight (5 and more aromatic rings) are mostly associated with the particle phase, whereas compounds with 2 to 3 rings are predominantly found in the gas phase (Guo et al., 2011). As demonstrated in Figure 4.5, the phase-distribution of PAHs in indoor air of the studied preschool was in agreement with findings of other studies (Possanzini et al., 2004).

Table 4.13. Indoor levels of PAHs in PM_{2.5}, PM₁, gas phase, and in air (i.e. total) at the studied preschool (ng/m³).

Indoor Compound	Particulate phase				Gas phase				Air							
	PM _{2.5}				PM ₁											
	Mean	25 th	Median	75 th	Mean	25 th	Median	75 th	Mean	25 th	Median	75 th				
Naphthalene	0.14	3.5×10 ⁻²	4.9×10 ⁻²	0.16	n.d.	n.d.	n.d.	n.d.	7.7	4.6	5.9	12.6	7.8	4.4	5.6	11
Acenaphthylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.8	2.0	2.8	4.6	3.8	2.0	2.8	3.9
Acenaphthene	0.23	0.13	0.26	0.33	n.d.	n.d.	n.d.	n.d.	0.96	0.39	0.59	1.4	1.1	0.20	0.67	1.2
Fluorene	2.5×10 ⁻²	1.2×10 ⁻²	2.0×10 ⁻²	2.9×10 ⁻²	2.5×10 ⁻²	1.0×10 ⁻²	1.9×10 ⁻²	2.8×10 ⁻²	5.6	3.1	4.9	6.7	5.6	2.9	4.7	6.5
Phenanthrene	0.13	6.1×10 ⁻²	0.11	0.17	0.11	5.5×10 ⁻²	7.7×10 ⁻²	0.17	9.6	7.5	8.6	11	9.8	7.3	8.7	11
Anthracene	4.2×10 ⁻²	1.7×10 ⁻²	2.7×10 ⁻²	5.8×10 ⁻²	3.6×10 ⁻²	1.1×10 ⁻²	2.7×10 ⁻²	5.5×10 ⁻²	0.15	4.3×10 ⁻²	0.16	0.20	0.20	0.13	0.18	0.21
Fluoranthene	0.37	0.20	0.34	0.46	0.35	0.23	3.9×10 ⁻²	0.44	0.52	0.48	0.54	0.56	0.88	0.70	0.78	0.89
Pyrene	0.29	0.12	0.24	0.40	0.19	6.2×10 ⁻²	9.9×10 ⁻²	0.34	0.72	0.45	0.64	0.92	1.0	0.60	0.94	1.1
Benz[a]anthracene	0.12	4.2×10 ⁻²	9.9×10 ⁻²	0.16	8.5×10 ⁻²	2.3×10 ⁻²	3.5×10 ⁻²	0.13	1.7	1.1×10 ⁻²	1.6×10 ⁻²	2.0×10 ⁻²	0.13	6.1×10 ⁻²	9.4×10 ⁻²	0.16
Chrysene	0.31	0.16	0.25	0.44	0.24	0.14	0.25	0.32	9.9×10 ⁻²	5.3×10 ⁻²	9.6×10 ⁻²	0.13	0.41	0.17	0.32	0.56
Benz[b]fluoranthene	0.96	0.44	0.76	1.1	0.69	0.25	0.38	0.91	0.13	8.9×10 ⁻²	0.12	0.16	1.1	0.60	0.79	1.2
Benz[k]fluoranthene	0.20	0.10	0.16	0.24	0.15	5.9×10 ⁻²	9.5×10 ⁻²	0.19	2.3×10 ⁻²	1.7×10 ⁻²	2.1×10 ⁻²	2.4×10 ⁻²	0.24	0.13	0.18	0.25
Benz[a]pyrene	0.36	0.17	0.26	0.40	0.29	0.11	0.16	0.39	8.9×10 ⁻²	4.9×10 ⁻²	6.5×10 ⁻²	0.11	0.45	0.28	0.32	0.48
Dibenzo[a,h]pyrene	6.4×10 ⁻²	2.1×10 ⁻²	5.8×10 ⁻²	0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.4×10 ⁻²	2.1×10 ⁻²	5.8×10 ⁻²	0.11
Dibenzo[a,h]anthracene	1.7	0.84	1.4	2.4	1.4	0.79	0.98	1.92	2.9	8.2×10 ⁻²	0.14	0.24	1.9	0.93	1.5	2.2
Benzol[ghi]perylene	0.50	0.25	0.39	0.58	0.46	0.21	0.25	0.52	5.5×10 ⁻²	7.5×10 ⁻²	0.11	0.12	0.59	0.30	0.51	0.67
Indeno[1,2,3-cd]pyrene	0.72	0.47	0.58	0.92	0.65	0.50	0.62	0.85	n.d.	n.d.	n.d.	n.d.	0.72	0.47	0.58	0.92
ΣPMHs	6.2	3.0	4.6	7.6	4.7	1.9	3.5	6.0	30	17	25	36	35	24	30	40
ΣPMHs _{arc}	4.6	2.2	3.6	5.9	3.5	1.0	2.5	3.7	82	52	6.1	13	13	7.6	9.8	15

n.d. – not detected.

Compounds with 2-3 aromatic rings, namely naphthalene, acenaphthylene, fluorene, and phenanthrene were almost entirely present in the gas phase (i.e. more than 99%). On the contrary, PAHs with 5 rings, namely benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, were predominantly bound to particles (i.e. more than 90%). Compounds with 6 aromatic rings such as dibenzo[a,l]pyrene and indeno[1,2,3-cd]pyrene were entirely present in particles. Finally, PAHs with 4 rings that included fluoranthene, pyrene, benz[a]anthracene and chrysene were distributed between the two phases with the proportions in the gas phase ranging between 13 and 71%. In that regard, it is necessary to point out that acenaphthene (3 aromatic rings) exhibited unexpected high distribution in the particle phase (i.e. 19%). No reason was identified which could justify this abundance in PM, though unlike other individual PAHs acenaphthene was detected only in 51% of the existent particulate samples and this might contribute to the apparent inconsistency. Overall, the PAH phase distribution of the studied preschool was similar to those of primary schools reported by Krugly et al. (2014); other studies available in literature that included characterization of PAHs in air (i.e. assessment of both gaseous and particulate phases) in educational settings (Table 4.1) did not specifically report these findings.

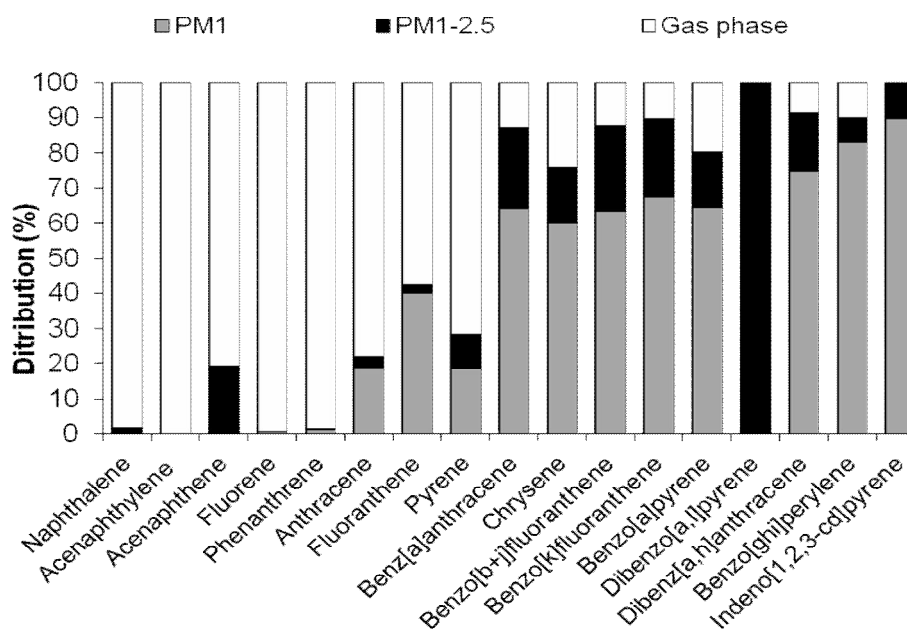


Figure 4.5. Distribution of PAHs between particulate and gaseous phases in indoor air of the studied preschool. The particle phase is further divided into PM₁ and PM_{1-2.5} fractions.

The results in Table 4.13 and Figure 4.5 also demonstrate that particulate PAHs were associated with particles of smaller sizes, i.e. $PM_{1.0}$. For all 18 individual compounds, the proportion in $PM_{1.0}$ (in comparison with $PM_{2.5}$) ranged between 65% (pyrene) and 95% (fluoranthene). On average, $PM_{1.0}$ -bound PAHs accounted for 76% for particulate Σ_{PAHs} whereas PAHs on particles bigger than $1\ \mu m$ (i.e. $PM_{1-2.5}$) accounted for 14% of PAH particulate content. Overall, these results confirmed the previously reported findings that particulate-bound PAHs are predominantly found in smaller fractions of PM (Klejnowski et al., 2010; Ladji et al., 2014). Due to their ability to deposit deep in the lower airways, the smaller classes of PM are more apt to cause respiratory toxicity and dysfunction. Furthermore, various chemical compounds bound to these small particles, such as PAHs, may eventually enhance (or at least contribute to) the PM-induced adverse health effects (Kim et al., 2013; Saravia et al., 2013).

The mean concentrations of the analysed ten carcinogenic PAHs (i.e. $\Sigma_{PAH_{scarc}}$) in indoor PM, gas phase, and in indoor air are reported also in Table 4.13. Dibenz[a,h]anthracene, a strong carcinogen with a TEF 5 times higher than benzo[a]pyrene (Okona-Mensah et al., 2005), was the most abundant carcinogen and accounted for 40% of $\Sigma_{PAH_{scarc}}$. Considering the protection of public health, it is important to stress that dibenz[a,h]anthracene exhibited the highest concentrations of all 18 PAHs in both $PM_{2.5}$ and $PM_{1.0}$ in indoor air of the studied preschool, being followed by other carcinogens: benzo[b+j]fluoranthene (20% of $\Sigma_{PAH_{scarc}}$) and indeno[1,2,3-cd]pyrene (19 and 16% of $\Sigma_{PAH_{scarc}}$ in $PM_{1.0}$ and $PM_{2.5}$, respectively). Finally, benzo[a]pyrene was the fifth most abundant particulate (and the fourth most abundant carcinogenic) PAH, contributing 8% of $\Sigma_{PAH_{scarc}}$ in both PM size fractions. Concerning the gas phase, naphthalene was the predominant carcinogenic gaseous PAH (94% of gaseous $\Sigma_{PAH_{scarc}}$) as the content of other carcinogenic compounds was much less significant (i.e. 0.5–2% of gaseous $\Sigma_{PAH_{scarc}}$).

The total concentration of the carcinogenic PAHs in indoor air of the studied preschool was $13\ ng/m^3$. The obtained results showed that 64% of $\Sigma_{PAH_{scarc}}$ existed in the gas phase whereas 36% was particle-bound. Despite this distribution, carcinogenic PAHs accounted for 27% of the gaseous PAH content whereas it was 74% of Σ_{PAHs} in both $PM_{2.5}$ and $PM_{1.0}$; similarly Jyethi et al. (2014) reported that carcinogenic PAHs accounted for approximately 75% of the particulate PAH content. Finally, in agreement with the findings on the distribution of PAHs (Figure 4.5), carcinogenic particulate PAHs were predominantly associated with $PM_{1.0}$ (76%).

The levels of PAHs in PM_{2.5} measured outdoors in the preschool yard are presented in Table 4.14. Overall, concentration levels of outdoor particulate PAHs were similar to those reported in earlier studies (Castro et al., 2011; Slezakova et al., 2013a, 2013b). The levels registered in ambient air of the selected preschool were much higher compared to other Portuguese and Italian cities (Alves et al., 2014; Cirillo et al., 2006).

Table 4.14. Concentrations of PM_{2.5}–bound PAHs in ambient (i.e. outdoor) air of the studied preschool (ng/m³).

Compound	Mean	25 th	Median	75 th
Naphthalene	0.76	5.5×10 ⁻²	6.9×10 ⁻²	0.20
Acenaphthylene	0.80	0.40	0.83	1.2
Acenaphthene	0.12	3.3×10 ⁻²	4.5×10 ⁻²	0.18
Fluorene	5.0×10 ⁻²	1.4×10 ⁻²	3.1×10 ⁻²	3.8×10 ⁻²
Phenanthrene	0.27×10 ⁻²	0.12	0.17	0.31
Anthracene	7.1×10 ⁻²	2.7×10 ⁻²	5.0×10 ⁻²	0.10
Fluoranthene	0.61	0.26	0.45	1.0
Pyrene	0.62	0.28	0.44	0.75
Benz[a]anthracene	0.25	7.1×10 ⁻²	0.13	0.27
Chrysene	0.60	0.27	0.33	0.76
Benzo[b+j]fluoranthene	1.3	0.39	0.86	1.7
Benzo[k]fluoranthene	0.29	0.11	0.18	0.33
Benzo[a]pyrene	0.49	0.15	0.31	0.57
Dibenzo[a,l]pyrene	9.8×10 ⁻²	5.7×10 ⁻²	8.2×10 ⁻²	0.12
Dibenz[a,h]anthracene	2.2	0.88	1.5	2.5
Benzo[ghi]perylene	0.70	0.32	0.42	0.76
Indeno[1,2,3-cd]pyrene	0.79	0.20	0.54	0.94
ΣPAHs	8.9	4.0	5.5	13
ΣPAHscarce	6.8	2.8	4.2	10

On the contrary, in close proximity of schools situated in Asian countries authors reported much higher concentrations of particle-bound PAHs (Jyethi et al., 2014; Ruchirawat et al., 2006, 2007) compared to this study. In general, the observed concentrations of outdoor particulate PAHs were higher than the indoor ones, but the compositional profiles were similar. The most abundant compounds were (in descending order): dibenz[a,h]anthracene (25% of ΣPAH_{scarce}), benzo[b+j]fluoranthene (approximately 15%), indeno[1,2,3-cd]pyrene (9%) and benzo[ghi]perylene (8%). Finally, ten carcinogenic PAHs accounted for 78% of the particulate PAHs, being similar percentage as indoors.

4.3.2.3 Indoor-to-outdoor ratios

I/O ratios of all 18 PM_{2.5}-bound PAHs are presented in Figure 4.6. The mean I/O ratios of all 18 PAHs were lower than unity suggesting that outdoor air is the dominant contributor to indoor PAH levels at this site. However, on some occasions all 18 PAHs exceeded unity, indicating contributions from indoor sources. Krugly et al. (2014) reported I/O ratio higher than unity for low molecular weight PAHs in indoor air of schools; these authors suggested an indoor origin due the respective activities of the occupants and/or volatilization from building materials. The potential indoor sources registered during the sampling in the studied preschool that could have resulted in elevated PAH content included combustion sources (candles on birthday cake during several occasions; Derudi et al., 2013), children artistic activities during classes (i.e. painting, sculpturing, wax melting, and other arts and crafts activities) and classroom cleaning (wood polishing) (Morawska et al., 2013).

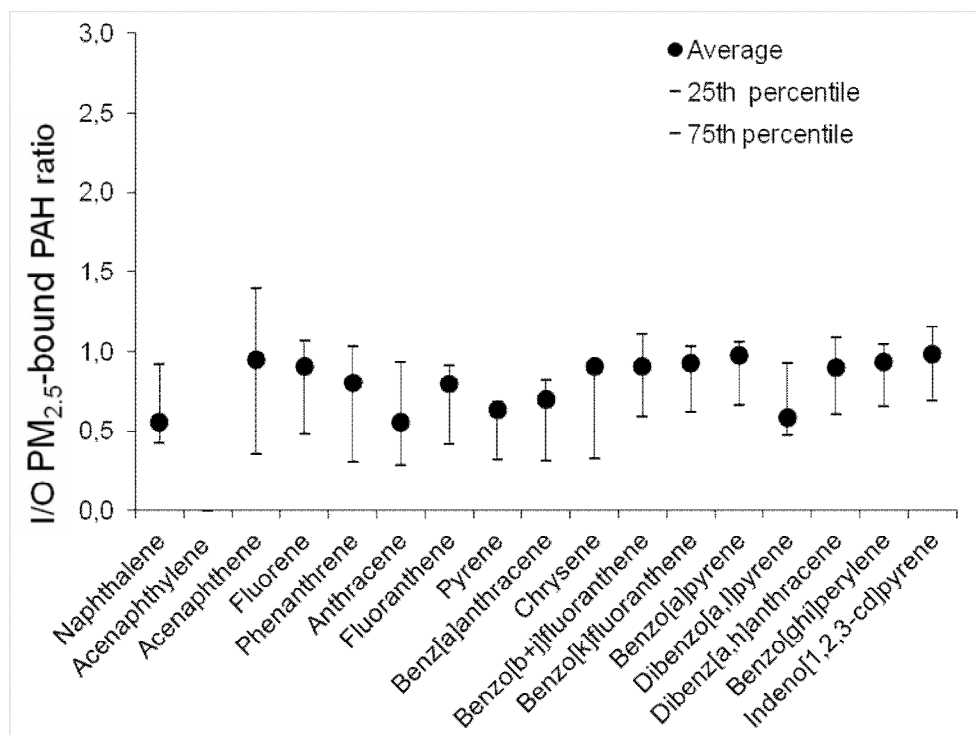


Figure 4.6. Indoor-to-outdoor (I/O) PM_{2.5}-bound PAH ratios at the studied preschool. Midpoints represent average values; whiskers are 25th and 75th percentiles.

4.3.2.4 Diagnostic concentration ratios

The ratio values of individual PAH concentrations were used as diagnostic tools to identify the possible origin of the PAHs. The diagnostic ratios were calculated based on the same particulate fraction ($PM_{2.5}$) for both indoor and outdoor PAHs and means are presented in Table 4.15. In the studied preschool, these diagnostic ratios in both indoors and outdoors were lower than 1, indicating a pyrogenic origin of the PAHs as reported in previous studies; Slezakova et al. (2013b) identified emissions from vehicle road transport as the main sources of PAHs in Oporto. The road traffic was the most likely source of PAHs at the studied preschool; in agreement, the ratio of benzo[a]anthracene to chrysene was lower than 0.35, indicating combustion of fuel and/or vehicular emissions (Krugly et al., 2014). The ratio value of fluoranthene to (fluoranthene plus pyrene) suggests potential origin from vehicular traffic (Kavouras et al., 1999; Yunker et al., 2002; Zencak et al., 2007) but also possibly from coal combustion (Krugly et al., 2014). Similarly, the ratio indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene+benzo[ghi]perylene), with values in range 0.35 to 0.70, indicates influence by diesel emissions (Kavouras et al. 2001; Rogge et al. 1993a; Sicre et al. 1987), as close to 0.56 it might suggest origin from coal combustion (Grimmer et al., 1983; Pio et al., 2001). Other ratios that were applied to the obtained PAHs levels in the studied preschool were indicative of vehicular influence. Ravindra et al. (2008) reported a ratio of benzo[a]pyrene to (benzo[a]pyrene plus chrysene) to distinguish between diesel (0.5) and gasoline (0.73) vehicle emissions. In addition, benzo[b]fluoranthene to benzo[k]fluoranthene ratios higher than 0.5 (Ravindra et al., 2008) and benzo[ghi]perylene/benzo[a]pyrene with values between 1.2 and 2.2 (Rogge et al., 1993a) also indicate diesel emissions. The comparison of the data in Table 4.15 with values of these three ratios, but also with ratios of benzo[b+k]fluoranthene and benzo[ghi]perylene shows that diesel motor emissions were a source of particulate PAHs at the studied preschool, as previously observed at primary schools (section 4.3.1) located in different location in Oporto city.. The ratio of fluorene/(fluorene+pyrene) is typically also used to differentiate between diesel and gasoline exhausts. Whereas values > 0.5 indicates contribution from diesel emissions, ratios < 0.5 indicate gasoline emissions (Ravindra et al., 2008; Rogge et al., 1993a); the present result shows very low values of this diagnostic ratio thus suggesting some presence of gasoline emissions.

Table 4.15. Diagnostic ratios for PM_{2.5}-bound PAHs at the studied preschool.

Ratio	This study		Value	Source	Reference
	Indoor	Outdoor			
^a ΣPAHs _{LMW} /ΣPAHs _{HMW}	0.24	0.57	> 1 < 1	Petrogenic Pyrogenic	Zhang et al. (2008)
Benzo[a]anthracene/Chrysene	0.38	0.42	> 0.35	Fuel combustion	Krugly et al. (2014)
Fluoranthene/(Fluoranthene + Pyrene)	0.54	0.50	0.40-0.5	Vehicular traffic	Kavouras et al. (1999); Yunker et al. (2002); Zencak et al. (2007); Rogge et al. (1993a); Oda et al. (2001)
			>0.5	Coal combustion	
			0.42, 0.52	Road dust	
Indeno[1,2,3-cd]pyrene/(Indeno[1,2,3-cd]pyrene + Benzo[ghi]perylene)	0.59	0.52	0.21-0.22 0.35-0.70 0.56 0.62 0.36	Gasoline cars Diesel emissions Coal combustion Wood combustion Road dust	Guo et al. (2003); Grimmer et al. (1983); Gogou et al. (1996); Kavouras et al. (2001); Khalili et al. (1995); Ravindra et al. (2008); Rogge et al. (1993a); Pio et al. (2001)
Benzo[a]pyrene/(Benzo[a]pyrene + Chrysene)	0.54	0.45	0.5 0.73	Diesel Gasoline	Ravindra et al. (2008)
Benzo[b]fluoranthene / Benzo[k]fluoranthene	4.72	4.64	>0.5	Diesel	Ravindra et al. (2008)
Benzo[ghi]perylene / Benzo[a]pyrene	1.38	1.44	1.2-2.2 2.5-3.3 0.86, 0.91	Diesel cars Gasoline cars Road dust	Rogge et al. (1993a,1993b); Oda et al. (2001)
Benzo[a]anthracene/(Benzo[a]anthracene + chrysene)	0.27	0.30	0.40, 0.38-0.64 0.76 0.43	Diesel Gasoline Wood combustion	Manoli et al. (2004); Sicre et al. (1987); Li and Kamens (1993)
Benzo[b+k]Fluoranthene/Benzo[ghi]perylene	1.36	1.75	1.60 0.33 2.18	Diesel cars Gasoline cars Wood combustion	Li and Kamens (1993)
Fluorene/(Fluorene + Pyrene)	0.08	0.07	>0.5 <0.5	Diesel Gasoline	Ravindra et al. (2008)
Phenanthrene/ (Phenanthrene+ Anthracene	0.75	0.79	>0.7	Lubricant oils and fossil fuels	Mirante et al. (2013)

^aTotal concentration of 2- and 3-ring PAHs (ΣPAHs_{LMW}), and 4- and 6-ring PAHs (ΣPAHs_{HMW})

Finally phenanthrene/(phenanthrene+anthracene) ratio exhibited values higher than 0.7, which are characteristic of contributions from lubricant oils and fossil fuels (Mirante et al., 2013). In general it is possible to conclude that most of the observed PAH diagnostic ratios indicated traffic (with a predominant contribution of diesel) and fossil fuel (with contribution of coal) combustion as the main sources of PAHs in the studied preschool.

4.3.2.5 Exposure estimation

The estimated dose rates of PAHs (indoor, outdoor and total) for different age-groups of children at the studied preschool are presented in Table 4.16. At the preschool, 3–5 years old children were divided into the classes according to their age. These age-classes had different daily schedules and activities which could have influenced the overall child dose rates of PAHs. For example the 3–years old children slept after lunch for 2–2.5 h whereas older children spent daily more times outdoors (0.75–1.75 h). The results in Table 4.16 show that total dose rates were not significantly different being approximately up to 1.4 (5-6 ringed PAHs) times higher for 4–5–years old children than for younger ones. Older children spent approximately twice more time outdoors (22% of their school time) than younger ones (9% of school time). In addition, older children performed more frequently physical activities such as exercising, running, and playing (both indoors and outdoors) which were associated with the highest breathing rates. In agreement with these findings, dose rates due to outdoor PAHs were approximately twice higher for older children than for younger ones. Specifically, the dose rates due PAHs outdoors contributed for older children between 7% (PAHs with 3 rings) till 56% (compounds with 5 rings) of the total PAHs school doses whereas for 3–years old children it was between 3% (3–ringed PAHs) and 34% (compounds with 5 rings).

Table 4.16. Mean dose rates of PAHs for 3– and 4–5 years old children at the studied preschool ($\text{ng kg}^{-1} \text{ day}^{-1}$).

3-years old children	Dose rate (ng kg ⁻¹ day ⁻¹)					
Compounds	Indoor			Outdoor		Total
	Particulate phase		Gas phase	Σ _{Indoor}	PM _{2.5}	
	PM _{2.5}	PM ₁				
2 rings	4.79×10 ⁴	4.69×10 ⁴	1.94×10 ⁵	1.99×10 ⁵	2.74×10 ⁴	2.01×10 ⁵
3 rings	8.06×10 ⁴	2.14×10 ⁴	4.42×10 ⁵	4.50×10 ⁵	1.40×10 ⁵	4.64×10 ⁵
4 rings	1.28×10 ⁵	9.46×10 ⁴	2.96×10 ⁵	4.24×10 ⁵	1.01×10 ⁵	5.25×10 ⁵
5 rings	4.86×10 ⁵	4.17×10 ⁵	4.25×10 ⁴	5.29×10 ⁵	2.68×10 ⁵	7.97×10 ⁵
6 rings	2.21×10 ⁵	1.78×10 ⁵	—	2.21×10 ⁵	1.02×10 ⁵	3.23×10 ⁵
Σ _{TEF-PAHs}	1.04×10 ⁶	8.10×10 ⁵	6.73×10 ⁵	7.77×10 ⁵	6.92×10 ⁵	8.46×10 ⁵
4–5 years old children						
Compounds						
2 rings	4.37×10 ⁴	4.28×10 ⁴	1.77×10 ⁵	1.81×10 ⁵	6.38×10 ⁴	1.87×10 ⁵
3 rings	7.35×10 ⁴	1.96×10 ⁴	4.03×10 ⁵	4.10×10 ⁵	3.28×10 ⁵	4.43×10 ⁵
4 rings	1.17×10 ⁵	8.63×10 ⁴	2.70×10 ⁵	3.87×10 ⁵	2.35×10 ⁵	6.22×10 ⁵
5 rings	4.44×10 ⁵	3.81×10 ⁵	3.88×10 ⁴	4.83×10 ⁵	6.26×10 ⁵	1.11×10 ⁶
6 rings	2.02×10 ⁵	1.62×10 ⁵	—	2.02×10 ⁵	2.38×10 ⁵	4.40×10 ⁵
Σ _{TEF-PAHs}	9.50×10 ⁵	7.39×10 ⁵	6.14×10 ⁵	7.09×10 ⁵	1.62×10 ⁶	8.71×10 ⁵

4.3.2.6 Health risks assessment

The results of TEF-adjusted concentrations for 18 PAHs at the studied preschool are presented in Table 4.17. These results demonstrate that total TEF-adjusted concentrations of 18 PAHs ($\Sigma_{\text{TEF-PAHs}}$) in $\text{PM}_{2.5}$ outdoors (14.1 ng/m^3) were similar to the indoors. Mean $\Sigma_{\text{TEF-PAHs}}$ in indoor air reached a value of 14.4 ng m^{-3} (in $\text{PM}_{2.5}$ and 0.270 ng/m^3 in gas phase) and was in the similar range as values reported in other studies (Krugly et al., 2014). Dibenz[a,h]anthracene with TEF of 5 was the largest contributor (59%) to $\Sigma_{\text{TEF-PAHs}}$ in indoor air. The concentration of dibenzo[a,l]pyrene were low in indoor air ($7.43 \times 10^{-2} \text{ ng/m}^3$, i.e. less than 0.2%), but due to its high TEF (100) it was the second largest contributor to $\Sigma_{\text{TEF-PAHs}}$ (35%) in indoor air of the studied preschool. These results emphasize the importance of the analysis and evaluation of these two potent carcinogens that are being discussed as possible surrogate compounds for PAH mixtures from various environments (Okona-Mensah et al., 2005). Concerning naphthalene, this PAH made the largest contribution to total PAH content in indoor air of the preschool (26% of Σ_{PAHs} ; Table 4.13), but its contribution to TEF-adjusted concentrations was less than 1% (Table 4.17). Nevertheless, in a view of WHO guidelines (WHO 2010) this gaseous PAH should be considered when assessing the health risks of PAHs. Furthermore, the high abundance of naphthalene in the gas phase indicates that this compound should be routinely monitored in indoor air. The values of $\Sigma_{\text{TEF-PAHs}}$ were used to estimate the corresponding lifetime lung cancer risks for the exposed populations. Considering that children and teaching staff spent approximately 7 h of their school daily time indoors and 1 h outdoors, the corresponding lung cancer risks were 3.71×10^{-4} in indoor air (3.66×10^{-4} in $\text{PM}_{2.5}$ and 5.2×10^{-6} in gaseous phase) and 5.11×10^{-5} for outdoors (i.e. in $\text{PM}_{2.5}$). Both estimated values exceeded WHO health-based guideline level of 10^{-5} , approximately 37 and 5 times higher for indoor and outdoor air, respectively (Boström et al., 2002). It is necessary to point out that unit risk guideline of benzo[a]pyrene recommended for indoor air that was used for calculation is based on epidemiological data from studies on coke-oven workers. It implies that benzo[a]pyrene represents the same proportion of carcinogenic activity of the PAH mixture as in the occupational exposure used to derive the unit risk (WHO 2010). Although this assumption is probably not correct, the associated uncertainties in risk estimates are unlikely to be large (WHO 2010).

Table 4.17. Toxicity equivalency factor (TEF)–adjusted mean concentrations of PAHs in indoor and outdoor air of the studied preschool (pg/m³).

Compound	TEF ^a	Indoor			Outdoor	
		Particulate phase (n=30)		Gas phase (n=15)	Air	PM _{2.5} (n=15)
		PM _{2.5}	PM ₁			
Naphthalene	0.001	0.317	0.3106	12.8	13.1	0.306
Acenaphthylene	0.001	n.d.	n.d.	4.55	4.55	1.15
Acenaphthene	0.001	0.348	n.d.	1.77	2.12	n.d.
Fluorene	0.001	3.76×10 ⁻²	2.57×10 ⁻²	8.88	8.91	8.74×10 ⁻²
Phenanthrene	0.001	0.148	0.116	14.0	14.2	0.336
Anthracene	0.01	0.488	0.372	2.41	2.90	0.639
Fluoranthene	0.001	0.487	0.371	0.572	1.06	0.553
Pyrene	0.001	0.310	0.217	1.15	1.46	0.506
Benz[a]anthracene	0.1	11.9	9.49	1.70	13.6	13.3
Chrysene	0.01	3.92	2.49	1.89	5.81	4.61
Benzo[b+] <i>l</i> fluoranthene	0.1	93.4	75.7	12.2	106	82.9
Benzo[k]fluoranthene	0.1	20.3	16.4	1.51	21.9	18.3
Benzo[a]pyrene	1	341	322	144	485	286
Dibenzo[a, <i>l</i>]pyrene	100 ^b	5 140	2 100	n.d	5 140	5 130
Dibenzo[<i>a,h</i>]anthracene	5	8 670	7 580	n.d	8 670	8 500
Benzo[ghi]perylene	0.01	4.61	4.85	n.d	4.61	5.01
Indeno[1,2,3- <i>cd</i>]pyrene	0.1	94.9	67.1	n.d	94.9	59.0
Σ TEF-PAHs	–	14 410	10 180	207	14 620	14 110

^aTEF values estimated by Nisbeth and LaGoy 1992 (Boström et al., 2002)

^bTEF value estimated by Muller, 1997 (Boström et al., 2002).

n.d. not detected.

The means of target carcinogenic risks associated with inhalation exposure to PAHs for two children age-groups (3 years and 4–5-years old) were estimated by USEPA methodology (USEPA 2014; Table 4.18). The results in Table 4.18 show that total and individual carcinogenic target risks of all PAHs, both in indoor air and outdoors, were below 10^{-6} and thus can be considered as negligible. Total carcinogenic risks due to indoor particulate-bound PAHs were higher than outdoor ones (13 and 4 times for 3- and 4–5-years old children, respectively), which was mostly due to the prolonged periods that children spent indoors (7.25 and 6.25 h indoors versus 0.75 and 1.75 h outdoors, respectively). Furthermore, indoor risk values were approximately 4 times higher for particulate-bound PAHs than for gaseous compounds. Overall, indoor exposure contributed 93% and 78% of the overall (i.e. both indoor and outdoor) school risks ($\Sigma TR_{\text{school}}$) for 3- and 4–5-years old children, respectively. Finally, considering different age groups, the total cancer risks of overall (i.e. both indoors and outdoors) school exposure ($\Sigma TR_{\text{school}}$) were approximately 2.1 times higher (but still negligible) for 4–5-years old children than for 3-years old. These differences were firstly caused by different exposure time (1 versus 2 years) and secondly, by different daily schedules. For comparison purposes, the health risks for two adult age categories of the school staff (25–54 and 55–64 years old) were also evaluated (Table 4.19). The values of total cancer risks of overall school exposure ($\Sigma TR_{\text{school}}$) ranged from 1.60×10^{-7} to 4.26×10^{-7} for 25–54- and 55–64-years old adults, respectively. Whereas these cancer risks were negligible (lower than 10^{-6}), they were 7–40 times higher than of children, mostly due to longer period (i.e. ED of 15 and 40 years versus 1–2 years) of the respective exposures. It is necessary to point out that dibenzo[a,l]pyrene was not considered for the evaluation of carcinogenic risks by USEPA methodology, because its chronic inhalation unit risk value is not available. Therefore, settling IUR value for dibenzo[a,l]pyrene is important for the respective risk analysis.

Finally, it is necessary to point out that apart from the PAH carcinogenicity, other health effects are relevant. Short-term exposure to PAHs has been reported to cause impaired lung function in asthmatics and thrombotic effects in people affected by coronary heart disease (Kim et al., 2013). Especially for children, Annesi-Maesano et al. (2007) reported an increased risk for flexural dermatitis in subjects exposed to high levels of traffic-related air pollution.

Table 4.18. Estimated target carcinogenic risks (TR) of PAHs for different age categories children.

Children 3–years old	TR _{indoor} PM _{2.5}	PM ₁	Gas phase	ΣTR _{indoor}	TR _{outdoor} PM _{2.5}	ΣTR _{school}
Naphthalene	3.18×10 ⁻¹¹	3.12×10 ⁻¹¹	1.29×10 ⁻⁹	1.32×10 ⁻⁹	3.18×10 ⁻¹²	1.32×10 ⁻⁹
Benz[a]anthracene	3.87×10 ⁻¹¹	3.09×10 ⁻¹¹	5.53×10 ⁻¹²	4.42×10 ⁻¹¹	4.46×10 ⁻¹²	4.87×10 ⁻¹¹
Chrysene	1.28×10 ⁻¹¹	8.11×10 ⁻¹²	6.15×10 ⁻¹²	1.89×10 ⁻¹¹	1.55×10 ⁻¹²	2.05×10 ⁻¹¹
Benzol[b+] <i>l</i> fluoranthene	3.04×10 ⁻¹⁰	2.46×10 ⁻¹⁰	3.97×10 ⁻¹¹	3.44×10 ⁻¹⁰	2.79×10 ⁻¹¹	3.71×10 ⁻¹⁰
Benzol[k]fluoranthene	6.61×10 ⁻¹¹	5.32×10 ⁻¹¹	4.24×10 ⁻¹²	7.04×10 ⁻¹¹	6.17×10 ⁻¹²	7.65×10 ⁻¹¹
Benzol[a]pyrene	1.11×10 ⁻⁹	1.05×10 ⁻⁹	4.67×10 ⁻¹⁰	1.58×10 ⁻⁹	9.63×10 ⁻¹¹	1.67×10 ⁻⁹
Dibenzo[a,h]anthracene	6.17×10 ⁻⁹	1.85×10 ⁻¹⁰	n.d.	6.17×10 ⁻⁹	6.24×10 ⁻¹⁰	6.80×10 ⁻⁹
Indeno[1,2,3-cd]pyrene	3.09×10 ⁻¹⁰	2.18×10 ⁻¹⁰	n.d.	3.09×10 ⁻¹⁰	1.98×10 ⁻¹¹	3.29×10 ⁻¹⁰
ΣPAHs	8.04×10 ⁻⁹	1.82×10 ⁻⁹	1.81×10 ⁻⁹	9.85×10 ⁻⁹	7.83×10 ⁻¹⁰	1.06×10 ⁻⁸
Children 4–5–years old	TR _{indoor} PM _{2.5}	PM ₁	Gas phase	ΣTR _{indoor}	TR _{outdoor} PM _{2.5}	ΣTR _{school}
Naphthalene	5.49×10 ⁻¹¹	5.38×10 ⁻¹¹	2.22×10 ⁻⁹	2.28×10 ⁻⁹	1.48×10 ⁻¹¹	2.29×10 ⁻⁹
Benz[a]anthracene	6.67×10 ⁻¹¹	5.32×10 ⁻¹¹	9.53×10 ⁻¹²	7.63×10 ⁻¹¹	2.08×10 ⁻¹¹	9.71×10 ⁻¹¹
Chrysene	2.20×10 ⁻¹¹	1.40×10 ⁻¹¹	1.06×10 ⁻¹¹	3.26×10 ⁻¹¹	7.24×10 ⁻¹²	3.98×10 ⁻¹¹
Benzol[b+] <i>l</i> fluoranthene	5.24×10 ⁻¹⁰	4.25×10 ⁻¹⁰	6.85×10 ⁻¹¹	5.92×10 ⁻¹⁰	1.30×10 ⁻⁹	1.89×10 ⁻⁹
Benzol[k]fluoranthene	1.14×10 ⁻¹⁰	9.15×10 ⁻¹¹	8.49×10 ⁻¹²	1.23×10 ⁻¹⁰	2.88×10 ⁻¹¹	1.51×10 ⁻¹⁰
Benzol[a]pyrene	1.91×10 ⁻⁹	1.80×10 ⁻⁹	8.05×10 ⁻¹⁰	2.72×10 ⁻⁹	4.49×10 ⁻¹⁰	3.17×10 ⁻⁹
Dibenzo[a,h]anthracene	1.06×10 ⁻⁸	9.27×10 ⁻⁹	n.d.	1.06×10 ⁻⁸	2.91×10 ⁻⁹	1.36×10 ⁻⁸
Indeno[1,2,3-cd]pyrene	5.32×10 ⁻¹⁰	3.76×10 ⁻¹⁰	n.d.	5.32×10 ⁻¹⁰	9.26×10 ⁻¹¹	6.25×10 ⁻¹⁰
ΣPAHs	1.39×10 ⁻⁸	1.21×10 ⁻⁸	3.13×10 ⁻⁹	1.70×10 ⁻⁸	4.83×10 ⁻⁹	2.18×10 ⁻⁸

n.d. – not detected

Table 4.19. Estimated target carcinogenic risks (TR) of PAHs for preschool staff.

Adults 25–54–years old	TR _{Indoor} PM _{2.5}	PM ₁	Gas phase	ΣTR _{Indoor}	TR _{outdoor} PM _{2.5}	ΣTR _{school}
Naphthalene	4.77×10 ⁻¹⁰	4.68×10 ⁻¹⁰	1.93×10 ⁻⁰⁸	1.98×10 ⁻⁸	4.77×10 ⁻¹¹	1.99×10 ⁻⁸
Benz[a]anthracene	5.81×10 ⁻¹⁰	4.63×10 ⁻¹⁰	8.30×10 ⁻¹¹	6.64×10 ⁻¹⁰	6.69×10 ⁻¹⁰	7.31×10 ⁻¹⁰
Chrysene	1.91×10 ⁻¹⁰	1.22×10 ⁻¹⁰	9.22×10 ⁻¹¹	2.84×10 ⁻¹⁰	2.33×10 ⁻¹¹	3.07×10 ⁻¹⁰
Benzof[b+j]fluoranthene	4.56×10 ⁻⁹	3.69×10 ⁻⁹	5.96×10 ⁻¹⁰	5.15×10 ⁻⁹	4.18×10 ⁻¹⁰	5.57×10 ⁻⁹
Benzof[k]fluoranthene	9.92×10 ⁻¹⁰	7.98×10 ⁻¹⁰	6.37×10 ⁻¹¹	1.06×10 ⁻⁹	9.25×10 ⁻¹¹	1.15×10 ⁻⁹
Benzof[a]pyrene	1.66×10 ⁻⁸	1.57×10 ⁻⁸	7.01×10 ⁻⁹	2.37×10 ⁻⁸	1.44×10 ⁻⁹	2.51×10 ⁻⁸
Dibenz[a,h]anthracene	9.26×10 ⁻⁸	2.78×10 ⁻⁹	n.d.	9.26×10 ⁻⁸	9.36×10 ⁻⁹	1.02×10 ⁻⁷
Indeno[1,2,3-cd]pyrene	4.63×10 ⁻⁹	3.27×10 ⁻⁹	n.d.	4.63×10 ⁻⁹	2.98×10 ⁻¹⁰	4.93×10 ⁻⁹
ΣPAHs	1.21×10 ⁻⁷	2.73×10 ⁻⁸	2.72×10 ⁻⁸	1.48×10 ⁻⁷	1.17×10 ⁻⁸	1.60×10 ⁻⁷
Adults 55–64–years old	TR _{Indoor} PM _{2.5}	PM ₁	Gas phase	ΣTR _{Indoor}	TR _{outdoor} PM _{2.5}	ΣTR _{school}
Naphthalene	1.27×10 ⁻⁹	1.25×10 ⁻⁹	5.16×10 ⁻⁸	5.28×10 ⁻⁸	1.27×10 ⁻¹⁰	5.30×10 ⁻⁸
Benz[a]anthracene	1.55×10 ⁻⁹	1.23×10 ⁻⁹	2.21×10 ⁻¹⁰	1.77×10 ⁻⁹	1.78×10 ⁻¹⁰	1.95×10 ⁻⁹
Chrysene	5.10×10 ⁻¹⁰	3.24×10 ⁻¹⁰	2.46×10 ⁻¹⁰	7.56×10 ⁻¹⁰	6.20×10 ⁻¹¹	8.18×10 ⁻¹⁰
Benzof[b+j]fluoranthene	1.22×10 ⁻⁸	9.85×10 ⁻⁹	1.59×10 ⁻⁹	1.37×10 ⁻⁸	1.12×10 ⁻⁹	1.49×10 ⁻⁸
Benzof[k]fluoranthene	2.65×10 ⁻⁹	2.13×10 ⁻⁹	1.70×10 ⁻¹⁰	2.82×10 ⁻⁹	2.47×10 ⁻¹⁰	3.06×10 ⁻⁹
Benzof[a]pyrene	4.44×10 ⁻⁸	4.18×10 ⁻⁸	1.87×10 ⁻⁸	6.31×10 ⁻⁸	3.85×10 ⁻⁹	6.69×10 ⁻⁸
Dibenz[a,h]anthracene	2.47×10 ⁻⁷	7.42×10 ⁻⁹	n.d.	2.47×10 ⁻⁷	2.50×10 ⁻⁸	2.72×10 ⁻⁷
Indeno[1,2,3-cd]pyrene	1.23×10 ⁻⁸	8.73×10 ⁻⁹	n.d.	1.23×10 ⁻⁸	7.93×10 ⁻¹⁰	1.31×10 ⁻⁸
ΣPAHs	3.22×10 ⁻⁷	17.28×10 ⁻⁸	7.25×10 ⁻⁸	3.94×10 ⁻⁷	3.13×10 ⁻⁸	4.26×10 ⁻⁷

n.d. – not detected

The authors pointed out that particles may enhance inflammatory reactions, which could be due to the intervention of PAHs contained in PM. Bae et al. (2010) also found evidence of a synergistic effect of exposure to high levels of PM, PAH and oxidative stress in schoolchildren. In order to fully understand the health implications, more studies concerning schoolchildren exposure to PAHs are needed.

4.3.3 Preschools from two different geographical regions

4.3.3.1 Particulate matter levels

The levels of PM in indoor and outdoor air of both preschools are presented in Table 4.20. At PS2 the indoor concentrations of PM_{2.5} and PM₁ were slightly lower than those detected at PS1, however PM_{2.5} levels at both preschools were below the available guidelines (25 µg/m³; Portuguese Regulation 2013). Statistical analysis of the results indicated that at both preschools indoor PM_{2.5} were not significantly higher than PM₁. On average majority of PM_{2.5} fraction was composed by PM₁; these particles accounted for 86% and 87% of PM_{2.5} at PS1 and PS2, respectively. Outdoors, PM₁ accounted for 62–80% of PM_{2.5} at PS1 and PS2, respectively. In addition, at both preschools indoor *versus* outdoor PM were not significantly different.

Table 4.20. Meteorological conditions and particulate matter (PM) levels at two studied preschools (PS1 and PS2).

	PS1	PS2
Meteorological parameters		
Mean (Min–Max)		
Indoor	Temperature: 25.4 °C (21.8–32.0) Relative humidity: 42.7% (19.6–71.20)	Temperature: 20.6 °C (14.4–29.4) Relative humidity: 54.1% (24.6–74.5)
Outdoor	Temperature: 25.6 °C (17.3–36.1) Relative humidity: 73.0% (17.0–89.6) Rain fall: 18 days; 3.6 mm (0.0–53) Atmospheric pressure: 1017.0 hPa (1002.4–1024.9)	Temperature: 20.6 °C (14.4–29.4) Relative humidity: 51.4% (24.7–77.3) Rain fall: 6 days; 4.2 mm (0.0–16) Atmospheric pressure: 1017.6 hPa (1009.8–1024.1)
PM		
Mean (Min – Max) (µg/m ³)		
Indoor	PM _{2.5} : 18.4 (8.3 –34.8) PM ₁ : 15.8 (3.0–26.1)	PM _{2.5} : 14.2 (5.8–28.1) PM ₁ : 12.4 (1.5–20.0)
Outdoor	PM _{2.5} : 19.3 (11.2–39.9) PM ₁ : 12.0 (6.3–30.8)	PM _{2.5} : 15.2 (8.6–22.6) PM ₁ : 12.2 (5.2–21.7)

4.3.3.2 PAH levels in indoor and outdoor air

The levels of indoor PAHs associated with particulate and gas phase are summarised in Table 4.21. Dibenzo[a,h]anthracene was the most abundant indoor particulate-bound PAH at both preschools which was in agreement with the previous studies on IAQ in Oporto Metropolitan Area (Castro et al., 2011; Slezakova et al., 2009, 2014). This PAH accounted (in both PM fractions) for 37–40% and 21% of particulate Σ_{PAHs} at PS1 and PS2, respectively. The dominance of this compound indicates emissions from motor light-duty gasoline vehicles (Ravindra et al., 2008). However, for other PAHs the profiles between both preschools differed. At PS1 other most abundant particulate PAHs were, by descending order, benzo[ghi]perylene (18% in $\text{PM}_{2.5}$, 19% in PM_1), benzo[b+j]fluoranthene (approximately 10% in $\text{PM}_{2.5}$, 12% in PM_1), and phenanthrene (6 and 5% in $\text{PM}_{2.5}$ and PM_1 , respectively). In addition, naphthalene was absent in both PM (as well as acenaphthylene and dibenzo[a,l]pyrene) but in gas phase it was the most abundant compound accounting for 44% of gaseous Σ_{PAHs} ; other abundant gaseous compounds were phenanthrene (34% of gaseous Σ_{PAHs}) and fluorene (16% of Σ_{PAHs}). These three PAHs accounted in total for 94% of indoor gaseous Σ_{PAHs} at PS1. Similarly, at PS2 these three compounds were also the most abundant gaseous PAHs (contributing 87% of gaseous Σ_{PAHs}) but the order of abundance was different: phenanthrene (48% of gaseous Σ_{PAHs}), naphthalene (25%), and fluorene (14%). Concerning particulate PAHs at PS2, acenaphthene (17% in $\text{PM}_{2.5}$, 23% in PM_1) and acenaphthylene (13% in $\text{PM}_{2.5}$, 17% in PM_1) were among the most abundant particulate PAHs, being followed by benzo[b+j]fluoranthene (10% in $\text{PM}_{2.5}$, 9% in PM_1). In addition, the overall data in Table 4.21 also indicate that, whereas total concentrations of PAHs in the gas phase and in air (i.e. sum of PAHs levels in particulate and gas phases) were rather similar at both preschools, the levels of particulate Σ_{PAHs} at PS2 were 2.9 and 3.6 times higher for $\text{PM}_{2.5}$ and PM_1 , respectively. Certainly, seasonal influences, meteorological conditions, as well as different emission sources and levels of urbanization of areas surrounding both preschools could account for the observed differences in PAH levels.

Up to this date, there are no air quality standards for PAHs in indoor air except for WHO recommendation for naphthalene (set as annual guideline value of $10 \mu\text{g}/\text{m}^3$; WHO, 2010). The means obtained at both preschools ($27.2 \pm 22.4 \text{ ng}/\text{m}^3$ at PS1 and $16.3 \pm 3.0 \text{ ng}/\text{m}^3$ at PS2) were well below the recommended guideline.

Table 4.21. Indoor levels of PAHs in PM_{2.5}, PM₁, gas phase, and in air (i.e. total) at the studied preschools PS1 and PS2 (ng/m³).

Preschool	PS1													
	Compound	Particulate phase				Gas phase				Air				
		PM _{2.5}		PM ₁		Mean		(Min–Max)		Mean		(Min–Max)		
		Mean	(Min–Max)	Mean	(Min–Max)	Mean	(Min–Max)	Mean	(Min–Max)	Mean	(Min–Max)	Mean	(Min–Max)	
	Naphthalene	n.d.	n.d.	n.d.	n.d.	27.2	13.6–62.7	27.2	13.6–62.7	27.2	13.6–62.7	27.2	13.6–62.7	
	Acenaphthylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
	Acenaphthene	2.50×10 ^{-2c}	2.38×10 ⁻² –2.64×10 ⁻²	n.d.	n.d.	0.538 ^a	0.351–0.695	0.562	0.375–0.721	0.562	0.375–0.721	0.562	0.375–0.721	
	Fluorene	3.82×10 ⁻³	2.07×10 ⁻³ –1.17×10 ⁻²	n.d.	n.d.	1.76×10 ⁻²	3.78–23.0	9.66	3.79–23.1	9.66	3.79–23.1	9.66	3.79–23.1	
	Phenanthrene	9.28×10 ⁻²	9.13×10 ⁻² –0.107	3.97×10 ⁻²	3.97×10 ⁻²	2.68×10 ⁻² –9.62×10 ⁻²	6.06–56.0	21.2	6.09–56.1	21.2	6.09–56.1	21.2	6.09–56.1	
	Anthracene	2.59×10 ⁻²	1.10×10 ⁻² –4.85×10 ⁻²	n.d.	n.d.	0.304 ^a	0.129–0.663	0.330	0.141–0.666	0.330	0.141–0.666	0.330	0.141–0.666	
	Fluoranthene	4.80×10 ⁻²	1.21×10 ⁻² –0.123	2.94×10 ⁻²	2.94×10 ⁻²	1.16×10 ⁻² –4.92×10 ⁻²	0.503–2.82	1.21	0.515–2.85	1.21	0.515–2.85	1.21	0.515–2.85	
	Pyrene	4.52×10 ^{-2a}	1.83×10 ⁻² –0.107	4.43×10 ⁻²	4.43×10 ⁻²	1.82×10 ⁻² –7.32×10 ⁻²	0.529–3.61	1.49 ^a	0.547–3.63	1.53	0.547–3.63	1.53	0.547–3.63	
	Benz[a]anthracene	2.23×10 ⁻²	1.25×10 ⁻² –6.33×10 ⁻²	2.20×10 ⁻²	2.20×10 ⁻²	9.28×10 ⁻³ –3.25×10 ⁻²	n.d.	n.d.	1.25×10 ⁻² –6.33×10 ⁻²	2.23×10 ⁻²	1.25×10 ⁻² –6.33×10 ⁻²	2.23×10 ⁻²	1.25×10 ⁻² –6.33×10 ⁻²	
	Chrysene	5.73×10 ⁻²	4.82×10 ⁻² –0.174	4.61×10 ⁻²	4.61×10 ⁻²	4.47×10 ⁻² –4.74×10 ⁻²	0.393–0.405	0.399 ^c	0.441–0.579	0.456	0.441–0.579	0.456	0.441–0.579	
	Benzo[b]fluoranthene	0.141	0.110–0.260	0.104	0.104	5.78×10 ⁻² –0.152	n.d.	n.d.	0.110–0.260	0.141	0.110–0.260	0.141	0.110–0.260	
	Benzo[k]fluoranthene	3.34×10 ⁻²	2.32×10 ⁻² –6.46×10 ⁻²	2.74×10 ⁻²	2.74×10 ⁻²	1.67×10 ⁻² –3.97×10 ⁻²	n.d.	n.d.	2.32×10 ⁻² –6.46×10 ⁻²	3.34×10 ⁻²	2.32×10 ⁻² –6.46×10 ⁻²	3.34×10 ⁻²	2.32×10 ⁻² –6.46×10 ⁻²	
	Benzo[a]pyrene	5.09×10 ⁻²	3.96×10 ⁻² –8.81×10 ⁻²	3.36×10 ⁻²	3.36×10 ⁻²	2.65×10 ⁻² –4.13×10 ⁻²	n.d.	n.d.	3.96×10 ⁻² –8.81×10 ⁻²	5.09×10 ⁻²	3.96×10 ⁻² –8.81×10 ⁻²	5.09×10 ⁻²	3.96×10 ⁻² –8.81×10 ⁻²	
	Dibenzo[a,h]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
	Dibenzo[a,h]anthracene	0.566	0.316–0.940	0.312	0.312	0.306–0.325	n.d.	n.d.	0.316–0.940	0.566	0.316–0.940	0.566	0.316–0.940	
	Benzo[ghi]perylene	0.250	0.244–0.289	0.159	0.159	0.110–0.254	n.d.	n.d.	0.244–0.289	0.250	0.244–0.289	0.250	0.244–0.289	
	Indeno[1,2,3-cd]pyrene	5.34×10 ⁻²	4.07×10 ⁻² –6.92×10 ⁻²	2.32×10 ⁻²	2.32×10 ⁻²	1.25×10 ⁻² –2.99×10 ⁻²	n.d.	n.d.	4.07×10 ⁻² –6.92×10 ⁻²	5.34×10 ⁻²	4.07×10 ⁻² –6.92×10 ⁻²	5.34×10 ⁻²	4.07×10 ⁻² –6.92×10 ⁻²	
	Σ _{PAHs}	1.45	1.18–2.23	0.857	0.857	0.964–1.624	61.9	23.9–149	63.3	25.1–149	63.3	25.1–149	63.3	25.1–149
	Σ _{PAHs+PC}	0.924	0.734–1.60	0.568	0.568	0.443–1.02	27.6	13.9–63.1	28.8	14.6–64.7	28.8	14.6–64.7	28.8	14.6–64.7

n.d. – not detected
Note: detection frequency of each compound was 100% unless otherwise indicated.
^a80% ≤ detection frequency < 100%.
^b60% ≤ detection frequency < 80%.
^c20% ≤ detection frequency < 60%.

Table 4.21. Indoor levels of PAHs in PM_{2.5}, PM₁₀, gas phase, and in air (i.e. total) at the studied preschools PS1 and PS2 (ng/m³) (continued).

Compound	PS2									
	Particulate phase					Gas phase				
	PM _{2.5}		PM ₁₀			Mean		(Min-Max)		Air
	Mean	(Min-Max)	Mean	(Min-Max)	Mean	Mean	Mean	(Min-Max)	Mean	(Min-Max)
Naphthalene	0.137	2.76×10 ⁻² –0.413	n.d.	n.d.	16.3	16.3	13.1–20.8	13.1–20.8	16.5	13.1–20.9
Acenaphthylene	0.568 ^c	0.430–0.745	0.535 ^b	0.402–0.647	0.500 ^c	0.500 ^c	0.376–0.751	0.376–0.751	1.04	0.806–1.40
Acenaphthene	0.717	0.598–2.90	0.704	0.293–1.04	2.92	2.92	0.457–8.04	0.457–8.04	3.63	1.06–8.22
Fluorene	2.03×10 ⁻²	8.93×10 ⁻³ –5.69×10 ⁻²	7.84×10 ^{-3b}	6.27×10 ⁻³ –1.03×10 ⁻²	9.12	9.12	5.09–14.3	5.09–14.3	9.14	5.10–14.3
Phenanthrene	0.178	6.66×10 ⁻² –0.373	6.85×10 ⁻²	2.15×10 ⁻² –0.138	31.4	31.4	25.6–35.2	25.6–35.2	31.6	25.7–35.3
Anthracene	1.04×10 ^{-2a}	4.98×10 ⁻³ –3.42×10 ⁻²	6.76×10 ^{-3b}	4.92×10 ⁻³ –8.59×10 ⁻³	0.472	0.472	0.330–0.636	0.330–0.636	0.482	0.335–0.641
Fluoranthene	6.13×10 ⁻²	2.56×10 ⁻² –0.151	5.94×10 ⁻²	2.55×10 ⁻² –9.68×10 ⁻²	2.58	2.58	2.28–3.22	2.28–3.22	2.64	2.31–3.37
Pyrene	0.138	6.03×10 ⁻² –0.202	0.123	5.86×10 ⁻² –0.193	1.73 ^a	1.73 ^a	1.41–2.03	1.41–2.03	1.87	1.47–2.16
Benz[a]anthracene	4.38×10 ⁻²	1.74×10 ⁻² –0.130	4.067×10 ⁻²	1.58×10 ⁻² –7.27×10 ⁻²	n.d.	n.d.	n.d.	n.d.	4.38×10 ⁻²	1.74×10 ⁻² –0.130
Chrysene	0.113	7.28×10 ⁻² –0.173	0.107	6.35×10 ⁻² –0.145	0.101 ^c	0.101 ^c	9.97×10 ⁻² –0.103	9.97×10 ⁻² –0.103	0.214	0.173–0.276
Benzofluoranthene	0.410	0.192–0.941	0.285	0.113–0.409	n.d.	n.d.	n.d.	n.d.	0.410	0.192–0.941
Benzokjfluoranthene	7.95×10 ⁻²	3.90×10 ⁻² –0.209	5.86×10 ⁻²	2.68×10 ⁻² –8.67×10 ⁻²	n.d.	n.d.	n.d.	n.d.	7.95×10 ⁻²	3.90×10 ⁻² –0.209
Benzofluorene	0.264	0.110–0.853	0.117	3.76×10 ⁻² –0.187	n.d.	n.d.	n.d.	n.d.	0.264	0.110–0.853
Dibenz[a,h]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenz[a,h]anthracene	0.883	0.341–2.58	0.680 ^b	0.299–0.921	n.d.	n.d.	n.d.	n.d.	0.883	0.341–2.58
Benzolghioperylene	0.304	0.163–0.866	0.174	3.57×10 ⁻² –0.299	n.d.	n.d.	n.d.	n.d.	0.304	0.163–0.866
Indeno[1,2,3-cd]pyrene	0.302 ^a	0.131–1.06	0.146 ^b	8.16×10 ⁻² –0.190	n.d.	n.d.	n.d.	n.d.	0.302	0.131–1.06
Σ _{PAHs}	4.23	2.60–7.77	3.11	2.59–5.44	65.1	65.1	50.7–83.7	50.7–83.7	69.4	51.4–84.1
Σ _{PAHsBGC}	2.23	1.25–6.05	1.43	0.325–1.97	16.4	16.4	13.2–20.9	13.2–20.9	18.7	14.5–24.9

n.d.: not detected

Note: detection frequency of each compound was 100% unless otherwise indicated.

^a80% ≤ detection frequency < 100%.

^b60% ≤ detection frequency < 80%.

^c20% ≤ detection frequency < 60%.

Krugly et al. (2014) reported gaseous PAHs in similar range of concentrations in indoor air of schools in Lithuania, whereas the levels of PM_{2.5}–bound PAHs (detected in PS1 and PS2) were similar to those of schools in Rome (Gatto et al., 2014; Romagnoli et al., 2014).

Particulate PAHs were mainly associated with particles of smaller sizes, i.e. PM₁ (55–75% particulate Σ_{PAHs} ; Table 4.21). This fraction is more apt to cause respiratory toxicity and dysfunction. As a consequence the presence of PM-bound PAHs may contribute to (or potentially even enhance) the PM-induced adverse health effects (Kim et al., 2013; Saravia et al., 2013).

Total concentrations of carcinogenic PAHs (i.e. Σ_{PAHscarc}) are reported in Table 4.21. Dibenz[a,h]anthracene was the most abundant carcinogenic particulate PAH, and at PS1 it accounted for 61% and 55% of Σ_{PAHscarc} of PM_{2.5} and PM₁, respectively; the corresponding abundance was slightly lower at PS2 with 40% of Σ_{PAHscarc} in PM_{2.5} and 47% in PM₁. Other abundant carcinogen were: benzo[b+j]fluoranthene (15–18% of Σ_{PAHscarc} at PS1; 18–20% of Σ_{PAHscarc} at PS2) and benzo[a]pyrene (6% of Σ_{PAHscarc} at PS1, 8–12% of Σ_{PAHscarc} for PM_{2.5} and PM₁ at PS2). Concerning the gas phase, naphthalene was the predominant carcinogenic gaseous PAH (99% of gaseous Σ_{PAHscarc} at both preschools). Furthermore, the obtained results showed that at PS1 97% of Σ_{PAHscarc} existed in the gas phase whereas 3% was particulate-bound; this proportion was 88% of Σ_{PAHscarc} in the gas phase and 12% particulate-bound at PS2. Despite this distribution, carcinogenic PAHs accounted for 25 and 45% of the gaseous PAH content at PS2 and PS1, respectively, whereas it was approximately between 50% and 65% Σ_{PAHs} for PM at both preschools. Finally, in agreement with the findings on PAH distributions (Figure 4.7), carcinogenic particulate PAHs were predominantly associated with PM₁ (61% and 64% at PS1 and PS2, respectively).

PAH levels measured in preschool yards (i.e. in ambient air) are presented in Table 4.22. Total concentrations (i.e. particulates and gas phase) of PAHs in outdoor air obtained at both preschools were much higher in comparison to other Italian cities (Ercolano, Pollica); Cirillo et al. (2006) reported PAHs in range up to 1.4 and 3.4 ng/m³ for outdoor air of schools in rural and urban Italian areas, respectively. On the contrary, Wilson et al. (2003) observed approximately 3 times higher outdoor levels of PAHs for schools in North Carolina (USA) than it was in the current study.

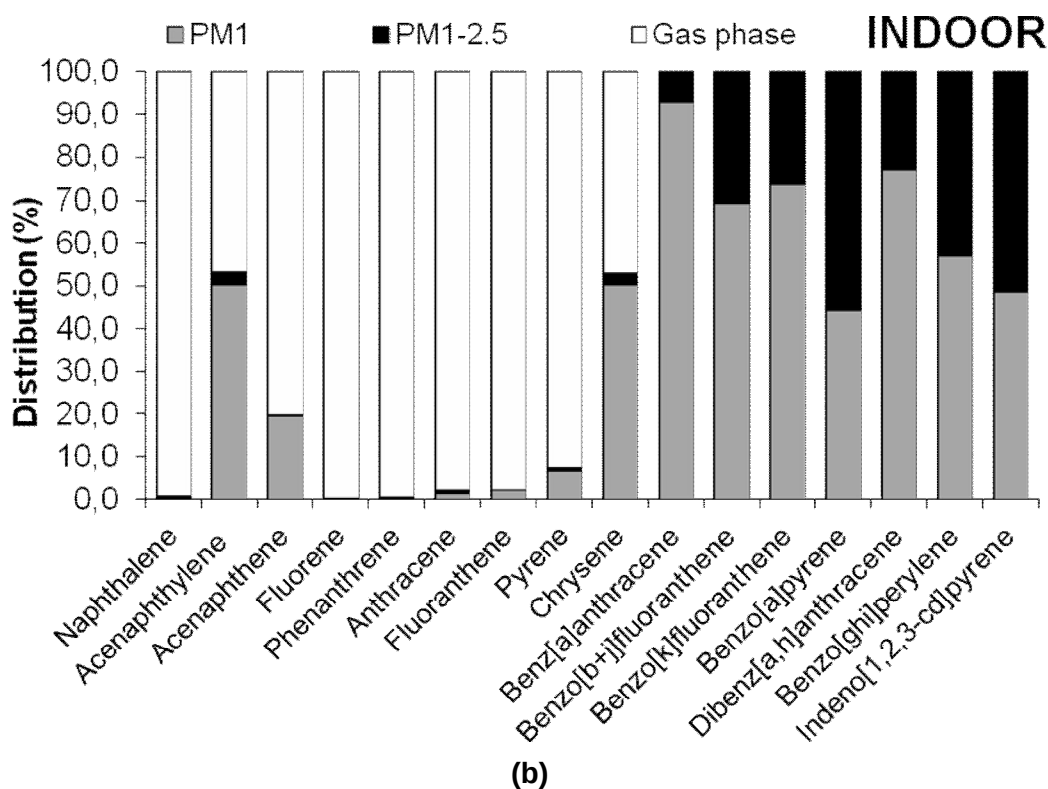
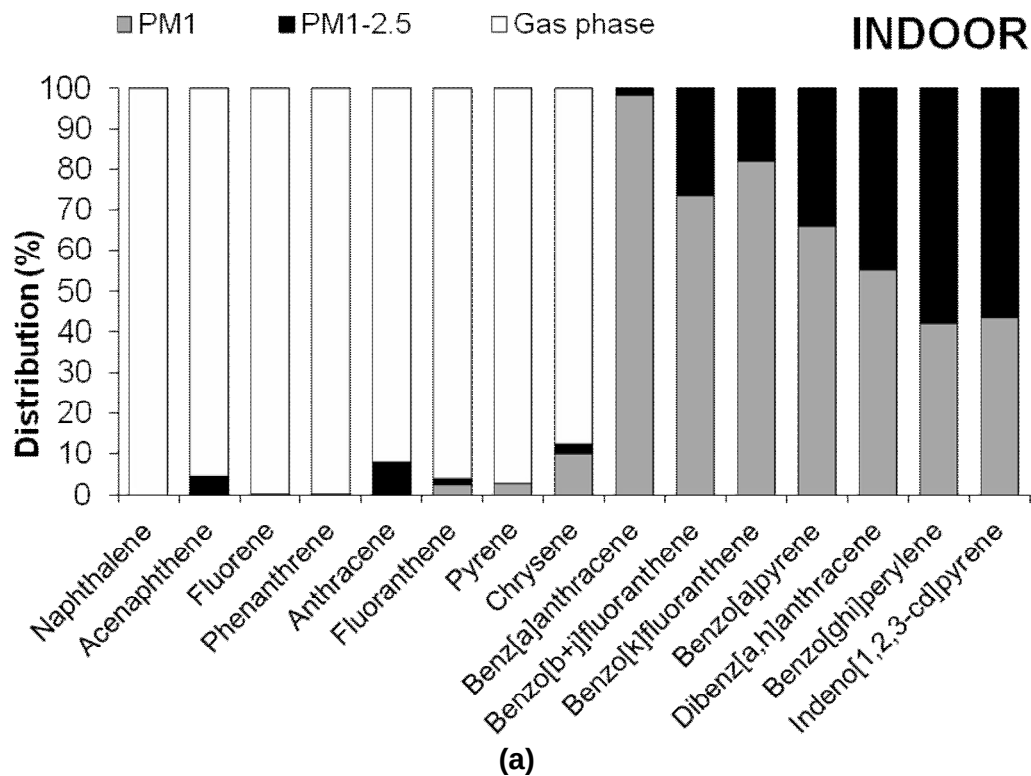


Figure 4.7. Distribution of PAHs between particulate and gaseous phases in indoor air of two preschools: (a) PS1 and (b) PS2. Particulate phase is further divided into PM₁ and PM_{1-2.5} fractions).

Table 4.22. Outdoor levels of PAHs in PM_{2.5}, PM₁₀, gas phase, and in air (i.e. total) of two studied preschools PS1 and PS2 (ng/m³).

Compound	PS1									
	Particulate phase					Gas phase				
	PM _{2.5}		PM ₁₀			Mean		(Min–Max)		Air
	Mean	(Min–Max)	Mean	(Min–Max)	Mean	Mean	(Min–Max)	Mean	(Min–Max)	
Naphthalene	4.26×10 ⁻²⁶	3.57×10 ⁻² –4.83×10 ⁻²	2.63×10 ⁻²⁶	2.46×10 ⁻² –2.71×10 ⁻²	9.55	9.59	0.784–20.9	9.59	0.820–20.9	
Acenaphthylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Acenaphthene	n.d.	n.d.	n.d.	n.d.	1.09 ^a	1.09	4.66×10 ⁻² –4.12	1.09	4.66×10 ⁻² –4.12	
Fluorene	4.88×10 ^{-3c}	2.66×10 ⁻³ –1.30×10 ⁻²	3.66×10 ^{-3c}	2.65×10 ⁻³ –4.87×10 ^{-3c}	3.36	3.37	0.217–5.41	3.37	0.220–5.43	
Phenanthrene	4.68×10 ⁻²	1.53×10 ⁻² –0.131	3.27×10 ⁻²	1.46×10 ⁻² –5.31×10 ⁻²	8.00	8.05	0.597–13.2	8.05	0.612–13.3	
Anthracene	8.21×10 ^{-3a}	3.21×10 ⁻³ –1.69×10 ⁻²	7.38×10 ⁻³	3.15×10 ⁻³ –1.01×10 ⁻²	0.532 ^a	0.541	4.97×10 ⁻² –0.839	0.541	5.29×10 ⁻² –0.856	
Fluoranthene	0.119	6.92×10 ⁻² –0.230	0.104	6.35×10 ⁻² –0.142	1.90 ^a	2.02	0.125–4.26	2.02	0.194–4.33	
Pyrene	0.123	5.69×10 ⁻² –0.252	9.44×10 ⁻²	5.38×10 ⁻² –0.131	1.95 ^a	2.07	0.159–4.17	2.07	0.216–4.42	
Benz[a]anthracene	5.23×10 ⁻²	2.61×10 ⁻² –0.120	4.32×10 ⁻²	2.32×10 ⁻² –6.65×10 ⁻²	n.d.	5.23×10 ⁻²	n.d.	5.23×10 ⁻²	2.61×10 ⁻² –0.120	
Chrysene	0.145	6.15×10 ⁻² –0.427	0.142	8.26×10 ⁻² –0.211	0.151 ^c	0.293	5.20×10 ⁻² –0.490	0.293	0.114–0.842	
Benz[b+j]fluoranthene	0.233	0.116–0.459	0.171	9.61×10 ⁻² –0.314	n.d.	0.233	n.d.	0.233	0.116–0.459	
Benz[k]fluoranthene	4.52×10 ⁻²	2.17×10 ⁻² –8.42×10 ⁻²	3.85×10 ⁻²	2.02×10 ⁻² –7.93×10 ⁻²	n.d.	4.52×10 ⁻²	n.d.	4.52×10 ⁻²	2.17×10 ⁻² –8.42×10 ⁻²	
Benz[a]pyrene	7.06×10 ^{-2a}	3.69×10 ⁻² –0.169	5.31×10 ⁻²	3.34×10 ⁻² –0.115	3.66×10 ^{-2b}	0.107	6.25×10 ⁻³ –7.02×10 ⁻²	0.107	4.3×10 ⁻² –0.239	
Dibenz[a,h]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Dibenz[a,h]anthracene	0.474	0.295–2.43	0.473	0.281–0.987	n.d.	0.474	n.d.	0.474	0.295–2.43	
Benz[ghi]perylene	0.172	9.20×10 ⁻² –0.674	0.156	9.14×10 ⁻² –0.312	n.d.	0.172	n.d.	0.172	9.20×10 ⁻² –0.674	
Indeno[1,2,3-cd]pyrene	8.53×10 ⁻²	3.11×10 ⁻² –0.190	5.82×10 ⁻²	2.88×10 ⁻² –0.143	n.d.	8.53×10 ⁻²	n.d.	8.53×10 ⁻²	3.11×10 ⁻² –0.190	
Σ PAHs	1.62	0.744–4.27	1.4	0.737–2.36	26.6	28.2	1.94–43.6	28.2	2.68–47.9	
Σ PAH _{toxic}	1.15	0.466–3.40	1.00	0.582–1.89	9.74	10.8	0.842–21.5	10.8	1.31–23.9	

n.d. – not detected
Note: detection frequency of each compound was 100% unless otherwise indicated.
^a80% ≤ detection frequency < 100%.
^b60% ≤ detection frequency < 80%.
^c20% ≤ detection frequency < 60%.

Table 4.22. Outdoor levels of PAHs in PM_{2.5}, PM₁, gas phase, and in air (i.e. total) of two studied preschools PS1 and PS2 (ng/m³) (continued).

Compound	PS2				Gas phase				Air	
	Particulate phase		PM ₁		Mean		(Min-Max)		Mean	(Min-Max)
	Mean	(Min-Max)	Mean	(Min-Max)	Mean	(Min-Max)	Mean	(Min-Max)		
Naphthalene	7.08×10 ^{-2a}	2.03×10 ⁻² –0.150	n.d.	n.d.	3.16	0.392–6.02	3.23	7.08×10 ⁻² –6.17		
Acenaphthylene	n.d.	n.d.	n.d.	n.d.	7.15 ^c	6.57–7.53	7.15	6.57–7.53		
Acenaphthene	0.979	0.383–2.30	0.708	0.274–1.03	0.900	5.14×10 ⁻² –3.44	1.88	0.434–3.90		
Fluorene	1.52×10 ⁻²	8.06×10 ⁻³ –3.06×10 ⁻²	1.00×10 ⁻²	3.82×10 ⁻³ –2.15×10 ⁻²	0.946	0.182–1.56	0.961	0.191–1.59		
Phenanthrene	0.158	4.79×10 ⁻³ –0.298	8.86×10 ⁻²	4.27×10 ⁻² –0.161	3.01	0.578–5.26	3.17	0.626–5.39		
Anthracene	1.07×10 ^{-2a}	4.14×10 ⁻³ –2.66×10 ⁻²	5.22×10 ^{-3a}	1.85×10 ⁻³ –1.07×10 ⁻²	0.172	8.48×10 ⁻² –0.320	0.183	8.89×10 ⁻² –0.324		
Fluoranthene	6.11×10 ⁻²	2.44×10 ⁻² –0.216	3.28×10 ^{-2a}	1.91×10 ⁻² –4.85×10 ⁻²	0.522	1.93×10 ⁻² –0.819	0.583	4.37×10 ⁻² –0.990		
Pyrene	0.249	0.122–0.749	0.215	0.118–0.302	0.904	0.230–1.41	1.15	0.352–1.54		
Benz[a]anthracene	0.109	5.10×10 ⁻³ –0.418	7.55×10 ^{-2b}	4.25×10 ⁻² –9.44×10 ⁻²	1.17×10 ^{-2b}	9.52×10 ⁻⁴ –2.26×10 ⁻²	0.121	5.20×10 ⁻² –0.539		
Chrysene	0.171	9.51×10 ⁻² –0.437	0.148	8.37×10 ⁻² –0.201	6.31×10 ^{-2b}	2.52×10 ⁻² –8.60×10 ⁻²	0.234	0.120–0.523		
Benz[b+g]fluoranthene	0.534	0.221–1.38	0.284	0.135–0.385	n.d.	n.d.	0.534	0.221–1.38		
Benz[k]fluoranthene	0.129	3.98×10 ⁻² –0.431	5.23×10 ⁻²	2.40×10 ⁻² –7.49×10 ⁻²	n.d.	n.d.	0.129	3.98×10 ⁻² –0.431		
Benz[a]pyrene	0.295	0.130–0.972	0.117	6.32×10 ⁻² –0.172	n.d.	n.d.	0.295	0.130–0.972		
Dibenz[a,h]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Dibenz[a,h]anthracene	1.07	0.487–3.20	0.503	0.226–0.699	n.d.	n.d.	1.07	0.487–3.20		
Benz[ghi]perylene	0.381	0.169–1.13	0.165	0.116–0.227	n.d.	n.d.	0.381	0.169–1.13		
Indeno[1,2,3-cd]pyrene	0.312	0.123–1.05	0.118 ^b	8.13×10 ⁻² –0.162	n.d.	n.d.	0.312	0.123–1.05		
Σ _{PAHs}	4.55	2.70–10.5	2.52	1.63–4.70	16.9	4.54–17.1	21.4	5.11–30.5		
Σ _{PAHs+BC}	2.69	1.33–7.82	1.30	0.590–1.71	3.23	0.512–6.12	5.92	1.84–11.3		

n.d. – not detected

Note: detection frequency of each compound was 100% unless otherwise indicated.

^a80% ≤ detection frequency < 100%.

^b60% ≤ detection frequency < 80%.

^c20% ≤ detection frequency < 60%.

Once again the most abundant particulate PAHs at PS1 were dibenz[a,h]anthracene (29 and 34% of Σ_{PAHs} for $\text{PM}_{2.5}$ and PM_1), benzo[b+j]fluoranthene (15 and 12% of Σ_{PAHs} for $\text{PM}_{2.5}$ and PM_1) and benzo[ghi]perylene (11%), whereas it was naphthalene (36% of Σ_{PAHs}), phenanthrene (30% of Σ_{PAHs}), and fluorene (approximately 13%) in the gas phase. Similarly to indoor air at PS2, the most abundant particulate PAHs in outdoor air were dibenz[a,h]anthracene (24% in $\text{PM}_{2.5}$, 20% in PM_1), acenaphthene (22% in $\text{PM}_{2.5}$ and 28% in PM_1) and benzo[b+j]fluoranthene (approximately 12%); the abundant gaseous compounds were acenaphthylene (43% of Σ_{PAHs}), naphthalene (19%), and phenanthrene (18% of Σ_{PAHs}). Finally, carcinogenic content of PAHs in outdoor air accounted for 38 and 28% Σ_{PAHs} at PS1 and at PS2, respectively, being similar to indoor air (45 and 27% at PS1 and PS2, respectively).

4.3.3.3 Phase distribution of PAHs

Phase-distribution of indoor PAHs was similar at both studied preschools (Figure 4.7) and in agreement with previously reported findings (Krugly et al., 2014). Compounds with 5 and 6 aromatic rings, namely benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene were entirely present in PM, whereas PAHs with 2 and 3 aromatic rings (naphthalene, fluorene and phenanthrene) existed almost entirely in the gas phase (>99%). In total, gaseous PAHs accounted for 98 and 94% of Σ_{PAHs} at PS1 and PS2, respectively. This high proportion of PAHs in gas phase clearly demonstrates that adequate assessment of PAHs exposure requires consideration of gaseous compounds, which are in the most studies neglected. PAHs with 4 rings (anthracene, fluoranthene, pyrene, and chrysene) were distributed between both phases with proportions in gas phase ranging from 47% (chrysene at PS2) to 97% (pyrene at PS1). Acenaphthylene and acenaphthene (3 aromatic rings) exhibited unexpectedly high distributions in particulate phases, especially at PS2 where 53 and 20%, respectively, were particulate-bound (opposed to 4% at PS1). No specific occurrence that could justify these abundances was identified during sample collection at PS2; the unusual distribution of these compounds might be caused by contribution from a particular emission source and/or seasonal influences; Vestenius et al. (2011) reported that PAHs concentration in particulate phase is closely related to the ambient temperature.

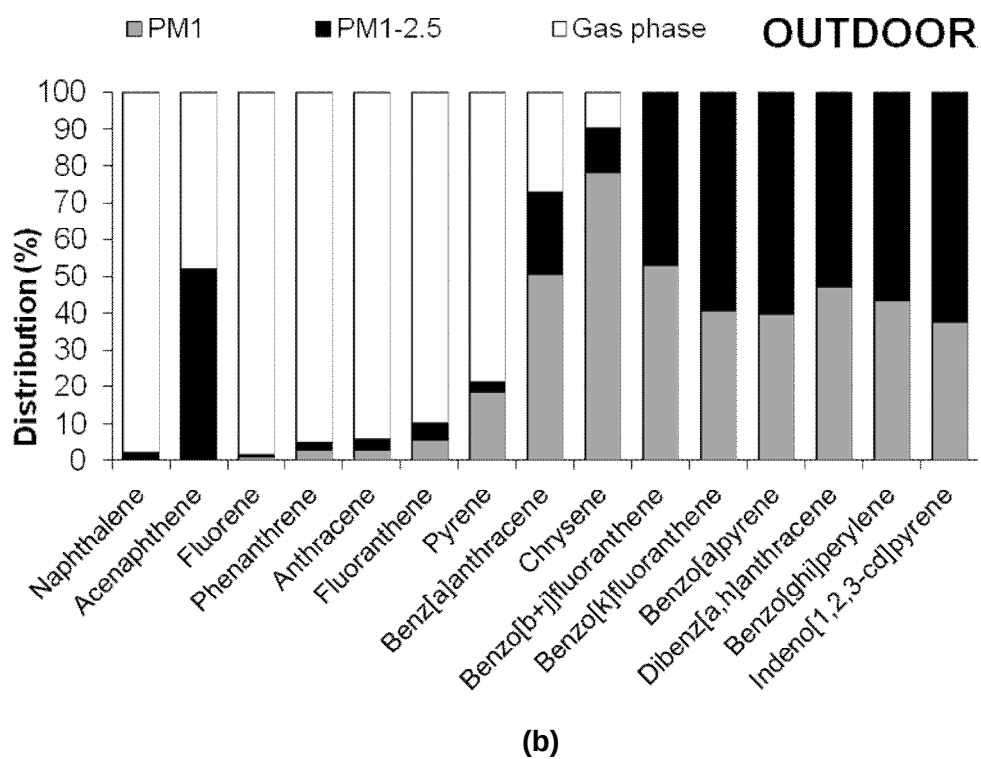
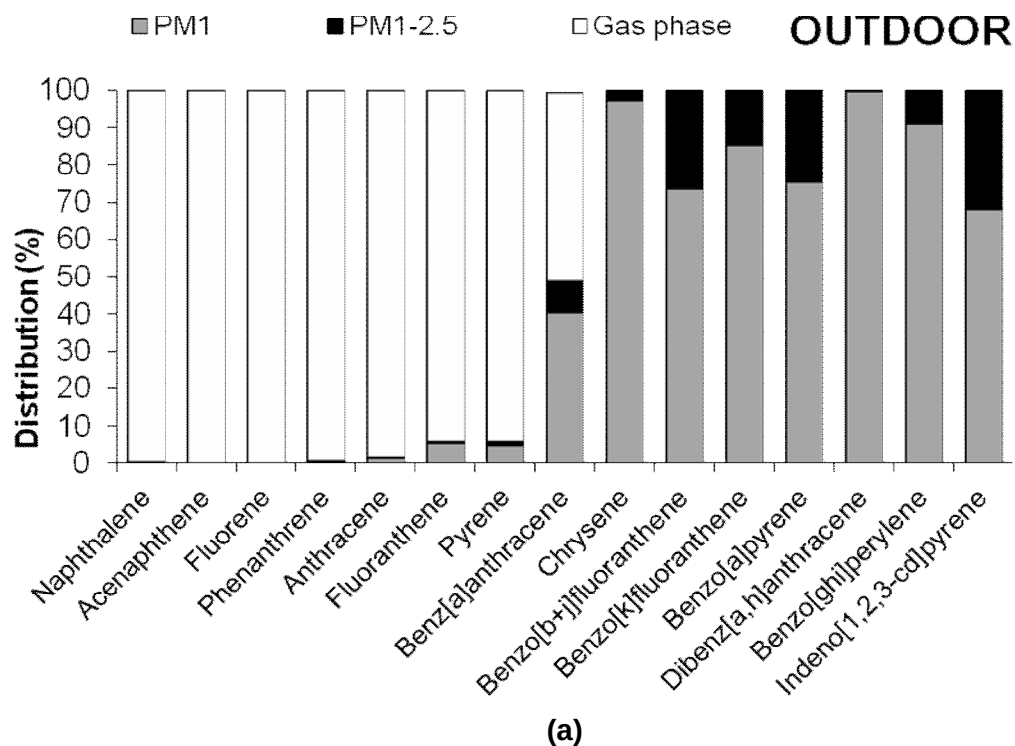


Figure 4.8. Distribution of PAHs between particulate and gaseous phases in outdoor air of two preschools: (a) PS1 and (b) PS2. Particulate phase is further divided into PM₁ and PM_{1-2.5} fractions (particles with aerodynamic diameter between 1.0 and 2.5 μ m).

Lower (both indoor/outdoor) temperatures at PS2 (Table 4.20) may resulted in higher amounts of these PAHs in particulate phase.

PM/gas phase distributions of outdoor PAHs at both preschools (Figure 4.8) were similar to those obtained in previous studies (Castro et al., 2011; Possanzini et al., 2004; Zhou et al, 2013) and to those of indoor air. Heavy molecular weight PAHs (with 5–6 rings) existed mostly in PM, while lighter congeners were mostly abundant in gas phase.

4.3.3.4 Indoor-to-outdoor ratios

I/O ratios of PM_{2.5}-bound PAHs at two preschools are presented in Figure 4.9. It is clear that I/O ratios varied between the compounds and both locations. At both preschools I/O ratios of lighter PAH congeners (naphthalene, acenaphthene, fluorene, and phenanthrene) exceeded unity (with particularly high exceedances at PS2). These results indicated a potential contribution of these PAHs from an indoor source.

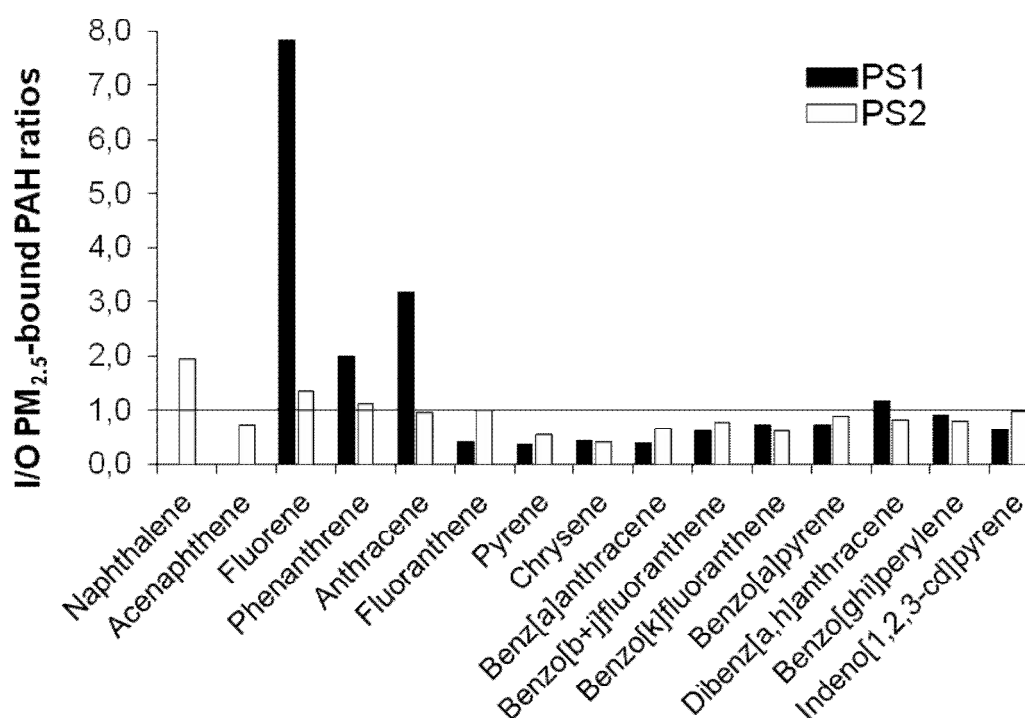


Figure 4.9. Indoor-to-outdoor (I/O) PM_{2.5}-bound PAH ratios at the studied preschools PS1 and PS2.

Similarly, Krugly et al. (2014) reported higher I/O ratios for semivolatile PAHs in indoor air of schools; authors suggested contribution occupants' activities and/or evaporation from building materials. Indoor sources that were registered during sampling included: children artistic activities (painting, sculpturing, wax melting and other craft activities) and classroom cleaning (wood polishing) (Morawska et al., 2013). On several occasions, birthday celebrations took place in the respective indoor environments with combustion sources (candles on birthday cake) which could also cause the elevated PAH content (Derudi et al., 2013). A previous study suggested that heavy molecular PAHs occurring indoors are usually emitted from outdoor sources, such as combustion engines, rather than from indoor combustion processes. Wang et al. (2014) reported significantly higher levels of pyrene and chrysene in houses naturally ventilated for more than 4 h compared to those ventilated for less than 1 h per day. They also observed concentrations of PM_{2.5}- bound benz[a]anthracene, benzo[b+j]fluoranthene, benzo[k]fluoranthene (and other heavy molecular PAHs) negatively associated with floor level which suggested that at indoor PAHs in dwelling situated at/close to ground levels were mainly resulted from outdoor emissions (Wang et al., 2014). The results of this study support those findings. Ratios of PAHs with 4 rings (namely pyrene, chrysene, benz[a]anthracene) were lower than 0.5 at PS1 (and between 0.4–0.7 at PS2), showing that outdoor sources of these compounds were prevailing. In addition, at both preschools, PAHs with 5–6 rings (benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene exhibited I/O ratios <1, indicating an outdoor origin of the contaminants.

4.3.3.5 Diagnostic concentration ratios

Diagnostic ratios were calculated based on the same particulate fraction (PM_{2.5}) for both indoor and outdoor PAHs (Table 4.23). At PS1, these diagnostic ratios, both indoors and outdoors, were lower than 1, indicating pyrogenic origin of PAHs. Traffic was most likely the source of PAHs at this preschool, which was situated in a close vicinity of busy streets. In agreement with these findings, ratio of benz[a]anthracene to chrysene was >0.35, indicating combustion of fuel and/or vehicular emissions (Krugly et al., 2014).

Table 4.23. Diagnostic ratios for PM_{2.5}-bound PAHs at the studied preschools.

Ratio	This study				Value	Source	Reference
	PS1		PS2				
	Indoor	Outdoor	Indoor	Outdoor			
^a ΣPAHs _{LMW} /ΣPAH _{SHMW}	0.13	0.15	1.70	0.96	>1 <1	Petrogenic Pyrogenic	Zhang et al. (2008)
Benz[a]anthracene/Chrysene	0.39	0.36	0.39	0.64	>0.35	Fuel combustion	Krugly et al. (2014)
Fluoranthene/(Fluoranthene + Pyrene)	0.50	0.49	0.31	0.20	0.40-0.50 >0.5 0.42, 0.52	Vehicular traffic Coal combustion Road dust	Kavouras et al. (1999); Oda et al. (2001); Rogge et al. (1993a); Yunker et al. (2002); Zencak et al. (2007)
Indeno[1,2,3-cd]pyrene/(Indeno[1,2,3-cd]pyrene + Benzo[ghi]perylene)	0.12	0.35	0.50	0.45	0.21–0.22 0.35–0.70 0.56 0.62 0.36	Gasoline cars Diesel emissions Coal combustion Wood combustion Road dust	Gogou et al. (1996); Grimmer et al. (1983); Kavouras et al. (2001); Khalili et al. (1995); Pio et al. (2001); Ravindra et al. (2008); Rogge et al. (1993a)
Benzo[a]pyrene/(Benzo[a]pyrene + Chrysene)	0.47	0.33	0.70	0.63	0.5 0.73	Diesel Gasoline	Ravindra et al. (2008)
Benzo[b]fluoranthene/Benzo[k]fluoranthene	4.23	5.15	5.16	4.15	>0.5	Diesel	Ravindra et al. (2008)
Benzo[ghi]perylene/Benzo[a]pyrene	7.39	2.44	1.15	1.29	1.2–2.2 2.5–3.3 0.86, 0.91	Diesel cars Gasoline cars Road dust	Oda et al. (2001); Rogge et al. (1993a, 1993b)
Benz[a]anthracene/(Benz[a]anthracene+ chrysene)	0.23	0.50	0.28	0.39	0.40, 0.38–0.64 0.76 0.43	Diesel Gasoline Wood combustion	Li and Kamens (1993); Manoli et al. (2004); Sicre et al. (1987)
Benzo[b+k]Fluoranthene/Benzo[ghi]perylene	0.23	0.50	0.67	0.50	1.60 0.33 2.18	Diesel cars Gasoline cars Wood combustion	Li and Kamens (1993)
Fluorene/(Fluorene + Pyrene)	0.46	0.074	0.13	0.06	>0.5 <0.5	Diesel Gasoline	Ravindra et al. (2008)
Phenanthrene/(Phenanthrene + Anthracene)	0.78	0.85	0.94	0.94	>0.7	Lubricant oils and fossil fuels	Mirante et al. (2013)
^a Total concentration of 2– and 3–rings PAHs (ΣPAHs _{LMW}), and 4– and 6–rings PAHs (ΣPAHs _{SHMW})							

^aTotal concentration of 2- and 3-rings PAHs (ΣPAHs_{LMW}), and 4- and 6-rings PAHs (ΣPAHs_{SHMW})

Ratio values of fluoranthene to fluoranthene and pyrene obtained at PS2 suggested also potential origin from vehicular traffic (Kavouras et al., 1999; Yunker et al., 2002; Pongpiachan 2014) with possible contribution from coal combustion (Krugly et al., 2014). However, $\Sigma\text{PAHs}_{\text{LMW}}/\Sigma\text{PAHs}_{\text{HMW}}$ was 0.96 outdoors and >1 for indoors at PS2, indicating petrogenic origin. This result was somewhat surprising considering that pyrogenic origin of PAHs was expected. Benz[a]anthracene to chrysene ratio was >0.35 , pointing towards fuel combustion and/or vehicular emissions. Similarly, the indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene+benzo[ghi]perylene) ratio at PS2 were between 0.35 and 0.70 indicating contribution of diesel emissions (Kavouras et al. 2001; Rogge et al., 1993a, Tipmanee et al., 2012). Other ratios that were applied were also indicative of vehicular influence. Benzo[b]fluoranthene to benzo[k]fluoranthene ratios >0.5 (Ravindra et al., 2008) indicate diesel emissions, whereas ratio of benzo[a]pyrene to benzo[a]pyrene and chrysene distinguishes between diesel (0.5) and gasoline (0.73) vehicle emissions. The comparison of these ratios with data in Table 4.23 suggests that diesel motor emissions were a relevant source of PAHs at both preschools. In addition, ratios of benz[a]anthracene to benz[a]anthracene and chrysene in outdoor air of PS1 and PS2 ranged from 0.39 to 0.50, thus also indicating diesel combustion (between 0.38 and 0.64), as previously suggested in a preliminary study (section 4.3.2). These findings are in agreement with data from Portugal related with diesel consumption. Ratio of fluorene/(fluorene and pyrene) >0.5 also indicates contribution of diesel emissions, whereas ratio <0.5 indicates gasoline emissions (Ravindra et al., 2008; Rogge et al., 1993b); the present results show very low values thus suggesting some presence of gasoline emissions at both preschools (indoors and outdoors). Similar findings were also demonstrated by obtained values of benzo[b+k]fluoranthene and benzo[ghi]perylene ratios that pointed towards contribution of gasoline emissions at both preschools (Li and Kamens 1993). Finally, at both preschools, values of phenanthrene to phenanthrene and anthracene ratios were higher >0.7 , which are characteristic of contributions from lubricant oils and fossil fuels (Mirante et al., 2013).

4.3.3.6 Health risk assessment

Results of TEF-adjusted concentrations for 18 PAHs (both in PM and gas phase) at both preschools are presented in Table 4.24. Dibenz[a,h]anthracene was the largest contributor (approximately between 20–36 % of PM) to ΣPAHs in indoor and

outdoor air, and due to its high TEF (5) it remained the largest contributor to $\Sigma_{TEF-PAHs}$ (Okona-Mensah et al., 2005). This PAH accounted approximately for 95% and 93% of indoor and outdoor particulate (and total, i.e. air) $\Sigma_{TEF-PAHs}$ at PS1 and PS2, respectively. Benzo[a]pyrene was the second most abundant compound (2–3% of $\Sigma_{TEF-PAHs}$ at PS1, 4–5% at PS2). Concerning the gaseous PAHs, naphthalene and phenanthrene made the largest contributions to total indoor PAH content of both preschools (73–78% of Σ_{PAHs} at PS2 and PS1; Table 4.21), but their TEF-adjusted contributions were less than 1% in indoor air of both PS1 and PS2 (Table 4.24). However, in a view of existing WHO IAQ guidelines for naphthalene (WHO 2010), and considering its high abundance namely in the gas phase, routine monitoring of this PAH should be performed when assessing IAQ and the respective health risks.

The values of $\Sigma_{TEF-PAHs}$ were used to estimate the corresponding lifetime lung cancer risks for the exposed populations using WHO unit risk of 8.7×10^{-5} per ng m^{-3} of benzo[a]pyrene for lifetime (70 years) exposure (WHO 2010). Both groups (3–and 4–5 years old) of children, who attended the respective preschools, had different daily schedules (on average 6.75 h of school daily time was indoors and 1.25 h outdoors). The corresponding lung cancer risks were 7.89×10^{-5} (6.75×10^{-5} and 1.14×10^{-5} for indoor and outdoor air, respectively) at PS1 and 1.36×10^{-4} (1.10×10^{-4} for indoors and 2.62×10^{-5} for outdoors) at PS2. These estimated values exceeded WHO health-based guideline level of 10^{-5} (Boström et al., 2002) for both preschools (approximately 8 and 13 times for PS1 and PS2, respectively).

Target carcinogenic risks associated with inhalation exposure to PAHs for two different age categories of children (3 years and 4–5–years old) were estimated by USEPA methodology (Table 4.25). It is necessary to point out that the risk values were calculated using 24 h mean concentrations; child school exposure was though 8 h per day. At both preschools total and individual carcinogenic target risks of PAHs, both indoors and outdoors, were well below 10^{-6} and thus negligible. At both preschools, total carcinogenic risks due to indoor exposure to PAHs were higher than outdoor ones. For 3–years old children, indoor risks were 18 and 10 times higher than outdoor ones at PS1 and PS2, respectively, whereas for 4–5–years old children they were 7 and 4 times higher at PS1 and PS2, respectively. These differences were most likely due to the prolonged periods that children spent indoors (7.25 and 6.25 h indoors versus 0.75 and 1.75 h outdoors, respectively).

Table 4.24. Toxicity equivalency factor (TEF)–adjusted mean concentrations of PAHs in indoor and outdoor air of the studied preschools PS1 and PS2 (pg/m³).

Compound	TEF ^a	Indoor				Outdoor			
		Particulate phase		Gas phase		Particulate phase		Gas phase	
		PM _{2.5}	PM ₁	PM _{2.5}	PM ₁	PM _{2.5}	PM ₁	PM _{2.5}	PM ₁
Naphthalene	0.001	–	–	27.2	27.2	4.26×10 ⁻²	2.63×10 ⁻²	9.55	9.59
Acenaphthylene	0.001	–	–	–	–	–	–	–	–
Acenaphthene	0.001	2.50×10 ⁻²	–	0.538	0.563	–	–	1.09	1.09
Fluorene	0.001	3.82×10 ⁻²	1.76×10 ⁻²	9.66	9.70	4.88×10 ⁻³	3.66×10 ⁻³	3.36	3.37
Phenanthrene	0.001	9.28×10 ⁻²	3.82×10 ⁻²	21.2	21.3	4.68×10 ⁻²	3.27×10 ⁻²	8.00	8.05
Anthracene	0.01	0.259	–	3.04	3.30	8.21×10 ⁻²	7.38×10 ⁻²	5.32	5.41
Fluoranthene	0.001	4.80×10 ⁻²	2.94×10 ⁻²	1.16	1.21	0.119	0.105	1.90	2.02
Pyrene	0.001	4.52×10 ⁻²	4.43×10 ⁻²	1.49	1.53	0.123	9.44×10 ⁻²	1.95	2.07
Benz[a]anthracene	0.1	2.23	2.20	–	2.23	5.23	4.31	–	5.23
Chrysene	0.01	0.573	0.461	3.99	4.57	1.45	1.42	1.52	2.97
Benzo[b+j]fluoranthene	0.1	14.1	10.4	–	14.1	23.3	17.1	–	23.3
Benzo[k]fluoranthene	0.1	3.34	2.74	–	3.34	4.52	3.85	–	4.52
Benzo[a]pyrene	1	50.9	33.6	–	50.9	70.6	53.1	–	70.6
Dibenzo[a,l]pyrene	n.a.	–	–	–	–	–	–	–	–
Dibenzo[a,h]anthracene	5	2830	1560	–	2830	2370	2364	–	2370
Benzo[ghi]perylene	0.01	3.77	1.59	–	3.77	1.72	1.56	–	1.72
Indeno[1,2,3-cd]pyrene	0.1	5.34	2.31	–	5.34	8.53	5.82	–	8.53
Σ _{TEF-PAHs}	–	2911	1613	68.3	2979	2485	2451	33	2518

TEF estimated by Nisbeth and LaGoy (Boström et al., 2002)

n.a.= not available

TEF estimated by Nisbeth and LaGoy (Boström et al., 2002)
n.a. = not available

Table 4.25. Estimated target carcinogenic risks (TR) of PAHs in indoor and outdoor air of the studied preschools PS1 and PS2 for different age categories of children.

PS1	TR _{indoor}				TR _{outdoor}				TR _{school}			
Children 3–years old	PM _{2.5}	PM ₁	Gas phase	Σ TR _{indoor}	PM _{2.5}	PM ₁	Gas phase	Σ TR _{outdoor}	PM _{2.5}	PM ₁	Gas phase	Σ TR _{school}
Naphthalene	–	–	2.73×10 ⁻⁹	2.73×10 ⁻⁹	4.43×10 ⁻¹³	2.73×10 ⁻¹³	9.92×10 ⁻¹¹	9.96×10 ⁻¹¹	2.83×10 ⁻⁹	–	–	2.83×10 ⁻⁹
Benz[a]anthracene	7.26×10 ⁻¹²	7.14×10 ⁻¹²	4.83×10 ⁻¹⁰	4.90×10 ⁻¹⁰	1.76×10 ⁻¹²	1.45×10 ⁻¹²	–	1.76×10 ⁻¹²	4.92×10 ⁻¹⁰	–	–	4.92×10 ⁻¹⁰
Chrysene	1.86×10 ⁻¹²	1.50×10 ⁻¹²	–	1.86×10 ⁻¹²	4.89×10 ⁻¹³	4.77×10 ⁻¹³	5.06×10 ⁻¹³	9.95×10 ⁻¹³	2.85×10 ⁻¹²	–	–	2.85×10 ⁻¹²
Benzo[b+g]fluoranthene	4.60×10 ⁻¹¹	3.38×10 ⁻¹¹	1.30×10 ⁻¹⁰	1.76×10 ⁻¹⁰	7.83×10 ⁻¹²	5.77×10 ⁻¹²	–	7.83×10 ⁻¹²	1.84×10 ⁻¹⁰	–	–	1.84×10 ⁻¹⁰
Benzo[k]fluoranthene	1.09×10 ⁻¹¹	8.91×10 ⁻¹²	–	1.09×10 ⁻¹¹	1.52×10 ⁻¹²	1.29×10 ⁻¹²	–	1.52×10 ⁻¹²	1.24×10 ⁻¹¹	–	–	1.24×10 ⁻¹¹
Benzo[a]pyrene	1.66×10 ⁻¹⁰	1.09×10 ⁻¹⁰	–	1.66×10 ⁻¹⁰	2.37×10 ⁻¹¹	1.79×10 ⁻¹¹	–	2.37×10 ⁻¹¹	1.90×10 ⁻¹⁰	–	–	1.90×10 ⁻¹⁰
Dibenz[a,h]anthracene	2.01×10 ⁻⁹	1.11×10 ⁻⁹	–	2.01×10 ⁻⁹	1.73×10 ⁻¹⁰	1.74×10 ⁻¹⁰	–	1.74×10 ⁻¹⁰	2.18×10 ⁻⁹	–	–	2.18×10 ⁻⁹
Indeno[1,2,3-cd]pyrene	1.74×10 ⁻¹¹	7.53×10 ⁻¹²	–	1.74×10 ⁻¹¹	2.87×10 ⁻¹²	1.96×10 ⁻¹²	–	2.87×10 ⁻¹²	2.03×10 ⁻¹¹	–	–	2.03×10 ⁻¹¹
ΣPAHs	2.26×10 ⁻⁹	1.28×10 ⁻⁹	3.34×10 ⁻⁹	5.60×10 ⁻⁹	2.12×10 ⁻¹⁰	2.02×10 ⁻¹⁰	9.97×10 ⁻¹¹	3.12×10 ⁻¹⁰	5.91×10 ⁻⁹	–	–	5.91×10 ⁻⁹
Children 4–5–years old	PM _{2.5}	PM ₁	Gas phase	Σ TR _{indoor}	PM _{2.5}	PM ₁	Gas phase	Σ TR _{outdoor}	PM _{2.5}	PM ₁	Gas phase	Σ TR _{school}
Naphthalene	–	–	4.71×10 ⁻⁹	4.71×10 ⁻⁹	2.07×10 ⁻¹²	1.28×10 ⁻¹²	4.63×10 ⁻¹⁰	4.65×10 ⁻¹⁰	5.17×10 ⁻⁹	–	–	5.17×10 ⁻⁹
Benz[a]anthracene	1.25×10 ⁻¹¹	1.21×10 ⁻¹¹	8.32×10 ⁻¹⁰	8.44×10 ⁻¹⁰	8.20×10 ⁻¹²	6.77×10 ⁻¹²	–	8.20×10 ⁻¹²	8.52×10 ⁻¹⁰	–	–	8.52×10 ⁻¹⁰
Chrysene	3.21×10 ⁻¹²	2.59×10 ⁻¹²	–	3.21×10 ⁻¹²	9.78×10 ⁻¹³	2.22×10 ⁻¹²	2.38×10 ⁻¹²	3.36×10 ⁻¹²	6.57×10 ⁻¹²	–	–	6.57×10 ⁻¹²
Benzo[b+g]fluoranthene	7.93×10 ⁻¹¹	5.83×10 ⁻¹¹	2.24×10 ⁻¹⁰	3.03×10 ⁻¹⁰	3.66×10 ⁻¹¹	2.69×10 ⁻¹¹	–	3.66×10 ⁻¹¹	3.40×10 ⁻¹⁰	–	–	3.40×10 ⁻¹⁰
Benzo[k]fluoranthene	1.87×10 ⁻¹¹	1.54×10 ⁻¹¹	–	1.87×10 ⁻¹¹	7.09×10 ⁻¹²	6.05×10 ⁻¹²	–	7.09×10 ⁻¹²	2.58×10 ⁻¹¹	–	–	2.58×10 ⁻¹¹
Benzo[a]pyrene	2.86×10 ⁻¹⁰	1.88×10 ⁻¹⁰	–	2.86×10 ⁻¹⁰	1.11×10 ⁻¹⁰	8.34×10 ⁻¹¹	–	1.11×10 ⁻¹⁰	3.97×10 ⁻¹⁰	–	–	3.97×10 ⁻¹⁰
Dibenz[a,h]anthracene	3.46×10 ⁻⁹	1.91×10 ⁻⁹	–	3.46×10 ⁻⁹	8.11×10 ⁻¹⁰	8.10×10 ⁻¹⁰	–	8.11×10 ⁻¹⁰	4.27×10 ⁻⁹	–	–	4.27×10 ⁻⁹
Indeno[1,2,3-cd]pyrene	2.99×10 ⁻¹¹	1.30×10 ⁻¹¹	–	2.99×10 ⁻¹¹	1.34×10 ⁻¹¹	9.13×10 ⁻¹²	–	1.34×10 ⁻¹¹	4.33×10 ⁻¹¹	–	–	4.33×10 ⁻¹¹
ΣPAHs	3.89×10 ⁻⁹	2.20×10 ⁻⁹	5.77×10 ⁻⁹	9.66×10 ⁻⁹	9.90×10 ⁻¹⁰	9.46×10 ⁻¹⁰	4.65×10 ⁻¹⁰	1.46×10 ⁻⁹	1.11×10 ⁻⁸	–	–	1.11×10 ⁻⁸

Table 4.25. Estimated target carcinogenic risks (TR) of PAHs in indoor and outdoor air of the studied preschools PS1 and PS2 for different age categories of children (continued).

PS2	ΣTR_{indoor}			$\Sigma TR_{outdoor}$			ΣTR_{school}
Children 3–years old	PM _{2.5}	PM ₁	Gas phase	PM _{2.5}	PM ₁	Gas phase	
Naphthalene	–	–	1.64×10 ⁻⁹	7.36×10 ⁻¹³	–	3.29×10 ⁻¹¹	1.67×10 ⁻⁹
Benz[a]anthracene	1.42×10 ⁻¹¹	1.32×10 ⁻¹¹	–	3.67×10 ⁻¹²	2.54×10 ⁻¹²	3.92×10 ⁻¹³	1.83×10 ⁻¹¹
Chrysene	3.68×10 ⁻¹²	3.49×10 ⁻¹²	3.29×10 ⁻¹²	5.72×10 ⁻¹³	4.98×10 ⁻¹³	2.12×10 ⁻¹³	7.75×10 ⁻¹²
Benzo[b+g]fluoranthene	1.33×10 ⁻¹⁰	9.25×10 ⁻¹¹	–	1.80×10 ⁻¹¹	9.56×10 ⁻¹²	–	1.51×10 ⁻¹⁰
Benzo[k]fluoranthene	2.58×10 ⁻¹¹	1.91×10 ⁻¹¹	–	4.33×10 ⁻¹²	1.76×10 ⁻¹²	–	3.01×10 ⁻¹¹
Benzo[a]pyrene	8.59×10 ⁻¹⁰	3.80×10 ⁻¹⁰	–	9.91×10 ⁻¹¹	3.93×10 ⁻¹¹	–	9.58×10 ⁻¹⁰
Dibenz[a,h]anthracene	3.13×10 ⁻⁹	2.41×10 ⁻⁹	–	3.93×10 ⁻¹⁰	1.85×10 ⁻¹⁰	–	3.52×10 ⁻⁹
Indeno[1,2,3-cd]pyrene	9.83×10 ⁻¹¹	4.76×10 ⁻¹¹	–	1.05×10 ⁻¹¹	3.97×10 ⁻¹²	–	1.09×10 ⁻¹⁰
ΣPAHs	4.27×10 ⁻⁹	6.39×10 ⁻¹⁰	1.64×10 ⁻⁹	5.30×10 ⁻¹⁰	2.43×10 ⁻¹⁰	3.35×10 ⁻¹¹	6.48×10 ⁻⁹
Children 4–5–years old	PM _{2.5}	PM ₁	Gas phase	PM _{2.5}	PM ₁	Gas phase	
Naphthalene	–	–	2.83×10 ⁻⁹	3.43×10 ⁻¹²	–	1.53×10 ⁻¹⁰	2.99×10 ⁻⁹
Benz[a]anthracene	2.45×10 ⁻¹¹	2.28×10 ⁻¹¹	–	1.71×10 ⁻¹¹	1.18×10 ⁻¹¹	1.83×10 ⁻¹²	4.34×10 ⁻¹¹
Chrysene	6.35×10 ⁻¹²	6.02×10 ⁻¹²	5.67×10 ⁻¹²	2.68×10 ⁻¹²	2.32×10 ⁻¹²	9.90×10 ⁻¹³	1.57×10 ⁻¹¹
Benzo[b+g]fluoranthene	2.30×10 ⁻¹⁰	1.60×10 ⁻¹⁰	–	8.38×10 ⁻¹¹	4.46×10 ⁻¹¹	–	3.14×10 ⁻¹⁰
Benzo[k]fluoranthene	4.46×10 ⁻¹¹	3.28×10 ⁻¹¹	–	2.02×10 ⁻¹¹	8.21×10 ⁻¹²	–	6.48×10 ⁻¹¹
Benzo[a]pyrene	1.48×10 ⁻⁹	6.54×10 ⁻¹⁰	–	4.62×10 ⁻¹⁰	1.84×10 ⁻¹⁰	–	1.94×10 ⁻⁹
Dibenz[a,h]anthracene	5.40×10 ⁻⁹	4.16×10 ⁻⁹	–	1.83×10 ⁻⁹	8.61×10 ⁻¹⁰	–	7.23×10 ⁻⁹
Indeno[1,2,3-cd]pyrene	1.69×10 ⁻¹⁰	8.20×10 ⁻¹¹	–	4.89×10 ⁻¹¹	1.85×10 ⁻¹¹	–	2.18×10 ⁻¹⁰
ΣPAHs	7.35×10 ⁻⁹	5.12×10 ⁻⁹	2.84×10 ⁻⁹	2.47×10 ⁻⁹	1.13×10 ⁻⁹	1.56×10 ⁻¹⁰	1.28×10 ⁻⁸

Furthermore, at both preschools the indoor risks were approximately 1–3 times higher for particulate-bound PAHs than for gaseous compounds. Considering different age groups, the total cancer risks of overall school exposure (ΣTR_{school}) were approximately 2 times higher (yet still negligible) for 4–5-years old children than for 3–years old. These differences were caused by different exposure time (1 *versus* 2 years) and secondly, by different daily schedules. Finally, the indoor risks contributed at PS1 95%, and 87% of the overall (i.e. both indoor and outdoor) school risks (ΣTR_{school}) for 3– and 4–5–years old children, respectively. At PS2 these contributions were slightly lower with indoor exposure contributing 91% and 80% of the overall school risks (ΣTR_{school}) for 3– and 4–5–years old children, respectively. The especially high contributions of indoor risks emphasize the need for future studies on these types of environments. In addition, better understanding of the complexity of child (pre)school exposure and the respective health impacts will allow development and implementation of protective guidelines of IAQ standards in these environments that are so vital for child healthy development.

4.4 Conclusions

This work fill a gap by providing information on PM and PAH concentrations in (pre)school environments, the exposure levels and assessment of the contribution to total exposure of children. Overall, $PM_{2.5}$ and PM_1 levels were slightly higher indoors than outdoors; however no significant differences were found. PM_1 fraction accounted with 86–91% of $PM_{2.5}$. Generally, PM concentrations at (pre)schools fulfilled the limits of the current legislation for indoor air of public buildings (Portuguese Regulation 2013).

The total (i.e. gaseous and particulate) concentrations of 18 PAHs were higher indoors than outdoors among the different sampling campaigns performed at preschools. In total, gaseous PAHs accounted with more than 85% (indoors) and 77–91% (outdoors) of total air $\Sigma PAHs$. Furthermore, naphthalene and phenanthrene were the most predominant PAHs in the gas phase both indoors and outdoors. Naphthalene values were always well below the recommended WHO indoor guideline of $10 \mu g/m^3$ (WHO 2010). The high proportion of PAHs in the gaseous phase clearly demonstrates that adequate assessment of PAHs exposure requires consideration of the gaseous compounds, which are in the most of the studies neglected. $PM_{2.5}$ -bound PAHs ranged from 1.18 – $7.77 \text{ ng}/m^3$ and between 0.744 – $13.0 \text{ ng}/m^3$, respectively in the indoor and outdoor air of preschools, while at primary schools concentrations were higher (ranged

from 1.18 to 59.7 ng/m³ in the indoor air and between 0.744 to 63.3 ng/m³ for the outdoor air). In that regard, it was observed that urban primary schools situated in the most polluted site exhibited the highest levels of indoor PAHs, whereas the lowest indoor concentrations were found for a school situated in a residential area. Particulate PAHs were predominantly associated with PM₁ (54-76% particulate ΣPAHs) with 5-rings PAHs being the most abundant compounds. Dibenz[a,h]anthracene was the most abundant particulate-bound PAH at preschools, however it accounted with less than 3% for the total air ΣPAHs. At primary schools, dibenz[a,h]anthracene and acenaphthylene were the most abundant particulate PAHs in the indoor and outdoor air. The higher prevalence of dibenz[a,h]anthracene suggests emissions from motor light-duty gasoline vehicles (Ravindra et al., 2008). Benzo[a]pyrene, the only known carcinogen PAH, was found in PM_{2.5} at concentrations ranging from 3.96×10⁻² to 0.853 ng/m³ and 3.69×10⁻² to 0.972 ng/m³, respectively in the indoor and outdoor air of preschools. At primary schools, the determined maximum benzo[a]pyrene levels were higher reaching 3.53 ng/m³ indoors and 3.55 ng/m³ outdoors. PM_{2.5}-benzo[a]pyrene mean concentrations at preschools were always below the PM₁₀ annual mean guideline of 1 ng/m³ (Directive 2004/107/EC), while some exceedances were registered at 3 primary schools (two located in the city center and one inserted in a residential area with industrial influence). I/O ratios of lighter PAHs (2–3 rings) indicated a contribution from indoor sources whereas PAHs with 4–6 rings resulted mainly from penetration of outdoor emissions into the indoor air. In addition the use of different diagnostic ratios allowed the identification of pyrogenic (mostly traffic emissions) and petrogenic sources. However the predominance of one of these sources could not be established among all the schools studied.

Total carcinogenic risks related with indoor exposure to PAHs in preschool and primary schools were higher than for outdoor ones (10–18 times for 3 years old children, 3–7 times for 4–5-years old children, and 2-10 times for 8-10 years children), mostly due to the long periods that children spent indoors. The risk assessment based on USEPA methodology revealed that cancer risks of preschool children and staff who attended preschools were negligible, however WHO health-based guideline level of 10⁻⁵ was exceeded (37 and 5 times, respectively). Concerning primary schoolchildren, cancer risks of 8–10 years old school children and adults exceeded (up to 22 times, and 2–166 times for children and schools staff, respectively) the recommended USEPA guideline; WHO health-based guideline was exceeded 7–87 times, thus indicating a potential to cause PAH-related adverse health effects.

4.5 Ethical statement

Human subjects were not directly involved in this study. Full information about the measurements conducted in this work was provided to parents of all students, teaching staff and boards of directors of both preschools.

4.6 References

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**CHAPTER 5 – ENVIRONMENTAL AND INTERNAL DOSE
ASSESSMENT OF EXPOSURE TO POLYCYCLIC AROMATIC
HYDROCARBONS IN PRESCHOOL CHILDREN: LEVELS AND
IMPACT OF INDOOR AIR POLLUTION ON EXCRETION OF MAIN
URINARY MONOHYDROXYL METABOLITES**

Chapter 5. Environmental and internal dose assessment of exposure to polycyclic aromatic hydrocarbons in preschool children: levels and impact of indoor air pollution on excretion of main urinary monohydroxyl metabolites

The concentrations of eighteen PAHs in indoor and outdoor air of two preschools, and levels of six urinary biomarkers of exposure (OH-PAHs) in preschool children were assessed. Total levels (gaseous +TSP) of Σ PAHs were higher indoors than outdoors. Gaseous PAHs accounted for 93-95% of Σ PAHs in indoor air, 2-3 rings compounds being the most abundant. Σ PAHs_{car} represented 26-45% of Σ PAHs, naphthalene and dibenz[a,h]anthracene were the strongest contributors; benzo[a]pyrene accounted for less than 0.6% of carcinogenic Σ PAHs. Diagnostic ratios identified pyrogenic (mostly traffic emissions) and petrogenic sources, but the predominance of one of these sources could not be established. A similar distribution profile was observed between airborne PAHs and OH-PAHs. Urinary 1OHNaph+1OHAce represented more than 78% of Σ OH-PAHs, being followed by 2OHFlu, 1OHPy, and 1OHPhen. 3OHB[a]P (PAH biomarker of carcinogenicity) was not detected. The contribution of indoor airborne PAHs to the urinary levels of OH-PAHs was explored by Spearman correlation coefficients. Results suggest that children had preschool indoor air as their major exposure source of naphthalene and acenaphthene, while no conclusion was reached regarding fluorene, phenanthrene and pyrene.

5.1 Introduction

Since children are a vulnerable group to the harmful effects of poor air quality due to their evolving defence mechanisms and because they inhale a higher volume of air *per* body weight than adults (Yoon et al., 2011), a comprehensive exposure assessment which requires measurement of pollutants in external media and biomarkers is needed. Incorporating exposure biomarkers into studies is particularly relevant in children because absorbed dose for a given external exposure level may be very different from that of an adult due to different behaviours (playing on the floor and hand-to-mouth activity), physiology (dermal exposure is an important route in children), and metabolism (Weaver 1998). Some studies have been describing a significant decreasing trend in OH-PAHs with increasing age (Heudorf and Angerer 2001), with children generally presenting higher levels than adolescents or adults (Li et al., 2008). The expression of metabolizing enzymes has been shown to alter with age, revealing the high susceptibility of children to environmental carcinogens (Strolin and Baltes 2003).

Typically, children spend approximately 7-8 h of their time (i.e., 30%) at premises of the education settings (i.e. preschools or schools), which raises an interest in characterizing the levels of health relevant air pollutants, such as PAHs, in these environments. Among the available information (Table 4.1) only six works focused on preschool children (Fiala et al., 2001; Oliveira et al., 2015a, 2015b; Vyskocil et al., 2000; Wilson et al., 2000, 2003). Studies that assessed simultaneously environmental PAH levels and the urinary levels of OH-PAHs in preschool children (3-6 years old) are even scarcer (Fiala et al., 2001; Vyskocil et al., 2000; Wilson et al., 2000, 2003) with very limited information concerning European countries (Fiala et al., 2001), and other compounds than 1OHPy. Furthermore, no report that also differentiates between genders in preschool children was found.

Thus, the main aim of this study was to assess exposure of preschool children to PAHs (18 compounds: 16 USEPA priority PAHs, dibenzo[a,l]pyrene, and benzo[j]fluoranthene recommended by EU Directive 2004/107/EC), by both external (air) and internal dose (biomarkers of exposure, namely six urinary OH-PAHs: 1OHNaph, 1OHAce, 2OHFlu, 1OHPhen, 1OHPy, and 3OHB[a]P). Levels of PAHs in gas phase and TSP were concurrently measured indoors and in ambient air of two Portuguese preschools. The phase distribution of indoor PAHs was characterized and the impacts of outdoor PAH emissions to indoor levels was assessed. The possible

emission sources were identified. In addition, the contribution of PAH levels in preschools indoor air to the urinary concentrations of individual and total OH-PAHs was explored for the first time taking into consideration the differences between the genders.

5.2 Materials and Methods

5.2.1 Identification of the sampling sites

This work was conducted in two Portuguese preschools, namely PS1 and PS2 that were situated in Oporto Metropolitan Area and approximately at 150 km north–east of Oporto, in Chaves, respectively. Further descriptions of both preschools were described in detail in Chapter 4 at section 4.2.1.

5.2.2 Sample collection

5.2.2.1 Air

18 selected compounds in gas and TSP phases were concurrently sampled in indoor air and outdoors of PS1 and PS2 between May and June of 2015. Indoor sampling was performed in a common room and at a classroom that were widely used throughout day for educational and entertaining activities of preschool children at PS1 and PS2, respectively. The detailed descriptions of the indoor places are presented in Chapter 4, namely in Table 4.3 and Figure 4.2. The record of room occupancy, type of ventilation systems, and other potential source activities were also collected. Gaseous and TSP samples were collected daily for a period of 8 h when children were at present at premises of preschools (between 9 a.m. and 5 p.m.). Sampling was done by constant flow samplers (model Bravo H2; TCR TECORA, Italy) according to the methodology previously described in detail in section 4.2.2.2. Ambient air sampling was conducted simultaneously with the indoor ones in yards of preschools. The samplers were placed in an open area avoiding any barriers (such as fences, walls, vegetation that might interfere with data collection. After the sampling, all samples (both filters and PUF plugs) were stored in a freezer (– 20 °C) before further chemical analysis. During the sampling campaign, indoor T and RH were registered (22–32°C and 20–71% at PS1; 14–29°C and 25–74% at PS2).

5.2.2.2 Urine

A total of 43 children (3–5 years old) participated in this study (Table 5.1). A structured questionnaire that was adapted from validated questionnaires (ISAAC 2000; WHO 2002) was filled out by parents. Parents of all participants read and signed informed consent forms approved by the Ethic Committee of University of Porto. The questionnaire collected information on gender, age and factors reported associated with PAH exposures, namely smoking habits in the families, and the most consumed meals (boiled, roasted, and grilled) at home during the two days before urine collection. Since the majority of children involved in this study lunched at the preschools, all meals were also daily registered at each school. The selected children for this study were not exposed to tobacco smoke.

USEPA has recommended the collection of 24-hours urine void for daily evaluation of total exposure to toxic substances that are primarily eliminated in the urine (USEPA 1996). However, this sampling method is burdensome and almost impossible in young children. Thus in this study, parents were asked to collect two spot-urine specimens of their child: one in the morning immediately after the subject woke up (first morning) and the other at night before the subject went to bed (last night) according to previous studies (Fiala et al., 2001; Tsai et al., 2003; van Wijnen et al., 1996; Vyskocil et al., 2000). Urine samples were collected in sterilized polycarbonate containers, and frozen at -20 °C until analysis.

Table 5.1. Characterization of preschool children participating in the study.

Characteristic	PS1	PS2
Total number (n)	27	16
Gender		
Boys (n; %)	17; 63	5; 31
Girls (n; %)	10; 37	11; 69
Age (years)		
3 (n; %)	6; 22	1; 6
4 (n; %)	4; 15	8; 50
5 (n; %)	17; 63	7; 44

5.2.3 PAHs and OH-PAHs chromatographic analysis

Extraction and quantification of PAHs from TSP and PUF plugs were performed accordingly with the validated analytical procedures (Castro et al., 2009, 2011) as previously described in chapter 4 (sections 4.2.3-4.2.4).

The extraction and quantification of urinary OH-PAHs was done according to Chetianukornkul et al. (2006) with slight modifications. Briefly a 10 mL aliquot of urine was adjusted to pH 5.0 with 0.5 mol/L HCl, buffered with 20 mL of 0.1 mol/L acetate buffer at pH 5.0, and then purged with nitrogen flow for 30 min in order to remove the interference of oxygen before the incubation step. 20 µL of 1.0 g/L of the antioxidant tert-butylhydroquinone (TBHQ, purity > 98.0%; Sigma-Aldrich, Steinheim, Germany) and 80 µL of enzyme 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). were added to the sample solution and incubated for 120 minutes at 37 °C (Binder KBWF, Tuttlingen, German), under constant stirring (120 rpm, Bunsen AO.400 orbital shaker, Madrid, Spain) and in the absence of light.

The hydrolyzed urine samples were loaded into Sep-Pak® Light Plus C18 (55-105 µm, 125 Å, 360 mg; Waters; Sigma-Aldrich, Steinheim, Germany) that were preconditioned with 5 mL of methanol and 10 mL of water. After elution of the urine sample, cartridges were sequentially washed with 10 mL of water, and 10 mL of 20:80 methanol/water. The C18 cartridges were completely dried under vigorous air flow and washed with 10 mL of n-hexane. OH-PAHs compounds retained in the C18 cartridges were eluted with 20 mL of 10:90 methanol/ethyl acetate. After elution, extracts were evaporated till dryness at room temperature (Büchi R200 rotavapor and a Büchi Vac V-500 pump), redissolved in 500 µL of methanol and filtered through a 0.22 µm PTFE syringe filter (Teknokroma, Barcelona, Spain) before the injection in the HPLC.

Chromatographic analysis of the PAHs and OH-PAHs compounds were performed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with PAD and FLD detectors on line. Separation of the compounds was done in a C18 column (CC 150/4 Nucleosil 100–5 C18 PAH, 150 × 4.0 mm; 5 µm particle size; Macherey–Nagel, Duren, Germany) maintained at a room temperature (20 ± 1 °C). Each OH-PAH compound was detected at its optimum excitation/emission wavelength pair: 232/337 nm for 1OHNaph and 1OHAce, 265/335 nm for 2OHFlu, 263/363 nm for 1OHPhen, 242/388 nm for 1OHPy and 308/432 nm for 3OHB[a]P.

External calibrations with PAHs and OH-PAHs mixed standards in acetonitrile and methanol, respectively, were performed using at least 6 calibration points. Calibration curves were linearly fitted with correlation coefficients always higher than 0.9979. RSD values ranged from 1.8% (dibenzo[a,l]pyrene) to 9.1% (naphthalene) and 0.9% (chrysene) to 9.8% (naphthalene) for PAHs extraction from filters and PUFs, respectively. Regarding urinary OH-PAHs, RSD values varied between 1.3% (2OHFlu) to 6.4% (1OHPhen) and from 1.3% (1OHNaph+1OHAce and 2OHFlu) to 8.1% (1OHNaph+1OHAce, 2OHFlu, and 1OHPy), respectively for intra- and inter-day precision. For PAHs, LODs between 1.0 pg/m³ (for anthracene, benzo[k]fluoranthene, chrysene, benz[a]anthracene, phenanthrene and indeno[1,2,3-cd]pyrene) and 148 pg/m³ (for acenaphthylene) were obtained, with corresponding LOQs in the range 3.4-492 pg/m³. Regarding OH-PAHs, LODs and LOQs ranged between 0.0008 µg/L urine (for 2OHFlu) to 0.195 µg/L urine (for 1OHNaph+1OHAce), and from 0.0028 µg/L urine to 0.650 µg/L urine, respectively.

The OH-PAH levels were normalized with individual creatinine levels. Creatinine measurements were performed according to the Jaffe colorimetric method (Kanagasabapathy and Kumari 2000).

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

5.2.4 Statistical analysis

Statistical analysis was performed using SPSS (IBM SPSS Statistics 20). Median values of PAHs and OH-PAHs were compared through the nonparametric Mann–Whitney U test, since normal distribution was not observed by Kolmogorov–Smirnov with Lilliefors correction and Shapiro–Wilk’s tests. Statistical significance was defined as $p \leq 0.05$. Spearman correlation coefficients (r) were calculated to examine the relation between individual OH-PAH with each other and with total OH-PAHs, and between PAH concentrations in indoor air and urinary OH-PAHs for each school.

5.3 Results and Discussion

5.3.1 PAHs

5.3.1.1 Levels in indoor and outdoor air of preschools

The indoor concentration ranges of TSP were 20.3-46.2 (mean 33.2) $\mu\text{g}/\text{m}^3$ and 14.6-30.6 (mean 22.6) $\mu\text{g}/\text{m}^3$, respectively at PS1 and PS2. The indoor concentrations of individual and ΣPAHs in gas phase, TSP, as well as the total levels in air (sum of PAHs in TSP and gas phase) at each school are presented in Table 5.2. Among 18 PAHs considered in this study, dibenz[a,h]anthracene was the most abundant in TSP at both preschools with indoor concentrations accounting for 31% of particulate ΣPAHs at PS1 and 25% at PS2. The higher prevalence of this PAH in indoor air suggests infiltrations of ambient air emissions originating from motor light-duty gasoline vehicles exhausts (Ravindra et al., 2008). These findings are in agreement with previous studies on indoor air quality in Oporto Metropolitan Area (Castro et al., 2011; Oliveira et al., 2015b). The other more abundant PAHs in indoor air were at PS1, by descending order, benzo[b+j]fluoranthene and benzo[ghi]perylene. Together with dibenz[a,h]anthracene, these TSP-bound PAHs represented 49% of particulate ΣPAHs in PS1 (Table 5.2) whereas other individual PAHs contributed less than 10% of the particulate ΣPAHs . At PS2, benzo[b+j]fluoranthene, acenaphthene and benzo[ghi]perylene were the prevalent particulate PAHs, with dibenz[a,h]anthracene accounting for 55% of ΣPAHs . During the sampling campaign no specific occurrence was found that can justify the high abundance of acenaphthene. The higher abundance in indoor air may possibly result from infiltration of outdoor air (i.e. emissions from industries in the geographical area since it is used in manufacture of dyes, plastics, pigments, pharmaceuticals and pesticides). In addition, acenaphthene was described as one of the predominant PAHs found in total (particle and gas phase) of diesel emissions (ATSDR 1995).

Concerning the gas phase, naphthalene was the most abundant PAH indoors at PS1 (44%). Indoor sources of naphthalene include the use of pest repellents or deodorants and, to lesser extents, vehicle emissions (Batterman et al., 2012). WHO recommends a guideline value of 10 $\mu\text{g}/\text{m}^3$ (WHO 2010) for naphthalene annual concentration in indoor air. Levels measured at both preschools (13.6-62.7 ng/m^3 for PS1 and 13.1-20.8 ng/m^3 for PS2) were well below that guideline.

Table 5.2. Indoor and outdoor levels of PAHs (median and range; ng/m³) in total suspended particles (TSP), gas phase, and in air (i.e. total) at the studied preschools (PS1 and PS2).

Compound	Indoor air		Outdoor air		Total air	Gas phase	TSP	Gas phase	TSP	Total air
	TSP	Gas phase	TSP	Gas phase						
Naphthalene	1.84×10 ^{-2a}	14.4 (13.6 - 62.7)	1.84×10 ^{-2a}	8.59 ^b (1.84×10 ⁻² - 20.9)	14.4 ^c (13.6 - 62.7)	14.4	1.84×10 ^{-2a}	8.59 ^b (1.84×10 ⁻² - 20.9)	1.84×10 ^{-2a}	8.61 [#] (3.68×10 ⁻² - 20.9)
Acenaphthylene	0.209 ^a	0.209 ^d (0.209 - 0.513)	0.209 ^a	0.209 ^a	0.418 (0.417 - 0.721)	0.418	0.209 ^a	0.209 ^a	0.209 ^a	0.418 (0.417 - 1.69)
Acenaphthene	1.41×10 ^{-2d} (1.41×10 ⁻² - 8.03×10 ⁻²)	0.536 ^b (1.41×10 ⁻² - 0.695)	1.41×10 ^{-2d} (1.41×10 ⁻² - 9.93×10 ⁻²)	0.531 ^b (1.41×10 ⁻² - 4.12)	0.550 [#] (2.83×10 ⁻² - 0.771)	0.550 [#]	1.41×10 ^{-2d} (1.41×10 ⁻² - 9.93×10 ⁻²)	0.531 ^b (1.41×10 ⁻² - 4.12)	0.545 [#] (2.83×10 ⁻² - 4.13)	0.545 [#] (2.83×10 ⁻² - 4.13)
Fluorene	3.13×10 ^{-3c} (2.12×10 ⁻³ - 4.27×10 ⁻³)	5.58 (3.78 - 23.0)	4.02×10 ^{-3b} (1.63×10 ⁻³ - 0.124)	4.25 (0.217 - 5.41)	5.58 ^c (3.69 - 23.0)	5.58	4.02×10 ^{-3b} (1.63×10 ⁻³ - 0.124)	4.25 (0.217 - 5.41)	4.25 [#] (0.219 - 5.42)	4.25 [#] (0.219 - 5.42)
Phenanthrene	4.65×10 ⁻² (2.76×10 ⁻³ - 7.58×10 ⁻²)	10.3 (6.06 - 56.0)	4.61×10 ^{-2b} (1.41×10 ⁻³ - 0.532)	8.41 (0.596 - 13.2)	10.3 (6.10 - 56.1)	10.3	4.61×10 ^{-2b} (1.41×10 ⁻³ - 0.532)	8.41 (0.596 - 13.2)	8.41 [#] (0.605 - 13.3)	8.46 [#] (0.605 - 13.3)
Anthracene	2.80×10 ^{-3c} (2.12×10 ⁻³ - 3.60×10 ⁻³)	0.187 ^b (2.12×10 ⁻² - 0.663)	8.42×10 ^{-3b} (2.12×10 ⁻³ - 4.73×10 ⁻²)	0.535 ^c (2.12×10 ⁻² - 0.839)	0.191 (4.71×10 ⁻³ - 0.665)	0.191	8.42×10 ^{-3b} (2.12×10 ⁻³ - 4.73×10 ⁻²)	0.535 ^c (2.12×10 ⁻² - 0.839)	0.543 [#] (4.24×10 ⁻³ - 0.852)	0.543 [#] (4.24×10 ⁻³ - 0.852)
Fluoranthene	3.68×10 ⁻² (2.79×10 ⁻² - 6.75×10 ⁻²)	0.568 ^b (2.83×10 ⁻³ - 2.82)	0.151 ^b (2.83×10 ⁻³ - 0.666)	1.82 ^b (2.83×10 ⁻² - 4.26)	0.605 [#] (3.64×10 ⁻² - 2.88)	0.605 [#]	0.151 ^b (2.83×10 ⁻³ - 0.666)	1.82 ^b (2.83×10 ⁻² - 4.26)	1.97 [#] (4.86×10 ⁻² - 4.41)	1.97 [#] (4.86×10 ⁻² - 4.41)
Pyrene	4.09×10 ⁻² (3.61×10 ⁻² - 6.40×10 ⁻²)	0.762 ^b (2.83×10 ⁻³ - 3.61)	0.141 ^b (2.83×10 ⁻³ - 0.706)	1.77 ^b (2.83×10 ⁻² - 4.17)	0.803 (4.53×10 ⁻² - 3.67)	0.803	0.141 ^b (2.83×10 ⁻³ - 0.706)	1.77 ^b (2.83×10 ⁻² - 4.17)	1.91 [#] (4.92×10 ⁻² - 4.32)	1.91 [#] (4.92×10 ⁻² - 4.32)
Benz[a]anthracene	2.85×10 ⁻² (2.38×10 ⁻² - 3.27×10 ⁻²)	2.12×10 ^{-3a} (1.41×10 ⁻³ - 0.405)	5.71×10 ^{-2b} (2.12×10 ⁻³ - 0.447)	2.12×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	3.06×10 ^{-2#} (2.60×10 ⁻² - 3.48×10 ⁻²)	3.06×10 ^{-2#}	5.71×10 ^{-2b} (2.12×10 ⁻³ - 0.447)	2.12×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	5.92×10 ⁻² (4.24×10 ⁻³ - 0.449)	5.92×10 ⁻² (4.24×10 ⁻³ - 0.449)
Chrysene	8.34×10 ⁻² (7.43×10 ⁻² - 0.111)	1.41×10 ^{-3d} (1.41×10 ⁻³ - 0.405)	0.217 ^b (1.41×10 ⁻³ - 1.42)	1.41×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	8.48×10 ⁻² (7.57×10 ⁻² - 0.512)	8.48×10 ⁻²	0.217 ^b (1.41×10 ⁻³ - 1.42)	1.41×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	0.218 (3.69×10 ⁻² - 1.89)	0.218 (3.69×10 ⁻² - 1.89)
Benz[b]fluoranthene	0.158 (0.131 - 0.218)	7.07×10 ^{-3a} (1.41×10 ⁻³ - 0.405)	0.290 ^b (7.07×10 ⁻³ - 2.25)	7.07×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	0.165 [#] (0.138 - 0.225)	0.165 [#]	0.290 ^b (7.07×10 ⁻³ - 2.25)	7.07×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	0.297 (1.41×10 ⁻² - 2.26)	0.297 (1.41×10 ⁻² - 2.26)
Benz[k]fluoranthene	3.55×10 ⁻² (3.06×10 ⁻² - 5.16×10 ⁻²)	1.41×10 ^{-3a} (1.41×10 ⁻³ - 0.405)	6.50×10 ^{-2b} (1.41×10 ⁻³ - 0.664)	1.41×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	3.69×10 ⁻² (3.33×10 ⁻² - 5.30×10 ⁻²)	3.69×10 ⁻²	6.50×10 ^{-2b} (1.41×10 ⁻³ - 0.664)	1.41×10 ^{-3a} (1.41×10 ⁻³ - 0.490)	6.64×10 ⁻² (2.83×10 ⁻³ - 0.665)	6.64×10 ⁻² (2.83×10 ⁻³ - 0.665)
Benz[a]pyrene	5.09×10 ⁻² (4.80×10 ⁻² - 8.22×10 ⁻²)	2.12×10 ^{-3a} (1.41×10 ⁻³ - 0.405)	9.01×10 ^{-2b} (2.12×10 ⁻³ - 1.23)	1.01×10 ^{-2d} (2.12×10 ⁻³ - 7.02×10 ⁻²)	5.30×10 ^{-2#} (5.01×10 ⁻² - 8.4×10 ⁻²)	5.30×10 ^{-2#}	9.01×10 ^{-2b} (2.12×10 ⁻³ - 1.23)	1.01×10 ^{-2d} (2.12×10 ⁻³ - 7.02×10 ⁻²)	0.100 (4.24×10 ⁻³ - 1.30)	0.100 (4.24×10 ⁻³ - 1.30)
Dibenzo[a,h]pyrene	5.66×10 ^{-3a}	5.66×10 ^{-3a}	5.66×10 ^{-3a}	5.66×10 ^{-3a}	1.13×10 ⁻²	1.13×10 ⁻²	5.66×10 ^{-3a}	5.66×10 ^{-3a}	1.13×10 ⁻²	1.13×10 ⁻²

^a When concentration was below the LOD, the value of the respective LOD/2 was used (Hornung and Reed 1990).

^c Statistically significant ($p \leq 0.05$) between indoor and outdoor PAH concentrations at each school.

^d Statistically significant ($p \leq 0.05$) between schools.

Note: Detection frequency of each compound was 100% unless otherwise indicated. ^b80% ≤ detection frequency < 100%; ^c60% ≤ detection frequency < 80%; ^d15% ≤ detection frequency < 60%.

Table 5.2. Indoor and outdoor levels of PAHs (median and range: ng/m³) in total suspended particles (TSP), gas phase, and in air (i.e. total) at the studied preschools (PS1 and PS2) (continued).

Compound	Indoor air		Outdoor air		Total air	Gas phase	TSP	Total air	Gas phase	TSP	Total air
	TSP	Gas phase	TSP	Gas phase							
PS1											
Dibenz[a,h]anthracene	0.521 (0.413 - 0.802)	4.24×10 ^{-3a}	0.525 (0.418 - 0.807)	0.639 ^b (4.24×10 ⁻³ - 6.90)	4.24×10 ^{-3 a}	0.643 (8.49×10 ⁻³ - 6.91)					
Benzof[ghi]perylene	0.152 (0.146 - 0.254)	2.83×10 ^{-3a}	0.155 (0.149 - 0.256)	0.287 ^b (2.83×10 ⁻³ - 2.02)	2.83×10 ^{-3 a}	0.290 [#] (5.66×10 ⁻² - 2.02)					
Indeno[1,2,3-cd]pyrene	0.117 (6.45×10 ⁻² - 0.211)	1.41×10 ^{-3a}	0.118 (6.59×10 ⁻² - 0.212)	0.150 ^b (0.106 - 0.947)	1.41×10 ^{-3 a}	0.151 (0.108 - 0.949)					
ΣPAHs	1.68 (1.36 - 1.99)	32.7 (24.1 - 149)	34.4 (26.1 - 150.5)	2.81 (0.281 - 17.3)	28.3 (2.18 - 43.7)	31.1 [#] (2.79 - 46.9)					
ΣPAHs _{carc}	1.10 (0.851 - 1.40)	14.4 (13.6 - 63.1)	15.5 (15.0 - 64.2)	1.85 (4.38×10 ⁻² - 13.3)	8.61 (4.38×10 ⁻² - 20.9)	10.5 (0.733 - 23.0)					
PS2											
Naphthalene	1.84×10 ^{-2a}	15.2 (13.1 - 20.8)	15.2 (13.1 - 20.8)	1.84×10 ^{-2a}	3.72 (0.392 - 6.02)	3.74 [#] (0.411 - 6.03)					
Acenaphthylene	0.209 ^a	0.292 ^d (0.209 - 0.751)	0.501 (0.417 - 0.959)	0.209 ^a	0.209 ^d (0.209 - 7.53)	0.418 (0.417 - 7.74)					
Acenaphthene	0.175 (0.133 - 0.512)	0.539 (0.457 - 8.04)	0.714 [#] (0.668 - 8.19)	0.461 (0.244 - 1.49)	0.129 (5.14×10 ⁻² - 3.41)	0.590 [#] (0.327 - 3.86)					
Fluorene	5.03×10 ^{-3c} (2.12×10 ⁻³ - 9.74×10 ⁻³)	8.02 (5.08 - 14.3)	8.03 (5.09 - 14.3)	4.35×10 ^{-3b} (2.12×10 ⁻³ - 1.28×10 ⁻²)	1.12 (0.183 - 1.56)	1.12 [#] (0.185 - 1.58)					
Phenanthrene	8.81×10 ⁻² (5.31×10 ⁻² - 0.114)	33.6 (25.6 - 35.2)	33.7 (25.7 - 35.3)	6.60×10 ⁻² (2.80×10 ⁻² - 0.111)	3.39 (0.577 - 5.26)	3.46 [#] (0.615 - 5.37)					
Anthracene	4.85×10 ^{-3c} (2.12×10 ⁻³ - 7.74×10 ⁻³)	0.502 (0.330 - 0.636)	0.507 (0.336 - 0.638)	2.12×10 ^{-3d} (2.12×10 ⁻³ - 8.20×10 ⁻³)	0.159 ^b (2.12×10 ⁻³ - 0.320)	0.161 [#] (4.24×10 ⁻³ - 0.324)					
Fluoranthene	3.49×10 ⁻² (3.15×10 ⁻² - 3.89×10 ⁻²)	2.44 (2.28 - 3.22)	2.47 [#] (2.31 - 3.26)	4.42×10 ^{-2b} (2.83×10 ⁻³ - 0.311)	0.647 ^b (2.83×10 ⁻³ - 0.819)	0.691 [#] (3.60×10 ⁻² - 0.991)					
Pyrene	0.131 (8.50×10 ⁻² - 0.150)	1.80 ^b (2.83×10 ⁻³ - 2.03)	1.93 [*] (8.99×10 ⁻² - 2.11)	0.132 (4.75×10 ⁻² - 0.188)	0.957 ^b (2.83×10 ⁻³ - 1.41)	1.09 [#] (0.188 - 1.54)					

^a When concentration was below the LOD; the value of the respective LOD/2 was used (Hornung and Reed 1990).

^{*} Statistically significant (p<0.05) between indoor and outdoor PAH concentrations at each school.

[#] Statistically significant (p<0.05) between schools.

Note: Detection frequency of each compound was 100% unless otherwise indicated. ^b80% ≤ detection frequency < 100%, ^c60% ≤ detection frequency < 80%, ^d15% ≤ detection frequency < 60%.

Table 5.2. Indoor and outdoor levels of PAHs (median and range; ng/m³) in total suspended particles (TSP), gas phase, and in air (i.e. total) at the studied preschools (PS1 and PS2) (continued).

Compound	Indoor air		Outdoor air		Total air	Gas phase	TSP	Gas phase	TSP	Total air
	TSP	Gas phase	TSP	Gas phase						
Benz[a]anthracene	4.21×10 ⁻² (3.68×10 ⁻² - 4.96×10 ⁻²)	2.12×10 ^{-3a}	2.92×10 ⁻² (1.10×10 ⁻² - 0.132)	2.12×10 ^{-3a}	4.42×10 ^{-2#} (3.90×10 ⁻² - 5.18×10 ⁻²)	2.12×10 ^{-3a}	2.92×10 ⁻² (1.10×10 ⁻² - 0.132)	2.12×10 ^{-3a}	2.92×10 ⁻² (1.10×10 ⁻² - 0.132)	3.13×10 ⁻² (1.31×10 ⁻² - 0.141)
Chrysene	7.94×10 ⁻² (5.08×10 ⁻² - 8.86×10 ⁻²)	1.41×10 ^{-3a}	0.131 (3.19×10 ⁻² - 0.180)	1.41×10 ^{-3a}	8.08×10 ⁻² (5.22×10 ⁻² - 9.00×10 ⁻²)	1.41×10 ^{-3a}	0.131 (3.19×10 ⁻² - 0.180)	2.63×10 ^{-2c}	2.63×10 ^{-2c}	0.157 (3.33×10 ⁻² - 0.260)
Benzo[b]fluoranthene	0.239 (0.149 - 0.256)	7.07×10 ^{-3a}	0.226 (6.72×10 ⁻² - 0.454)	7.07×10 ^{-3a}	0.246# (0.156 - 0.263)	7.07×10 ^{-3a}	0.226 (6.72×10 ⁻² - 0.454)	7.07×10 ^{-3a}	7.07×10 ^{-3a}	0.233 (7.43×10 ⁻² - 0.461)
Benzo[k]fluoranthene	4.39×10 ⁻² (2.77×10 ⁻² - 5.17×10 ⁻²)	1.41×10 ^{-3a}	4.54×10 ⁻² (1.22×10 ⁻² - 0.114)	1.41×10 ^{-3a}	4.53×10 ⁻² (2.92×10 ⁻² - 5.31×10 ⁻²)	1.41×10 ^{-3a}	4.54×10 ⁻² (1.22×10 ⁻² - 0.114)	1.41×10 ^{-3a}	1.41×10 ^{-3a}	4.68×10 ⁻² (1.36×10 ⁻² - 0.115)
Benzo[a]pyrene	9.57×10 ⁻² (5.34×10 ⁻² - 0.107)	2.12×10 ^{-3a}	8.54×10 ⁻² (2.93×10 ⁻² - 0.263)	2.12×10 ^{-3a}	9.78×10 ^{-2#} (5.56×10 ⁻² - 0.109)	2.12×10 ^{-3a}	8.54×10 ⁻² (2.93×10 ⁻² - 0.263)	2.12×10 ^{-3a}	2.12×10 ^{-3a}	8.75×10 ⁻² (3.14×10 ⁻² - 0.265)
Dibenzo[a,h]pyrene	5.66×10 ^{-3a} (0.473 - 0.649)	5.66×10 ^{-3a}	5.66×10 ^{-3a} (0.388 - 0.971)	5.66×10 ^{-3a}	1.13×10 ⁻² (0.477 - 0.654)	5.66×10 ^{-3a}	5.66×10 ^{-3a} (0.388 - 0.971)	5.66×10 ^{-3a}	5.66×10 ^{-3a}	1.13×10 ⁻² (0.392 - 0.975)
Dibenzo[a,h]anthracene	0.158 (0.03×10 ⁻² - 0.184)	2.83×10 ^{-3a}	0.160 (3.92×10 ⁻² - 0.273)	2.83×10 ^{-3a}	0.161 (8.31×10 ⁻² - 0.187)	2.83×10 ^{-3a}	0.160 (3.92×10 ⁻² - 0.273)	2.83×10 ^{-3a}	2.83×10 ^{-3a}	0.163# (4.20×10 ⁻² - 0.276)
Benzo[ghi]perylene	9.81×10 ⁻² (6.76×10 ⁻² - 0.123)	1.41×10 ^{-3a}	0.103 (2.96×10 ⁻² - 0.238)	1.41×10 ^{-3a}	9.95×10 ⁻² (6.90×10 ⁻² - 0.124)	1.41×10 ^{-3a}	0.103 (2.96×10 ⁻² - 0.238)	1.41×10 ^{-3a}	1.41×10 ^{-3a}	0.104 (3.10×10 ⁻² - 0.240)
Indeno[1,2,3-cd]pyrene	1.90 (0.870 - 2.48)	60.8 (50.7 - 83.9)	3.37 (1.12 - 3.50)	60.8 (50.7 - 83.9)	62.7 (52.6 - 85.3)	60.8 (50.7 - 83.9)	3.37 (1.12 - 3.50)	11.7 (4.91 - 16.0)	11.7 (4.91 - 16.0)	15.1# (6.20 - 19.2)
ΣPAHs	1.10 (0.727 - 1.30)	15.2 (13.2 - 20.8)	1.02 (0.309 - 2.34)	15.2 (13.2 - 20.8)	16.3 (14.4 - 21.6)	15.2 (13.2 - 20.8)	1.02 (0.309 - 2.34)	3.78 (0.440 - 6.14)	3.78 (0.440 - 6.14)	4.80 (0.984 - 7.15)

^a When concentration was below the LOD, the value of the respective LOD/2 was used (Hornung and Reed 1990).

[#] Statistically significant (p≤0.05) between indoor and outdoor PAH concentrations at each school.

^c Statistically significant (p≤0.05) between schools.

Note: Detection frequency of each compound was 100% unless otherwise indicated. ^b80% ≤ detection frequency < 100%; ^c60% ≤ detection frequency < 80%; ^d15% ≤ detection frequency < 60%.

However, it is necessary to point out that at PS2, naphthalene was the second predominant compound (25%); phenanthrene concentrations were 2.2 times higher than naphthalene, ($p < 0.001$) and represented 55% of gaseous Σ PAHs. Phenanthrene is used in the manufacture of dyestuffs and it has been reported as one of the most abundant compounds in diesel exhaust vapour emissions (ATSDR 1995). Furthermore, naphthalene, phenanthrene and fluorene represented 93% of the indoor gaseous Σ PAHs at both preschools. Finally, gaseous PAHs contributed 95 and 93% of indoor total air levels of Σ PAHs whereas TSP-bound PAHs accounted for 5 and 7% of the total air Σ PAHs at PS1 and PS2, respectively; dibenz[a,h]anthracene, the most abundant TSP-bound PAH, accounted for less than 3% of the total air Σ PAHs of both preschools.

Only two studies were found in the literature regarding indoor PAH concentrations in the gas phase of schools. Σ PAHs levels reported in this study were slightly higher than ranges described by Krugly et al. (2014) and Oliveira et al. (2015b), particularly at PS1. Although the lighter PAH compounds are considered to be less toxic (except naphthalene, a possible human carcinogen), they are able to react with other pollutants (such as O_3 , nitrogen oxides, and sulfur dioxide) to form diones, nitro- and dinitro-PAHs, and sulfuric acids, which toxicity may be more significant (Kim et al., 2013).

In general, the levels of TSP-bound PAHs in ambient air (Table 5.2) were slightly, but not significantly, higher than indoors with the respective ranges of 13.5–58.8 (mean 39.5) $\mu\text{g}/\text{m}^3$ and 6.92–77.4 (mean 28.5) $\mu\text{g}/\text{m}^3$ at PS1 and PS2, respectively. Overall, at both preschools PAH profiles (both for particulate and gas phase) in ambient air were similar to those indoors (Table 5.2) thus suggesting that penetration of outdoor emissions was the predominant source to indoors. Once again dibenz[a,h]anthracene was the most abundant particulate PAH accounting in outdoor air for 23 and 11% of Σ PAHs at PS1 and PS2, respectively; naphthalene was the most predominant compound in the gas phase contributing 30% of Σ PAHs at PS1 and 32% at PS2. Finally, PAHs in gas phase contributed 91 and 77% of total outdoor air Σ PAHs.

At PS1 only levels of naphthalene and fluorene were significantly higher indoors than outdoors ($p = 0.002$), while at PS2 indoor concentrations of naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and chrysene were significantly higher than outdoor ones ($p \leq 0.001$). Contribution from potential indoor sources of PAHs, such as paints, candles, and wax melting, was registered on several occasions during the sampling campaign period at both preschools, though more frequently at

PS2. Overall, median PAH concentrations in total air were higher indoors than outdoors for both preschools, especially at PS2 (ca. 3 times higher), which is particularly health relevant. This pattern was also observed in previous studies (Cirillo et al., 2006; Wilson et al., 2000, 2003).

Inter-comparison between schools indicated that indoor levels of acenaphthene, fluoranthene, benz[a]anthracene, benzo[b+j]fluoranthene and benzo[a]pyrene were significantly higher at PS2 ($p \leq 0.016$) (Table 5.2). In addition, Σ PAHs and levels of naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[ghi]perylene in ambient air were significantly higher at PS1 than at PS2 ($p \leq 0.029$); outdoor levels of acenaphthene were statistically different ($p = 0.042$) but the median values obtained for both schools were similar.

Finally, median concentrations of Σ PAHs_{car} are also presented in Table 5.2. Globally, naphthalene, dibenz[a,h]anthracene and benzo[b+j]fluoranthene composed the majority of Σ PAHs_{car} at both preschools (Figure 5.1). Indoors these three PAHs represented 98% of total air Σ PAHs_{car} (90–94% in outdoor air). Once again the strong contribution of the gas phase is evidenced by naphthalene being its main compound (Figure 5.1). Dibenz[a,h]anthracene was the most abundant suspected carcinogen in TSP (25–31% in indoor air; 11–23% in outdoor air) and the second most abundant in the total air Σ PAHs_{car} (3% in the indoor air of both preschools; 6–8% for outdoor air); its TEF is 5 times higher than of benzo[a]pyrene (Okona-Mensah et al., 2005). Benzo[a]pyrene accounted for less than 0.6% of total indoor air Σ PAHs_{car} (1.8% for outdoor air) at both preschools. Dibenz[a,l]pyrene levels, which carcinogenic potency has been estimated to be approximately 100 times that of benzo[a]pyrene (Okona-Mensah et al., 2005), were below the detection limit of 5.66×10^{-3} ng/m³ (Table 5.2).

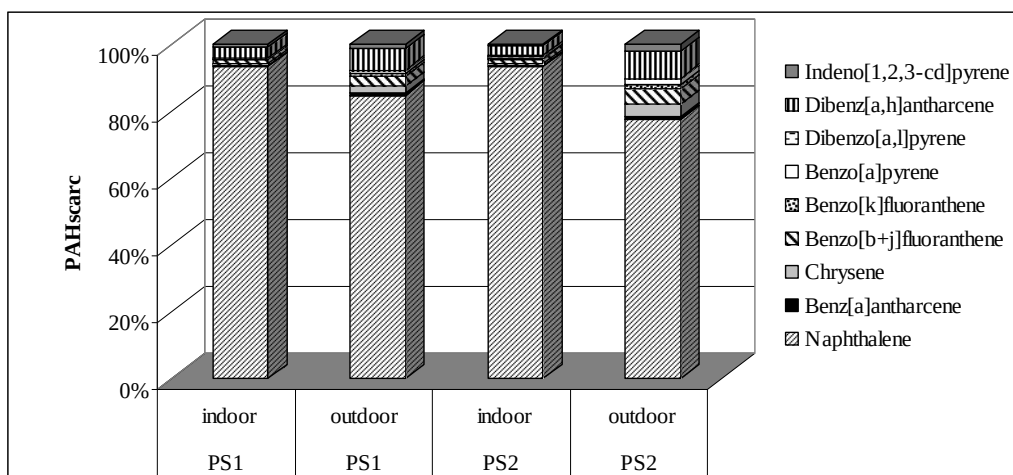


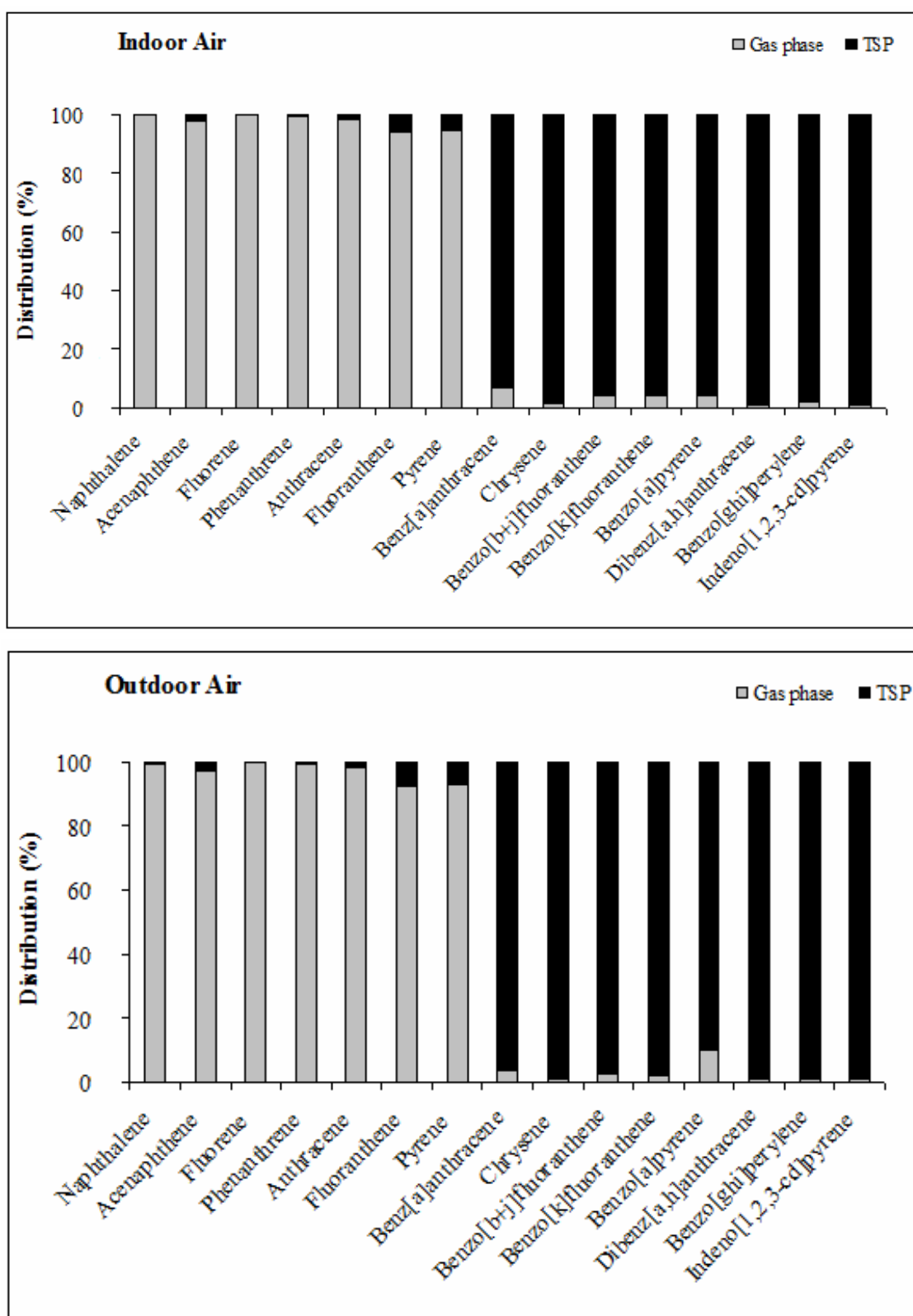
Figure 5.1. Distribution of the individual carcinogenic PAHs among the total carcinogenic PAHs (PAHs_{car}) in the indoor and outdoor total air of PS1 and PS2.

5.3.1.2 Phase distribution of PAHs

Phase distribution influences PAHs infiltration efficiency indoors, and the associated health risks since the routes of particulate or gaseous pollutants to the human body are different (Krugly et al., 2014). The distribution of PAHs in indoor and outdoor air of both schools was similar, with the exception of the higher contribution of acenaphthene to TSP at PS2 (indoor air: 3 *versus* 25% at PS1 and PS2 respectively; outdoors: 3 *versus* 78% at PS1 and PS2) (Figure 5.2). PAH adsorption/absorption also depends on the types of suspended particulates (e.g., soot, dust, fly-ash, pyrogenic metal oxides, pollens, etc.) existent in the indoor areas (Zhang and Tao 2009). Naphthalene, fluorene, phenanthrene, and anthracene (2-3 aromatic rings) existed almost entirely in the gas phase of both preschools (>98.5% at PS1 and >98.1% at PS2). Fluoranthene, pyrene, benz[a]anthracene, and chrysene (4 aromatic rings) were distributed between both phases, the first two mentioned congeners being predominantly found in the gas phase (proportions ranging from 88-99%) while the other two (benz[a]anthracene, and chrysene) existed mainly in the particulate phase (83-99%). The six dominant gaseous PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) represented more than 83% and 68% of total air Σ PAHs at PS1 and PS2, respectively, emphasizing the importance of the gaseous compounds. PAHs with 5 and 6 aromatic rings were almost exclusively bound to particulate phase (90-99% and 98-99%, respectively).

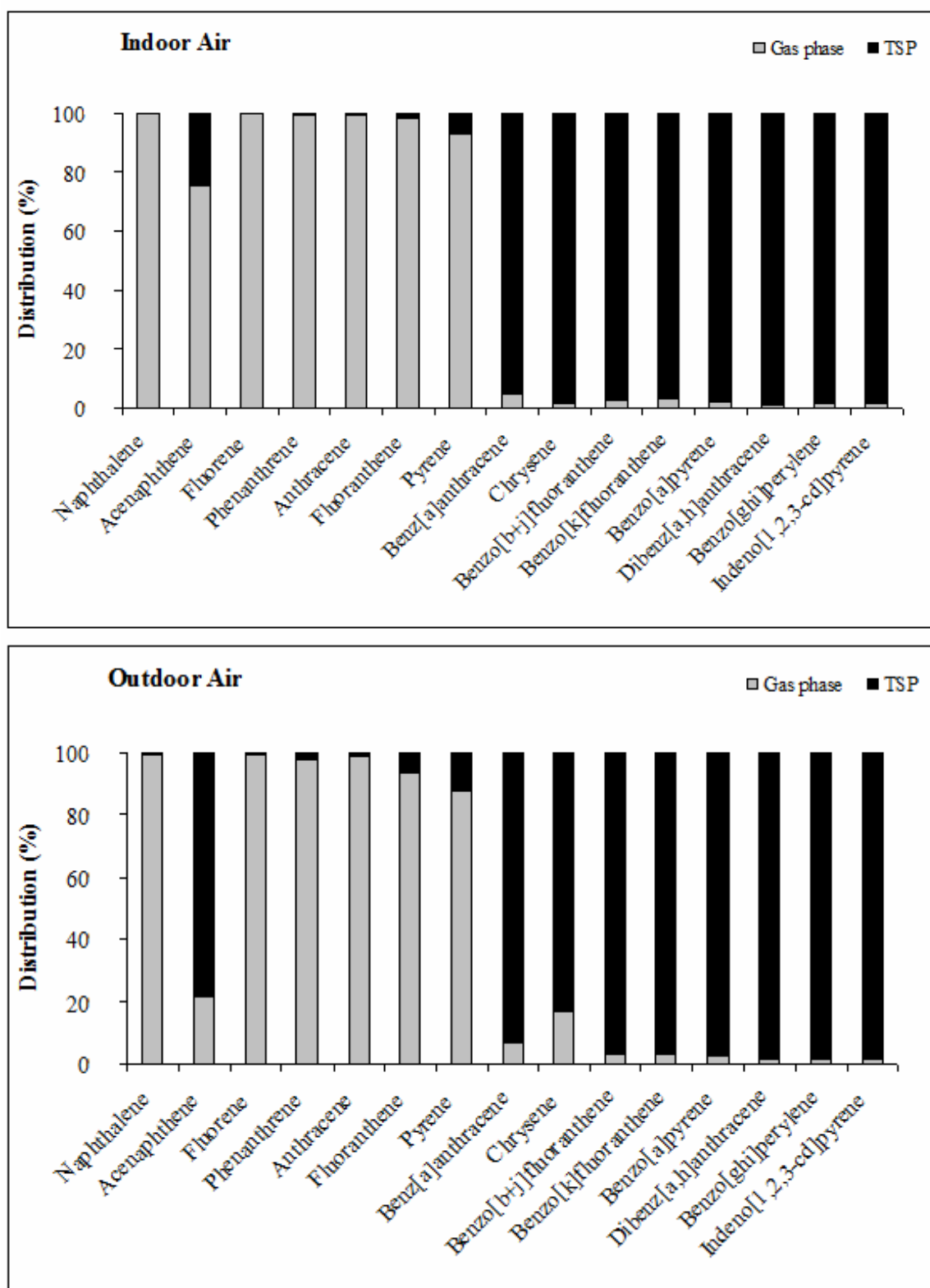
5.3.1.3 Indoor-to-outdoor ratios

I/O calculated for total air at PS1 and PS2 are shown in Figure 5.3. In general, the obtained values indicated that at both schools there were significant indoor emission sources affecting mainly the lighter compounds (2-3 rings), and also some 4 rings PAHs at PS2 (namely anthracene, fluoranthene, pyrene, and benz[a]anthracene). A similar pattern was also described by Krugly et al. (2014) at Lithuanian primary schools.



(a)

Figure 5.2. Distribution of PAHs between gas and particulate phases in indoor and outdoor air of the two selected Portuguese preschools: (a) PS1 and (b) PS2.



(b)

Figure 5.2. Distribution of PAHs between gas and particulate phases in indoor and outdoor air of the two selected Portuguese preschools: (a) PS1 and (b) PS2 (continued).

The main indoor sources of PAHs in school microenvironments are heating/combustion systems (non-existent/not used at the selected preschools during the sampling campaigns), re-suspension of indoor dust, emissions from cooking activities (in canteens), cleaning, some electronic equipment (such as printers), arts/crafts and other leisure activities (including birthday celebrations with candles that occurred frequently), and also re-emission and sorption of gaseous PAHs from indoor materials and surfaces (Krugly et al., 2014; Morawska et al., 2013). In addition, the presence of heavier congeners indoors can be attributed, namely at PS1, to infiltration of outdoor emissions (I/O ratios <1). Insufficient cleaning of indoor areas at PS2 and/or resuspension of indoor particles may have caused for the elevated levels of PAHs with a high number of aromatic rings inside the classroom. 5-6 rings PAHs are mostly non-degradable because they are strongly bound to particles and as a consequence they can persist indoors a longer time.

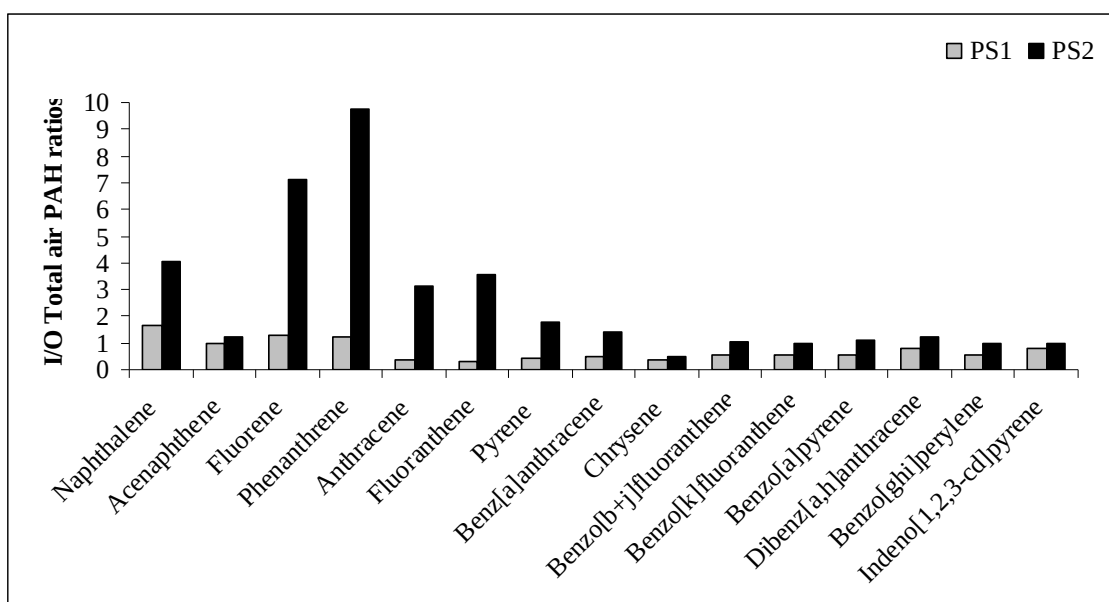


Figure 5.3. Indoor to outdoor (I/O) total air (gas and TSP-bound PAH) ratios at the studied preschools PS1 and PS2.

5.3.1.4 Diagnostic concentration ratios

Diagnostic ratios were calculated using the total air concentrations (Table 5.3) once it is important to evaluate the origin of PAHs in the respective environments. Pyrolytic sources are characterized by prevalence of 4-6 ring PAHs, while in petroleum sources the lowest molecular weight PAHs predominate.

Table 5.3. Diagnostic ratios for PAHs in air at the studied preschools.

Ratio	PS1		PS2		Value	Source	Reference
	Indoor	Outdoor	Indoor	Outdoor			
^a ΣPAHs _{LMW} /ΣPAHs _{HMW}	12.2	4.09	10.4	3.24	>1 <1	Petrogenic Pyrogenic	Zhang et al. (2008)
Benz[a]anthracene/Chrysene	0.36	0.27	0.55	0.29	>0.35	Fuel combustion	Krugly et al. (2014)
Fluoranthene/(Fluoranthene + Pyrene)	0.43	0.50	0.56	0.40	0.40-0.50 >0.5	Vehicular traffic Coal combustion	Kavouras et al. (1999); Yunker et al. (2002); Zencak et al. (2007)
					0.42, 0.52	Road dust	
Indeno[1,2,3-cd]pyrene/(Indeno[1,2,3-cd]pyrene + Benz[ghi]perylene)	0.43	0.34	0.38	0.39	0.21-0.22 0.35-0.70 0.56 0.62 0.36	Gasoline cars Diesel emissions Coal combustion Wood combustion Road dust	Gogou et al. (1996); Grimmer et al. (1983); Kavouras et al. (2001); Khalili et al. (1995); Pio et al. (2001); Ravindra et al. (2008)
Benzo[a]pyrene/(Benzo[a]pyrene + Chrysene)	0.38	0.32	0.55	0.34	0.5 0.73	Diesel Gasoline	Ravindra et al. (2008)
Benzo[b]fluoranthene/Benzo[k]fluoranthene	4.48	4.48	5.44	4.98	>0.5	Diesel	Ravindra et al. (2008)
Benz[a]anthracene/(Benz[a]anthracene + Chrysene)	0.27	0.21	0.35	0.23	0.40, 0.38-0.64 0.76	Diesel Gasoline	Li and Kamens (1993); Manoli et al. (2004); Sicre et al. (1987)
					0.43	Wood combustion	
Benzo[b+k]fluoranthene/Benzo[ghi]perylene	1.31	1.26	1.83	1.72	1.60	Diesel cars	
					0.33	Gasoline cars	Li and Kamens (1993)
					2.18	Wood combustion	
Fluorene/(Fluorene + Pyrene)	0.87	0.69	0.81	0.52	>0.5 <0.5	Diesel Gasoline	Ravindra et al. (2008)
Phenanthrene/(Phenanthrene + Anthracene)	0.98	0.94	0.99	0.96	>0.7	Lubricant oils and fossil fuels	Mirante et al. (2013)

^aTotal concentration of 2- and 3-ring PAHs (ΣPAHs_{LMW}), and 4-, 5- and 6-ring PAHs (ΣPAHs_{HMW}).

Altogether, the reached values suggested that the predominance of one of these sources cannot be established. High values of the concentration ratio of low molecular weight compounds to high molecular weight compounds, and phenanthrene/(anthracene+phenanthrene) >0.7 indicate prevailing contribution of petrogenic pollution. These results may be due to the emissions of the petrochemical complex and the petrol station in the surrounding areas of PS1 and PS2, respectively. However, the other diagnostic ratios are characteristics of pyrogenic inputs. In particular, they suggest influence of vehicular traffic and specifically of diesel emissions (benzo[b]fluoranthene/benzo[k]fluoranthene >0.5 ; indeno[1,2,3-cd]pyrene/(indeno[1,2,3-cd]pyrene + benzo[ghi]perylene) >0.35 and <0.7 ; fluoranthene/(fluoranthene + pyrene) >0.40 and <0.50 ; fluorene/(fluorene + pyrene) >0.5 (Ravindra et al., 2008). These findings are in agreement with patterns of fuel consumption in Portugal: diesel is the most used car fuel accounting for 78.5% of the total national car fuel consumption (NIS 2015). In that regard, it is necessary to point out that overall higher ratio values were reached indoors of both schools emphasizing the impact of traffic on IAQ. This is not surprising since both schools were situated in a close vicinity of busy streets. It should be though remarked that limited ventilation was conducted in both preschools during the sampling (Chapter 4, Table 4.3) which might have led to reduced exchanged air rates and thus resulted in increased indoor air pollution. Finally, it is worth mentioning that discrimination between pyrolytic and petrogenic sources are a complex task because diagnostic ratios may be altered in different proportions during phase transfers and environmental degradation.

5.3.2 Urinary OH-PAHs in preschool children

PAH exposures occur through multiple routes with air and food being the main ones for children (excluding the contribution of passive and thirdhand tobacco smoke) (Kim et al., 2013). The use of biomarkers is especially relevant in children exposure assessment since there is also significant potential for dermal absorption and particle-bound PAHs ingestion (i.e., from hand-to-mouth activities, young children also tend to breathe through their mouths) when compared with adults. These differences may result in greater toxicity in children than in adults from similar external environmental exposures.

Results (median and range) from OH-PAH analysis in urine samples presented for each preschool and children gender (due to marked differences in physiology and

metabolism between males and females) are exhibited in Table 5.4. Besides the overall median values, the results for different collection period (i.e. first morning and last night urine) are also presented. Urinary OH-PAH values were normalized by the creatinine levels. No urine sample was excluded due to the creatinine concentrations (> 30 mg/dL and < 300 mg/dL, WHO 1996). Creatinine values among genders (at PS1: 30.2-157 mg/dL for boys, 30.9-95.9 mg/dL for girls; PS2: 43.4-103 mg/dL and 47.8-133 mg/dL for boys and girls, respectively) were evaluated and no significant differences were found ($p>0.05$). Thus it can be assumed that creatinine-adjusted OH-PAH concentrations are not influenced by urinary creatinine levels.

Based on data collected from questionnaires, only children whose diets at home were exclusive of barbecue and deep-fried foods within 2 days before urine sample collection were considered. Thus it was assumed that preschool children had similar levels of PAHs exposure through food consumption. Detection rates were above 82 and 92% for 1OHNaph+1OHAce and 1OHPy, respectively, while 2OHFlu and 1OHPhen presented detection rates higher than 94%.

Urinary 3OHB[a]P (metabolite of the PAH biomarker of carcinogenicity) was never detected being in accordance with the low levels found in air and with some previous studies (Fan et al., 2012; Miller et al., 2010; Wilson et al., 2000). It is documented that the detection frequency of metabolites with five or more aromatic rings in urine drops drastically (Li et al., 2008, 2012) since these compounds are mainly excreted through faeces (Likhachev et al., 1992). Furthermore, PAHs may be deposited in human adipose tissues without being metabolized immediately and thus contributing to a low level steady state excretion (ATSDR 1995).

Some toxicological studies have investigated the kinetics of urinary OH-PAH excretions from different exposure routes in adults; no information regarding children exists. The half-life of urinary 1OHPy excretion rates varies between 6 and 35 h after inhalation exposure (Brzeznicki et al., 1997), and 4.4 (Buckley and Lioy, 1992) to 12 h (Viau et al., 1995) after ingestion exposure; 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 1-OHPhen were also reported for ingestion exposure (Li et al., 2012). Data characterizing inhalation exposures are rare. Urinary levels of OH-PAHs were inversely related to the size of compounds, i.e., the higher the molecular weight the lower the detected OH-PAH concentrations.

Table 5.4. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine) measured in urine samples of preschool children collected at two preschools (PS1 and PS2).

Compound**	< LOD		< LOD	
	n (%)	($\mu\text{mol/mol}$ creatinine)	n (%)	($\mu\text{mol/mol}$ creatinine)
PS1				
1OHNaph+1OHAce	-	4.42 ^a ($5.11 \times 10^{-2} - 14.4$)	-	3.90 ^a (0.178 – 7.46)
2OHFlu	-	0.216 ^b ($1.26 \times 10^{-2} - 1.34$)	-	0.124 ^b ($5.82 \times 10^{-2} - 0.866$)
1OHPhen	-	5.53 $\times 10^{-2}$ c + ($1.75 \times 10^{-2} - 0.301$)	-	5.63 $\times 10^{-2}$ c + ($3.80 \times 10^{-2} - 0.121$)
1OHPy	-	5.72 $\times 10^{-2}$ c ($1.93 \times 10^{-2} - 0.246$)	-	6.58 $\times 10^{-2}$ c + ($2.05 \times 10^{-2} - 0.128$)
$\Sigma\text{OH-PAHs}$		4.75 ^{ad} (0.240 – 15.4)		4.15 ^{ad} (0.345 – 7.71)
Morning				
1OHNaph+1OHAce	5 (18)	2.17 ^a ($5.11 \times 10^{-2} - 17.2$)	3 (19)	2.57 ^a ($5.72 \times 10^{-2} - 9.12$)
2OHFlu	-	0.293 ^b ($1.36 \times 10^{-2} - 0.852$)	-	0.156 ^{ab} ($8.50 \times 10^{-2} - 0.482$)
1OHPhen	1 (4)	6.41 $\times 10^{-2}$ c ($3.93 \times 10^{-4} - 0.340$)	-	8.14 $\times 10^{-2}$ c * + ($4.79 \times 10^{-2} - 0.189$)
1OHPy	2 (8)	9.11 $\times 10^{-2}$ c ($3.50 \times 10^{-4} - 0.329$)	-	0.114 ^{bc} * + ($1.71 \times 10^{-2} - 0.212$)
$\Sigma\text{OH-PAHs}$		2.62 ^{ad} (0.376 – 17.7)		2.92 ^{ad} (0.401 – 9.79)
Night				
1OHNaph+1OHAce	3 (11)	5.62 ^a ($5.11 \times 10^{-2} - 16.1$)	1 (6)	4.86 ^a (0.178 – 14.6)
2OHFlu	1 (4)	9.01 $\times 10^{-2}$ b ($1.13 \times 10^{-3} - 2.61$)	-	8.12 $\times 10^{-2}$ b ($6.10 \times 10^{-3} - 5.78$)
1OHPhen	-	4.17 $\times 10^{-2}$ c # ($1.60 \times 10^{-2} - 0.263$)	-	3.20 $\times 10^{-2}$ c * # + ($1.71 \times 10^{-2} - 5.21 \times 10^{-2}$)
1OHPy	-	3.63 $\times 10^{-2}$ c ($9.07 \times 10^{-3} - 0.163$)	-	2.24 $\times 10^{-2}$ c * + ($4.21 \times 10^{-3} - 6.56 \times 10^{-2}$)
$\Sigma\text{OH-PAHs}$		5.79 ^{ad} (0.105 – 18.8)		5.00 ^{ad} (0.290 – 20.4)

* 1OHNaph+1OHAce: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene;

1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene

¹ When concentration was below the LOD, the value of the respective LOD/ $\sqrt{2}$ was used (Hornung and Reed 1990).

* Statistically significant ($p \leq 0.05$) between morning and night hydroxyl-PAH concentrations for each school.

Statistically significant ($p \leq 0.05$) between boys and girls monohydroxyl-PAH concentrations for each school.

+ Statistically significant ($p \leq 0.05$) between schools for boys and girls monohydroxyl-PAH concentrations.

a, b, c, d Different letters correspond to statistically different means ($p \leq 0.05$) within each group.

Table 5.4. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine) measured in urine samples of preschool children collected at two preschools (PS1 and PS2) (continued).

Compound**	< LOD		< LOD	
	n (%)	($\mu\text{mol/mol}$ creatinine)	n (%)	($\mu\text{mol/mol}$ creatinine)
PS2				
1OHNaph+1OHAce	-	3.49 ^a (1.27 – 7.76)	-	3.73 ^a (8.51×10^{-2} – 9.40)
2OHFlu	-	0.324 ^b (0.104 – 0.910)	-	0.221 ^b (0.114 – 0.482)
1OHPhen	-	8.53×10^{-2} ^{bc +} (7.05×10^{-2} – 0.270)	-	0.138 ^{c +} (6.14×10^{-2} – 0.430)
1OHPy	-	0.117 ^{bc} (4.15×10^{-2} – 0.941)	-	0.184 ^{bc +} (4.94×10^{-2} – 0.276)
$\Sigma\text{OH-PAHs}$		4.02 ^{ad} (1.54 – 9.07)		4.27 ^{ad} (0.556 – 9.67)
Morning				
1OHNaph+1OHAce	2 (12)	0.664 [*] (0.104 – 3.49)	3 (19)	3.23 ^a (6.58×10^{-2} – 6.36)
2OHFlu	-	0.229 (5.29×10^{-2} – 1.24)	1 (6)	0.276 ^b (4.08×10^{-4} – 0.738)
1OHPhen	1 (6)	0.146 ^a (2.63×10^{-3} – 0.307)	-	0.171 ^{bc* +} (3.16×10^{-2} – 0.585)
1OHPy	1 (6)	0.179 ^a (2.34×10^{-3} – 0.941)	-	0.203 ^{bc* +} (5.05×10^{-2} – 0.514)
$\Sigma\text{OH-PAHs}$		1.22 ^{b*} (0.503 – 5.37)		3.88 ^{ad} (0.288 – 7.50)
Night				
1OHNaph+1OHAce	1 (6)	9.35 ^{a*} (1.87 – 15.42)	-	4.29 ^a (0.104 – 18.7)
2OHFlu	1 (6)	0.350 ^b (8.49×10^{-2} – 0.583)	-	0.174 ^b (1.28×10^{-2} – 0.642)
1OHPhen	-	7.99×10^{-2} ^{bc} (2.50×10^{-2} – 0.144)	-	8.57×10^{-2} ^{bc* +} (3.15×10^{-2} – 0.276)
1OHPy	-	0.111 ^{bc} (3.18×10^{-2} – 0.168)	-	7.27×10^{-2} ^{c* +} (1.33×10^{-2} – 0.224)
$\Sigma\text{OH-PAHs}$		9.89 ^{ad*} (2.02 – 16.3)		4.62 ^{ad} (0.708 – 18.9)

* 1OHNaph+1OHAce: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene;

1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene

[†] When concentration was below the LOD, the value of the respective LOD/ $\sqrt{2}$ was used (Hornung and Reed 1990).

^{*} Statistically significant ($p \leq 0.05$) between morning and night hydroxyl-PAH concentrations for each school.

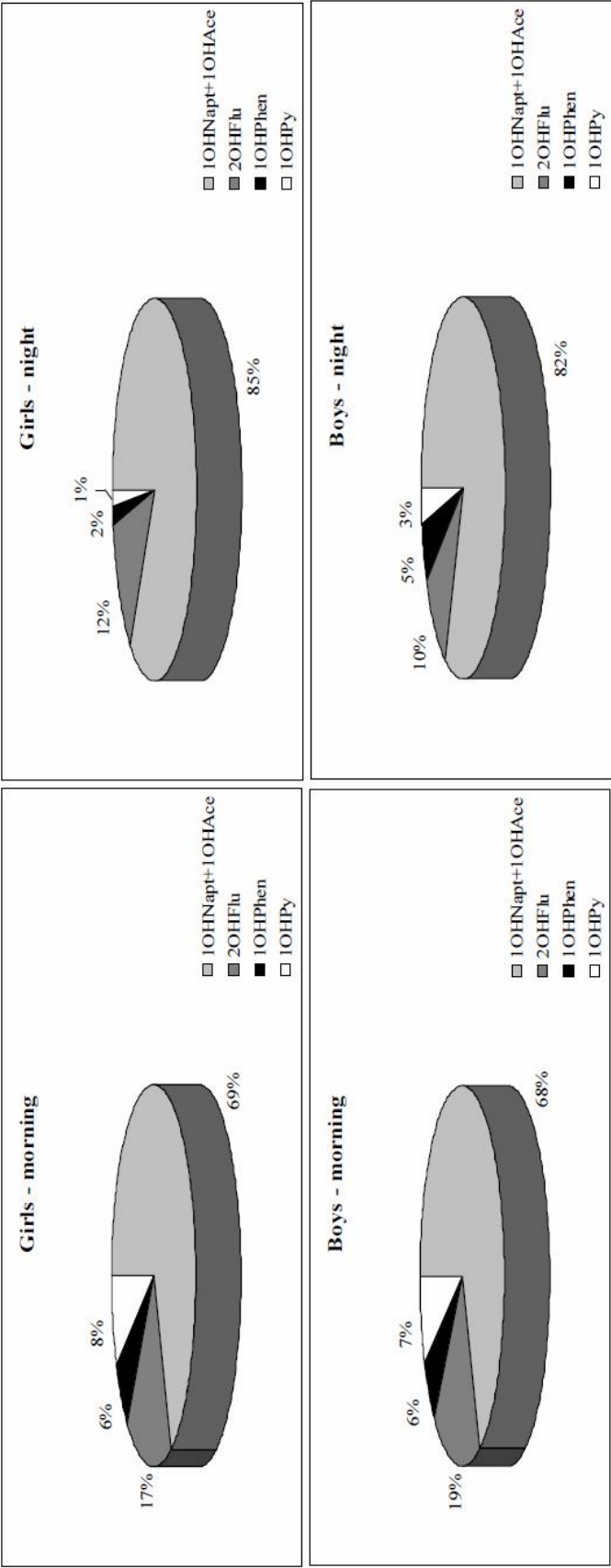
[#] Statistically significant ($p \leq 0.05$) between boys and girls monohydroxyl-PAH concentrations for each school.

⁺ Statistically significant ($p \leq 0.05$) between schools for boys and girls monohydroxyl-PAH concentrations.

^{a, b, c, d} Different letters correspond to statistically different means ($p \leq 0.05$) within each group.

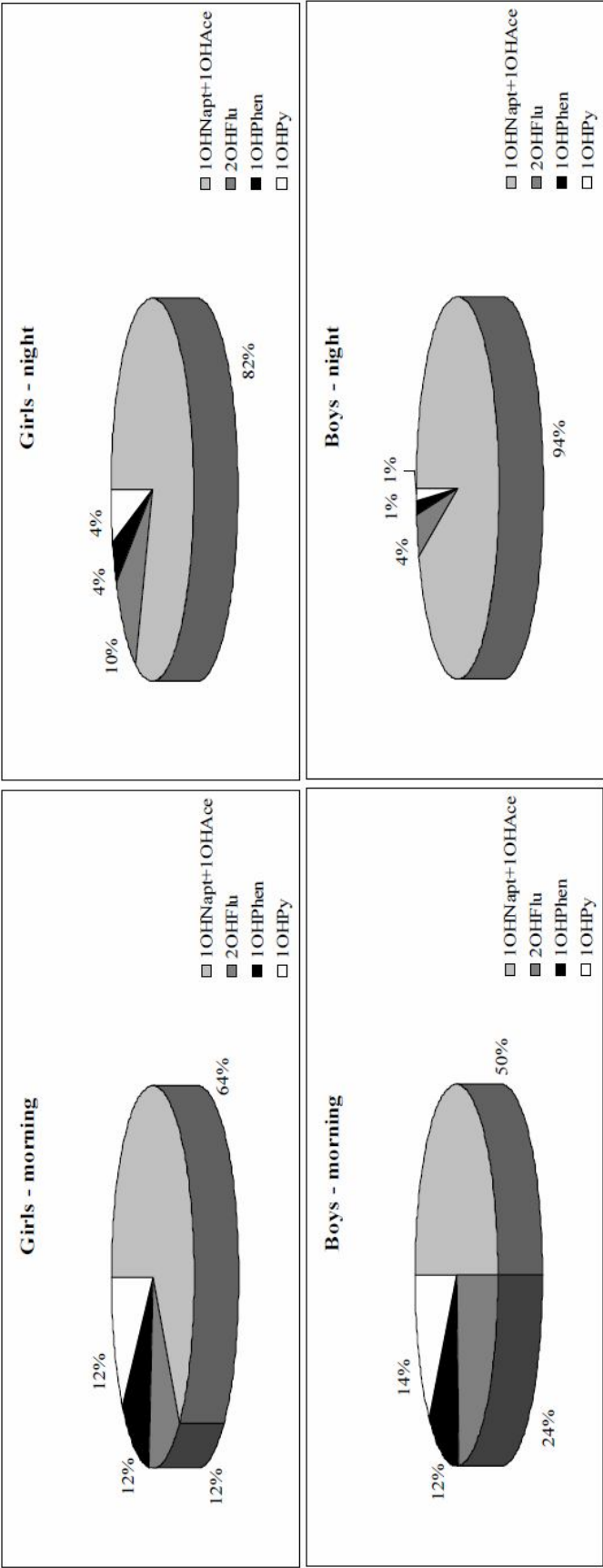
Overall, 1OHNaph+1OHAce were by far the most abundant OH-PAHs (78% for girls at PS2 to 84% for boys at PS2), being followed by 2OHFlu (8% for boys at PS2 to 11% for girls at PS1), 1OHPy (3% for girls at PS1 to 7% for girls at PS2), and 1OHPhen (3% for boys at PS2 to 5% for girls at PS2) at both preschools. This distribution profile follows the same pattern of variation as the distribution of PAHs in air of both preschools (Table 5.2). PAHs with 2-3 aromatic rings were the most abundant ones (91-94% and 63-73% of total air Σ PAHs in indoor and outdoor air of both preschools, respectively). 1OHNaph+1OHAce were predominantly detected at higher concentrations in last night than in first morning urines. An inverse trend was found for other metabolites (2OHFlu, 1OHPhen, and 1OHPy), which were elevated in the first morning urines (Table 5.4, Figure 5.4).

The inter-comparison between preschools indicated that children from PS2 presented slightly higher concentrations of 2OHFlu, 1OHPhen, and 1OHPy for boys and girls. However, statistically differences were only found for urinary 1OHPhen ($p=0.019$) in boys, and 1OHPhen and 1OHPy levels in girls ($p\leq 0.006$). These results are in accordance with the higher levels of fluorene (8.03 ng/m³ *versus* 5.58 ng/m³ at PS2 and PS1, respectively), phenanthrene (33.7 ng/m³ *versus* 10.3 ng/m³ at PS2 and PS1, respectively) and pyrene (1.93 ng/m³ *versus* 0.803 ng/m³ at PS2 and PS1, respectively) detected in indoor air at PS2 (Table 5.2). In the selected preschools, children spent ca. 84% (6.75 h in a total of 8 h) of their daily time indoors. Taken into consideration differences between genders, it can be observed that girls presented slightly higher individual OH-PAH concentrations in the first morning urine (except for 2OHFlu at PS1; Table 5.4) while in last night urines a contrary tendency was observed, with boys having OH-PAH levels slightly higher than girls (except for 1OHPhen at PS2). These results reflect differences in absorption, bioavailability, metabolic pathway and excretion rates (Li et al., 2008). Additional research is needed to confirm and explore these results since available information is almost inexistent. Among the available studies regarding OH-PAHs in children, considering a similar age category as in this report (3-5 years old), few works explored the possible differences between gender (Hansen et al., 2005; Miller et al., 2010; Mucha et al., 2006; van Wijnen et al., 1996) and simultaneously the contents of morning and early afternoon urines (van Wijnen et al., 1996). van Wijnen et al. (1996) reported slightly higher creatinine adjusted 1OHPy concentrations in girls compared to boys while the studies of Hu et al. (2011) and Mucha et al. (2006) were inconclusive.



(a)

Figure 5.4. Distribution of 1-hydroxybaphthalene + 1-hydroxyacenaphthene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, and 1-hydroxypyrene in the first morning and last night urine samples of girls and boys: (a) PS1 and (b) PS2.



(b)

Figure 5.4. Distribution of 1-hydroxybaphthalene + 1-hydroxyacenaphthene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, and 1-hydroxypyrene in the first morning and last night urine samples of girls and boys: (a) PS1 and (b) PS2 (continued).

No reference standard guidelines are established for urinary OH-PAHs. Still, Jongeneelen (2001) proposed in his pioneer study a reference value of 0.24 $\mu\text{mol/mol}$ creatinine of 1OHPy for non-smokers and non-occupational exposed groups, and a no-biological effect level of 1.4 $\mu\text{mol/mol}$ creatinine in exposed workers. Among the evaluated subjects, seven children (one boy from PS1, and five girls and one boy from PS2) presented urinary 1OHPy concentrations higher than the proposed guideline reaching a maximum value of 0.94 $\mu\text{mol/mol}$ creatinine (first morning urine of a male child from PS2). With the exception of one sample, the respective urinary 1OHPy concentrations were significantly reduced (by 2-8 times) in the respective last night urine samples. The available studies on children urinary concentrations of OH-PAHs are summarised in Table 5.5. Due to the strong age-dependence of metabolism and creatinine excretion, only studies involving children with a comparable age group were considered. By far 1OHPy is the most characterized metabolite among children being the only determined in the majority (94%) of the studies. In general, the range of 1OHPy concentrations observed in the present study for both preschools were in agreement with the levels reported for children from Canada (Vyskocil et al., 2000), Czech Republic (Fiala et al., 2001), China (Hu et al., 2011; Fan et al., 2012), Denmark (Hansen et al., 2005) Germany (Heudorf and Angerer, 2001; Wilhelm et al., 2007), Spain (Freire et al., 2009), Ukraine (Mucha et al., 2006), and USA (Wilson et al., 2000, 2003) but were lower than those reported for Japanese (Mori et al., 2011) and Dutch (van Wijnen et al., 1996) children (Table 5.5). No studies that included 1OHAce has been found worldwide, and in European children 2OHFlu has never been assessed. The levels of 2OHFlu determined in this work were 4 (boys from PS2) to 11 (girls from PS1) times higher than those described for Chinese children from a non-polluted area (Fan et al., 2012); no further comparison can be made as the available data from USA were not normalized by creatinine levels (Table 5.5). Regarding urinary 1OHPhen, 2 (girls at PS2) to 6 (boys at PS1) times lower levels were observed compared with the concentrations reported for children living in homes with parquet flooring that contained high levels of PAHs in the parquet glue (Heudorf and Angerer 2001).

Table 5.5. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine except when indicated otherwise) reported in children (n = number of enrolled students) of similar age categories as in the present study (3-5 years old) (continued).

Continent	Country, city	n (age, years)	Note	Metabolite** ($\mu\text{mol/mol}$ creatinine)				Reference
				1OHNaph	2OHFlu	1OHPhen	1OHPy	
Europe	Denmark, Copenhagen	101 (3-13)	Urban residences					Hansen et al. (2005)
			Boys				0.11; 0.07 (0.01 – 0.53)	
			Girls				0.10 (0.01 – 0.63)	
			Rural residences					
			Boys				0.06; 0.07 (0.01 – 1.66)	
			Girls				0.06; 0.08 (0.01 – 0.54)	
	Germany, Dortmund Hörde, Duisburg	948 (5-9)	Live in the vicinity of a coke oven plant				0.157 (0.030-1.07)	Wilhelm et al. (2007) ^a
			Rural town				0.081 (0.026-0.559)	
	Germany, Frankfurt am Main	327 (0-6)	Homes with parquet flooring containing high levels of PAHs in the parquet glue					Heudorf and Angerer (2001) ^a
						0.316 $\pm$ 0.218 [*] (nd – 1.68)	0.066 $\pm$ 0.082 [*] (nd – 0.741)	

**1OHNaph+1OHAc: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene; 3OHBAp: 3-hydroxybenzo[a]pyrene

nd - not detected

* concentrations expressed as mean $\pm$ SD

^a concentrations originally expressed as $\mu\text{g/g}$ creatinine and converted to $\mu\text{mol/mol}$ creatinine: concentration reported/(molecular weight of OH-PAH/molecular weight of creatinine);

^b concentration corresponds to the sum of metabolites 1- and 3OHBAp;

^c concentration corresponds to the sum of metabolites 1- and 2OHNaph.

Table 5.5. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine except when indicated otherwise) reported in children (n = number of enrolled students) of similar age categories as in the present study (3-5 years old) (continued).

Continent	Country, city	n (age, years)	Note	Metabolite** ($\mu\text{mol/mol}$ creatinine)			Reference
				1OHNaph	2OHFlu	1OHPhen	
Europe	The Netherlands	644 (1-6)	Polluted areas			0.34 $\pm$ 0.38* (nd – 2.50) 0.43 $\pm$ 0.40* (0.03 – 1.58)	van Wijnen et al. (1996)
	The Netherlands	644 (1-6)	Boys			0.19	van Wijnen et al. (1996)
			Girls			0.22	
Asia	Spain, Granada	174 (4)	Urban			0.060 $\pm$ 0.040* 0.054 $\pm$ 0.055*	Freire et al. (2009)
	Ukraine, Mariupol	48 (3)	Live near a steel mill and cooking facility			0.62 $\pm$ 0.45* 0.74 $\pm$ 0.53*	Mucha et al. (2006)
	Ukraine, Kiev	42 (3)	Control area			0.31 $\pm$ 0.21* 0.37 $\pm$ 0.19*	
	Afghanistan, Kabul, Ningarhar	13 (2-9)	Urban and rural areas	1343 (46.6 – 6865) ng/L urine		3167 (71.4 – 16288) ng/L urine	Henat et al. (2012)
	China, Guangzhou	35 (4-6)	Non-polluted area	1.38 (0.73 – 13.20)		0.10 (nd – 0.41)	Fan et al. (2012)

* 1OHNaph+1OHAc: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene; 3OHBaP: 3-hydroxybenzo[a]pyrene
nd - not detected

* concentrations expressed as mean $\pm$ SD

^a concentrations originally expressed as $\mu\text{g/g}$ creatinine and converted to $\mu\text{mol/mol}$ creatinine: concentration reported/(molecular weight of OH-PAH/molecular weight of creatinine);

^b concentration corresponds to the sum of metabolites 1- and 3OHBaP;

^c concentration corresponds to the sum of metabolites 1- and 2OHNaph.

Table 5.5. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine except when indicated otherwise) reported in children (n = number of enrolled students) of similar age categories as in the present study (3-5 years old) (continued).

Continent	Country, city	n (age, years)	Note	Metabolite** (μmol/mol creatinine)				Reference	
				1OHNaph	2OHFlu	1OHPhen	1OHPy	3OHB[a]P	
Asia	China, Taiwan	369 (1-13)	Communities located in close vicinity to a power plant				0.194 ± 0.143 [*]		Hu et al. (2011)
							0.186 ± 0.148 [*]		
							0.113 ± 0.082 [*]		
							0.122 ± 0.089 [*]		
	China, Southern Taiwan	40 (2-6)	Homes with smoking parents						Tsai et al. (2003) ^a
		Morning			0.011 (0.001 – 0.53)				
		Night			0.015 (0.002 – 1.33)				
	Japan, Kanagawa	134 (3-6)	Kindergarten				0.066 (0.02 – 1.25)		Mori et al. (2011) ^a
North America	Canada, Montreal	23 (3-6)	Kindergartens						Vyskocil et al. (2000)
			Morning						
			Polluted area			0.20 [*] (0.002 – 0.77)			
			Non polluted area			0.12 (0.01 – 0.41)			

^a 1OHNaph+1OHAce: 1-hydroxybaphthalene + 1-hydroxyxycenaphthene; 2OHFlu: 2-hydroxyfluorene; 10HPhen: 1-hydroxyphenanthrene; 10HPy: 1-hydroxypyrene; 3OHBaP: 3-

hydroxybenzo[*a*]pyrene

nd - not detected

* concentrations expressed as mean $\pm$ SD

^a concentrations originally expressed as µg/g creatinine and converted to µmol/mol creatinine: concentration reported/(molecular weight of OH-PAH/molecular weight of creatinine);

^b concentration corresponds to the sum of metabolites 1- and 3OHBA[P];

^c concentration corresponds to the sum of metabolites 1- and 2OHNaph

Table 5.5. Monohydroxyl-PAH concentrations (median and range, expressed as $\mu\text{mol/mol}$ creatinine except when indicated otherwise) reported in children (n = number of enrolled students) of similar age categories as in the present study (3-5 years old) (continued).

Continent	Country, city	n (age, years)	Note	Metabolite** ($\mu\text{mol/mol}$ creatinine)	1OHFlu	1OHPhen	1OHPy	3OHBA[a]P	Reference
North America	Canada, Montreal	23 (3-6)	Kindergartens						Vyskocil et al. (2000)
			Evening						
			Polluted area				0.19 ^c (0.03 – 0.77)		
			Non polluted area				0.13 ^c (0.03 – 0.26)		
	USA, Harlem, Bronx, Washington	113 (5)	Dominican	2967 ^c (113 – 138000) ng/L urine					Orjuela et al. (2012)
			African American	2871 ^c (82 – 67700) ng/L urine					
			Boys	2490 (1875 – 3306) ng/L urine	290 (240 – 350) ng/L urine	167 (139 – 201) ng/L urine	153 (125 – 187) ng/L urine	nd	
			Girls	3327 (2570 – 4306) ng/L urine	289 (246 – 340) ng/L urine	165 (141 – 192) ng/L urine	167 (141 – 197) ng/L urine	nd	
	USA, North Carolina	9 (2-5)	Day-care centers	1.37 (0.26-3.03)			0.127 ^c (0.03 – 0.32)	0.033 ^c (<0.02 – 0.11) ^b	Wilson et al. (2003) Wilson et al. (2000)
			Day-care centers	(0.72 – 3.3) ^c			(0.029 – 0.27)	(<0.017 – 0.11) ^b	

* 1OHFlu: 1-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene; 3OHBA: 3-hydroxybenzo[a]pyrene
nd - not detected
* concentrations expressed as mean $\pm$ SD
^a concentrations originally expressed as $\mu\text{g/g}$ creatinine and converted to $\mu\text{mol/mol}$ creatinine: concentration reported/(molecular weight of OH-PAH/molecular weight of creatinine);
^b concentration corresponds to the sum of metabolites 1- and 3OHBA[a]P;
^c concentration corresponds to the sum of metabolites 1- and 2OHFlu

5.3.3 Correlations between airborne PAHs and urinary OH-PAHs

At both preschools, the possible contribution of total (Σ PAHs) and selected indoor airborne compounds (naphthalene, acenaphthene, naphthalene + acenaphthene, fluorene, phenanthrene, pyrene) to respective total (Σ OH-PAHs) and individual metabolites (1OHNaph+1OHAc, 2OHFlu, 1OHPhen, and 1OHPy) excretion in children was estimated through the determination of Spearman correlation coefficients (r). As stated previously, since children spent majority of their school daily time indoors, and only 1.25 h outdoors, correlations were only conducted between PAHs in indoor air and OH-PAH levels. Overall the strongest correlations were reached between Σ PAHs in preschool indoor air and urinary Σ OH-PAHs (varying from $r=0.218$ for girls at PS2 to $r=0.700$ for boys at PS2), and between naphthalene and acenaphthene with 1OHNaph+1OHAc (ranging from $r=0.264$ for girls at PS2 to $r=0.500$ for boys at PS2, and $r=0.533$ (girls at PS1)). Still, statistical significance ($p \leq 0.05$) was not reached. No evident relationships ($r \leq 0.300$) were detected for the other PAH and corresponding OH-PAH, including 1OHPy (proposed as the biomarker of PAHs exposure; Mucha et al., 2006) and pyrene. These findings are in agreement with Fiala et al. (2001) that concluded that 1OHPy seems to be a non-sensitive marker of environmental inhalation exposure to pyrene (except in the presence of heavy air pollution). These results reflect the impact of the indoor levels of lighter PAHs, and specifically of naphthalene and acenaphthene, on urinary Σ OH-PAHs, and thus on preschool children total PAHs exposure.

This influence is even more pronounced if only last night urine data are used; Spearman correlation coefficients reached 0.800 between Σ PAHs and Σ OH-PAHs (for boys at PS2; $p=0.200$) and 0.600 between naphthalene and acenaphthene with 1OHNaph+1OHAc (for boys at PS2; $p=0.400$). Overall, PS2 presented stronger correlations than PS1, which is in agreement with the higher PAH concentrations in indoor air. These assumptions were further corroborated by analysing the Spearman correlation coefficients between individual OH-PAH and Σ OH-PAHs. It is expected that individual OH-PAH levels correlate between each other if originating from the same major source. Urinary 1OHNaph+1OHAc from children (boys and girls) of both preschools strongly correlated with Σ OH-PAHs ($r \geq 0.983$, $p \leq 0.001$ at PS1; $r \geq 0.991$, $p \leq 0.001$ at PS2). However urinary 1OHNaph+1OHAc were weakly correlated with the other metabolites, which may be explained by the different routes of their individual PAH to the human body. Naphthalene and acenaphthene are the more volatile

compounds and most of their environmental levels enter in the human body mainly in a gaseous form and thus 1OHNaph+1OHAc concentrations probably reflect the contribution of air. It seems that children had preschool indoor air as their major exposure source of naphthalene and acenaphthene. Fluorene, phenanthrene and pyrene are absorbed through the respiratory tract but also by the gastrointestinal tracts, and by the skin. Generally, the metabolites 2OHFlu, 1OHPhen, and 1OHPy strongly correlated with each other ($r \leq 0.641$, $p \leq 0.136$ at PS1; $r \leq 0.975$, $p \leq 0.322$ at PS2); more specifically, Spearman correlation coefficients between 1OHPy and 2OHFlu were $r \geq 0.377$ ($p \leq 0.136$) and $r \geq 0.432$ ($p \leq 0.285$), for boys and girls at PS1 and PS2, respectively; between 1OHPy and 1OHPhen values were $r = 0.562$ ($p = 0.019$) for boys at PS1, and $r \geq 0.794$ ($p \leq 0.005$) for boys and girls at PS2. These findings suggest the existence of additional and significant common sources of exposure (food, home air and/or outdoor air) to fluorene, phenanthrene and pyrene. It can be also concluded that sole use of 1OHPy as the biomarker of PAHs exposure can lead to underestimation of total PAHs body burden, and relevant complementary information may be attained by analysing 2- and 3-ring OH-PAHs together with 1OHPy.

5.4 Conclusions

This study filled a gap by providing information on PAHs in preschool environments, the exposure levels and assessment of the contribution to total exposure of 3-5 old children. Overall, the total (i.e. gaseous and particulate) concentrations of 18 PAHs were higher indoors than outdoors. Gaseous PAHs accounted for 93-95% (indoors) and 77-91% (outdoors) of the total air Σ PAHs, while dibenz[a,h]anthracene, the most abundant TSP-bound PAH, accounted for less than 3%. The use of different diagnostic ratios allowed identification of pyrogenic (mostly traffic emissions) and petrogenic sources. However, the predominance of one of these sources could not be established for both preschools.

Preschool children internal dose was assessed by six biomarkers of exposure; urinary 1OHNaph+1OHAc were the most predominant metabolites, being followed by 2OHFlu, 1OHPy, and 1OHPhen. This distribution profile followed the same pattern of variation as the distribution of PAHs in air of both schools. This study highlighted the need to consider the gas phase (which has been neglected) for adequate assessment of environmental PAHs exposure, and the need to use urinary concentrations of 2- and 3-ring OH-PAHs together with 1OHPy to assess individual's recent total exposure to

PAHs. It is recommended that total body burden of PAHs should not be assessed exclusively by 1OHPy biomonitoring, as it has been performed in the large majority of studies. Overall, the obtained results revealed the impact of naphthalene and acenaphthene levels in indoor air of preschools on urinary Σ OH-PAHs, and thus on children total PAHs exposure. It seems that exposure to the levels in preschool indoor air represented the major exposure source for naphthalene and acenaphthene, while no conclusion was reached regarding fluorene, phenanthrene and pyrene.

Results for a larger number of schools, participants and number of (air and urine) samples are needed to draw more meaningful and statistically more significant conclusions. Also, a more comprehensive monitoring of all possible sources would be precious. Still, this study has provided a quantitative support in understanding the impacts of environmental PAHs exposure and its influences on preschool children.

5.5 Ethical statement

The study proposal was reviewed and approved by Ethic Committee of University of Porto. Full information about the measurements conducted in this work was provided to parents of all students, teaching staff and boards of directors of both preschools.

5.6 References

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CHAPTER 6 – PART I: GLOBAL CONCLUSIONS

6.1 Global conclusions

The work performed in the first part of this thesis characterized PM, its elemental composition and PAH profiles at (pre)school (micro)environments and assessed the potential health risks for (pre)school children.

The determined PM₁₀ and PM_{2.5} mean concentrations were below the established limits of the current legislation for indoor and outdoor air (Portuguese Regulation 2013), except during the preliminary study at an Oporto preschool (PM_{2.5} levels of 27 and 29 µg/m³ in the indoor and outdoor air, respectively). PM₁₀ was composed by 62-67% of PM_{2.5} while PM_{2.5} was constituted by 86-91% of PM₁. These findings highlight the importance of analysing the fine fraction when environmental or personal exposure is assessed.

Calcium, sodium, aluminium, and potassium were the most abundant elements in indoor PM (82–84% of the analysed content); carcinogenic elements (1–2% of the indoor analysed content) were mostly PM_{2.5}-bound (83–91%).

Overall PAH concentrations (PM_{2.5}-bond ranged from 2.82-54.1 ng/m³ indoors and between 6.35-48.3 ng/m³ outdoors at primary schools; ΣPAHs (gas and particulate phase) varied from 25.1-149 ng/m³ and 2.68-47.9 ng/m³, respectively in the indoor and outdoor air of preschools; these concentrations were in lower range with other scarce studies available in the literature (Eiguren-Fernandez et al., 2007; Krugly et al., 2014; Wilson et al., 2000, 2003). Naphthalene was one of the most abundant PAHs in the preschool total air (gas and particulate phase), however its concentrations were always well below the recommended limit of 10 µg/m³ for the indoor air (WHO 2010). PM_{2.5}-benzo[a]pyrene mean concentrations at preschools were always below the PM₁₀ annual mean guideline of 1 ng/m³ (Directive 2004/107/EC), while some exceedances were registered (indoors and outdoors) at some primary schools (two located in the city center and one inserted in a residential area with industrial influence). These results confirm the relevance of urban planning in order to reduce children's exposure at schools. Dibenz[a,h]anthracene was found to be one of the most abundant particulate PAHs in indoor and outdoor air of the characterized pre- and primary schools evidencing the impact of emissions from motor light-duty gasoline vehicles in air quality. At preschools particulate PAHs were mainly associated with PM₁ (54-76% particulate ΣPAHs) with 5-ring PAHs being the most abundant compounds. The total (i.e. gaseous and particulate) concentrations of PAHs were predominantly higher indoors than outdoors, being the penetration of outdoor emissions one of the most relevant sources. In addition, I/O ratios of lighter PAHs (2–3 rings) indicated also

contribution from indoor sources, namely of naphthalene, acenaphthylene, acenaphthene and phenanthrene. Indoor levels of naphthalene and acenaphthene seem to contribute significantly to total urinary OH-PAHs of preschool children. Still, more studies involving a higher number of students and more schools are needed to further explore these findings. The assessment of urinary monohydroxyl-PAHs also revealed that, the use of other compounds rather than only 1OHPy, is necessary to better characterize the children internal dose of PAHs.

Regarding potential health risks due to elemental composition of PM, non-carcinogenic and carcinogenic risks from preschool exposure were acceptable for children; for adults carcinogenic risks exceeded (4–11 times) the USEPA recommend value of 10^{-6} being 8–40 times higher than for children. Total carcinogenic risks due to exposure to PAHs in the indoor air of pre- and primary schools were higher than in outdoor air, which was attributed to the longer periods that children spend indoors. Overall cancer risks of children and staff attending at (pre)schools exceeded from 5–37 and 7–87 times the WHO health-based guideline of 10^{-5} .

6.2 References

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PART II – FIREFIGHTERS’ OCCUPATIONAL EXPOSURE

**CHAPTER 7 – PARTICULATE MATTER AND POLYCYCLIC
AROMATIC HYDROCARBONS AT FIRE STATIONS:
FIREFIGHTERS EXPOSURE MONITORING AND
BIOMONITORING AND ASSESSMENT OF THE CONTRIBUTION
TO TOTAL INTERNAL DOSE**

Chapter 7. Particulate matter and polycyclic aromatic hydrocarbons at fire stations: firefighters exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose

This work characterizes levels of eighteen PAHs in the breathing air zone of firefighters during their regular work shift at eight Portuguese fire stations, and the firefighters' total internal dose by six urinary OH-PAHs. Σ PAHs concentrations varied widely (46.4–428 ng/m³), mainly due to site specificity (urban/rural) and characteristics (age and layout) of buildings. Airborne PAHs with 2–3 rings were the most abundant (63.9–95.7% Σ PAHs). Similarly, urinary 1OHNaph and 1OHAce were the predominant metabolites (66–96% Σ OH-PAHs). Naphthalene contributed the most to carcinogenic Σ PAHs (39.4–78.1%) in majority of firehouses; benzo[a]pyrene, the marker of carcinogenic PAHs, accounted with 1.5–10%. Statistically positive significant correlations ($r \geq 0.733$, $p \leq 0.025$) were observed between Σ PAHs and urinary Σ OH-PAHs for firefighters of four fire stations suggesting that, at these sites, indoor air was their major exposure source of PAHs. Firefighter's personal exposure to PAHs at Portuguese fire stations were well below the existent occupational exposure limits. Also, the quantified concentrations of post-shift urinary 1OHPy in all firefighters were clearly lower than the benchmark level (0.5 μ mol/mol) recommended by ACGIH.

7.1 Introduction

Firefighting is among the most hazardous yet the least studied occupations in terms of exposures and their relationship to occupational diseases. Forest fires release significant amounts of trace gases and aerosols into the atmosphere. Depending on meteorological conditions, fire emissions can reduce air quality and visibility, even in a long distance from an emission source (Dempsey 2013; Hurteau et al., 2014). The most frequent pollutants released to the atmosphere by fires are CO, PM, NO₂, and VOCs, including PAHs.

Firefighters are chronically exposed to complex mixtures of particles, smoke and products of incomplete combustion. Firefighting, along with construction, mining and agriculture, ranks among the most dangerous professions; its occupational exposure being regarded as possible carcinogen to humans (IARC 2010; NIOSH 2007). However, no occupational exposure limits or guidelines are available for firefighters.

The term 'firefighters' encompasses several types of fire scenarios such as wildland, municipal, industrial, aviation, military, and oil wells, being the wildland and the municipal fires the most described ones. Wildland firefighters have the primary responsibility to suppress fires in wildland fuels such as forests, grasslands, and brush. Wildland fires refer to wildfires and prescribed burns. During the last decades, the number of forest fires has been increasing in many countries around the world with more potent incidents and longer fire seasons. USA, Australia, and the Southeastern Europe are the geographical areas that have been continuously affected by strong fire incidents. The United Nation International Strategy for Disaster Reduction estimated that 3 to 4 million km² of land is affected by wildfire annually, with 18 000 km² occurring in Europe (Youssof et al., 2014). The Mediterranean region is one of the most affected regions by wildfires in Europe with nearly 500 000 ha, on average being burned annually by 65 000 wildfires in southern countries (European Commission 2010; Youssof et al., 2014). Prescribed burning, in combination with mechanical thinning, is a successful method to reduce the buildup of fuels on forest floors and contributes to the reduction of the risk of catastrophic wildfires. Regarding firefighting activities there are two distinct phases: knockdown and overhaul. During knockdown, firefighters control and extinguish the fire. After extinction, some reignitions may occur. At overhaul, the remaining small fires are extinguished, however the environment is not as hot or as smoky as during knockdown, but it still contains products of combustion from small fires or smoldering material. Overhaul can last several hours or even days

which increases firefighters exposure to combustion products. Occupational exposures of municipal and wildland firefighters widely differ. Approximately 90% of municipal structural fires are extinguished within 5 to 10 minutes, or abandoned and fought from the outside (IARC 2010). These fires present an average duration of heavy physical activity of approximately 10 minutes. The knockdown of large forest fires may last hours or days. Wildland firefighters go to fires more frequently and spend more time at fires during a season than do municipal firefighters during an entire year. In addition, all of their exposure occurs during the wildfire season. Wildland firefighters are engaged in heavy work activity while fighting fires, because they carry a considerable amount of equipment and use hand tools to help fire combat. Some typical tasks include hiking, fireline construction, chainsaw work, and brush removal (IARC 2010). In addition, firefighters are exposed to heat stress, falling hazards and/or can suffer accidents with hand and power tools; burns and being struck by falling rocks and trees are the most frequent ones. Wildland firefighters face more difficulties to control the forest fires due to adverse meteorological conditions (temperature, moisture, and wind speed and direction), difficulties to access fire front line and sometimes the lack of staff and/or adequate means such as helicopters. The number of runs and the time spent at fires varies tremendously, depending on the geographic location, the social and economic environment, staffing, etc. (IARC 2010).

In prior decades, firefighters were known as “smoke eaters”. However, respiratory protection devices for firefighters have existed for over a century (IARC 2010). A modern positive-pressure type self-containing breathing apparatus (SCBA) came into more widespread use during 1960s and 1970s. Since then some improvements have been achieved in order to increase protection. Nowadays pressure-demand SCBAs are commonly used by municipal firefighters (IARC 2010, Fent et al., 2013). When properly fitted, worn, and maintained, SCBA have the highest assigned protection factor of any other related equipment. Pressure-demand SCBA is considered the most adequate device for firefighters' protection given the levels of contaminants in fire atmosphere (Fent et al., 2013). However SCBA are typically not used by wildland firefighters and during the overhaul phase. Additionally, during potentially hazardous yet tolerable situations such as outside fires of vehicles, brush, detached garages, and in smaller structures SCBA may also not be used (Fabian et al., 2014). In these situations firefighters may inhale unknown concentrations of residual gases and/or smoke particles contaminated with absorbed chemicals. PM and PAHs are among the most relevant air pollutants released by fires. Table 7.1 presents the existent guidelines of PM and PAHs occupational exposure limits established by the

NIOSH and the Occupational Safety and Health Administration (OSHA). However it is worth to mention that occupational exposure limits have been developed for workers generally exposed to a single substance and engaged in light levels of work, which is not the case of firefighters (IARC 2010). The amount of inhaled chemicals is greater for a firefighter with heavy work levels than for a worker engaged in regular levels of activity. This fact need to be taken into consideration when comparing exposure levels to occupational exposure limits.

7.1.1 Firefighters occupational exposure to particulate matter

Table 7.2 presents the existent studies (organized by geographical region) on firefighters' occupational exposure to PM. Among the 15 studies published during the last decade, 53% were conducted in USA (California, Colorado, Florida, Ohio, Oregon, South Carolina, and Washington), 27% in Australia and the other 20% in Portugal. Overall, total PM ranged between 0.92 mg/m³ for line and mop-up firemen during a wildfire combat (Gaughan et al., 2014a) to 8.64 mg/m³ during the initial fire attack of a prescribed burn (Reinhardt and Ottmar 2010). In addition, Baxter et al. (2014) and McNamara et al. (2012) reported number concentrations of total PM, PM_{2.5}, PM_{1.0-2.5}, PM_{0.5-1.0}, and PM_{0.3-0.5} rather than mass concentration (Table 7.2). Concerning fine particles, firefighters were exposed to respirable PM ranging from 0.02 mg/m³ (Reisen et al., 2011) to a maximum of 280 mg/m³ during Australian prescribed burns (Reisen et al., 2006). Occupational exposure of firefighters to PM_{2.5} ranged between 0.005 mg/m³ (base camp; McNamara et al., 2012) and 2.67 mg/m³ (prescribed burns; Neitzel et al., 2009). Regarding occupational exposure, NIOSH defined a permissible exposure limits (PEL) of 15 and 5 mg/m³ for total and respirable particles, respectively, not otherwise regulated (Table 7.1). Considering these guidelines, studies conducted in Australia that assessed firefighters exposure during prescribed burns (Reisen and Brown 2009; Reisen et al. 2006), as well as those of fuel reduction, slash or heap burns, experimental and prescribed burns, and wildfires (Reisen et al., 2011) presented concentrations higher than the established PEL for respirable particles. NIOSH also pointed out that dust from various types of wood (usually known as wood dust) is a potential occupational carcinogen. Consequently, NIOSH defined a recommended time-weighted average (TWA) exposure limit of 1 mg/m³ (NIOSH 2007).

Table 7.1. Summary of the existent guidelines for PM and PAHs occupational exposure limits (TLV: threshold limit value; TWA: time-weighted average for a normal 8-hours workday and a 40-hours workweek; IDLH: immediately dangerous for life or health concentration; REL: recommended airborne exposure limit averaged over a 10-hours work shift; PEL: permissible airborne exposure limit averaged over an 8-hours work shift; STEL: short-term exposure limit over a period of 15-minutes of exposure). Values are expressed in mg/m³ unless indicated otherwise.

Pollutant	Occupational exposure	
	Averaging time	Limit value
Particles not otherwise regulated*	8 h workshift of a 40 h workweek	OSHA PEL: TWA 15 (total particulate) ^(a)
	8 h workshift of a 40 h workweek	OSHA PEL: TWA 5 (respirable fraction) ^(a)
		TLV: TWA 0.2 ^(b,c)
Coal-tar pitch volatiles**	8 hours of a working day	NIOSH REL: 0.1 ^(c)
	10 hours of a work shift	As low as possible ^(d)
Polycyclic aromatic hydrocarbons	Maximum excursion	ACGIH TLV: 1 ^(b)
	Permitted excursion	ACGIH: 0.6 ^(b)
	30 minutes	IDLH: 1310 (250 ppm) ^(a)
	8 hours of a working day	NIOSH REL/OSHA PEL: TWA 50 (10 ppm) ^(a,b,c,e)
	15 minutes	NIOSH REL: STEL 75 (15 ppm) ^(a,b,e)
	15 minutes	OSHA PEL: STEL 11 ^(d)
Naphthalene	15 minutes	NIOSH REL/ACGIH TLV: STEL 75 ^(d)

* Particles not otherwise regulated corresponds to all PM, except PM₁₀ and PM_{2.5}

** Extractable as benzene soluble fraction of total particulate containing benz[a]anthracene, benz[b]fluoranthene, chrysene, anthracene, benzo[a]pyrene, phenanthrene, acridine, and pyrene.

(a) US National Institute for Occupational Safety and Health (NIOSH 2007)

(b) American Conference of Governmental Industrial Hygienists (ACGIH 2012)

(c) Agency for Toxic Substances and Disease Registry (ATSDR 2015)

(d) Fent et al. (2013)

(e) International Agency for Research on Cancer (IARC 2010)

Table 7.2. Review of firefighters' occupational exposure to PM (mean $\pm$ SD or range, expressed as mg/m³) during fire combat activities.

Continent	Country; city	Note	PM _{2.5}	Other PM fractions	Total PM	Reference
Europe	Portugal; Gestosa	Experimental forest fire	0.18 – 0.74			Miranda et al. (2011)
		Experimental forest fire, maximum daily average	0.47			Miranda et al. (2009)
		Experimental forest fire	0.12 – 2.2			Miranda et al. (2010)
North America	USA; California, Florida	Prescribed burns		Respirable PM _{3.5}	Total suspended particles	Reinhardt and Ottmar (2010)
		Overall shift				
		Initial attack; peak		0.022; 1.56	1.39; 1.81	
		Project fires; peak		0.50; 2.30	1.47; 4.17	
		Prescribed burns; peak		0.63; 6.90	–	
		At fires				
		Initial attack; peak		1.11; 2.46	5.32; 8.64	
		Project fires; peak		0.72; 2.93	1.72; 4.38	
		Prescribed burns; peak		1; 10.5	–	
		By work task				
USA; Cincinnati, Ohio		Burn boss		1.32		
		Lighting		0.75		
		Holding		1.56		
		Holding boss		1.81		
		Direct attack		4.04		
		Mop up		0.75		
		Sawyer		2.93		
		Engine		1.37		
		Metropolitan fire station A	0.02 – 0.05		4.67 – 69.29*	Baxter et al. (2014)
		Metropolitan fire station B	0.02 – 0.15		3.03 – 93.69*	
USA; Colorado		Overhaul scenes at live fire events	0.25 – 17.53		95.68 – 139.41*	
		Control environment	0.005		6.37 – 9.28*	
		Wildfire crew corporation				Gaughan et al. (2014a)
		Fire line construction			Total particulate	
		Mop-up			2.28 ± 1.43 (1.16 – 3.93)	
					1.86 ± 1.88 (0.92 – 3.12)	

*number of particles expressed as number/cm³

Table 7.2. Review of firefighters' occupational exposure to PM (mean $\pm$ SD or range, expressed as mg/m³) during fire combat activities (continued).

Continent	Country; city	Note	PM _{2.5}	Other PM fractions	Total PM	Reference
North America	USA; Colorado	Wildfire crew corporation Fire line construction			Respirable particles 0.45 ± 1.62 (0.24 – 0.88)	Gaughan et al. (2014a)
		Mop-up			0.38 ± 1.92 (0.20 – 0.72)	
		Firefighter job			Total particulate	
		Lead			1.16	
		Line			1.99 ± 1.51 (0.92 – 3.12)	
		Sawyer			2.80 ± 1.55 (1.71 – 3.93)	
		Swamper			2.27 ± 1.28 (1.78 – 2.93)	
					Respirable particles	
		Lead			0.88	
		Line			0.35 ± 1.58 (0.20 – 0.72)	
USA; Savannah River Site	USA; Savannah River Site	Swamper			0.41 ± 1.87 (0.22 – 0.78)	Adetona et al. (2013, 2011)
		Swamper			0.40 ± 1.23 (0.33 – 0.49)	
		Prescribed burn days				
		2003	0.21 (0.15 – 0.30)			
		2004	0.25 (0.18 – 0.33)			
		2005	0.35 (0.26 – 0.46)			
		Non-prescribed burn days				
		2003	0.023 (0.013 – 0.043)			
		2004	0.018 (0.012 – 0.027)			
		2005	0.012 (0.009 – 0.015)			
USA; Washington, Oregon, California	USA; Washington, Oregon, California	Prescribed burns	0.65 (0.29 – 1.31)			Hejl et al. (2013)
		Prescribed burns	1.20 ± 0.61 (0.63 – 2.67)			
		Base camps near wildfires				McNamara et al. (2012)
		Day – base camp personnel				
		Site 1 – Washington	0.017 ± 0.014			
		Site 2 – Washington	0.005 ± 0.003			
		Williams Creek	0.011 ± 0.005			
		Site 1 – La Brea	0.037 ± 0.015			
		Site 2 – La Brea	0.025 ± 0.003			

*number of particles expressed as number/cm³

Table 7.2. Review of firefighters' occupational exposure to PM (mean $\pm$ SD or range, expressed as mg/m³) during fire combat activities (continued).

Continent	Country; city	Note	PM _{2.5}	Other PM fractions	Total PM	Reference
North America	USA; Washington, Oregon, California	Night – base camp personnel and firefighters				McNamara et al. (2012)
		Site 1 – Washington	0.025 $\pm$ 0.014			
		Site 2 – Washington	0.011 $\pm$ 0.007			
		Williams Creek	0.012 $\pm$ 0.005			
		Site 1 – La Brea	0.044 $\pm$ 0.007			
		Site 2 – La Brea	0.036 $\pm$ 0.015			
		Base camps near wildfires				
		Site 1 – Washington		PM _{0.3–0.5}		
		Site 2 – Washington		14.04 (13.59 – 14.52)*		
		Williams Creek		12.47 (12.08 – 12.88)*		
		Site 1 – La Brea		9.24 (8.93 – 9.57)*		
		Site 2 – La Brea		82.75 (81.11 – 84.42)*		
				56.95 (56.08 – 57.84)*		
				PM _{0.5–1.0}		
		Site 1 – Washington		1.41 (1.36 – 1.46)*		
		Site 2 – Washington		0.84 (0.81 – 0.87)*		
		Williams Creek		0.57 (0.55 – 0.58)*		
		Site 1 – La Brea		10.34 (10.14 – 10.53)*		
		Site 2 – La Brea		4.90 (4.77 – 5.02)*		
				PM _{1.0–2.5}		
		Site 1 – Washington		0.30 (0.29 – 0.32)*		
		Site 2 – Washington		0.08 (0.08 – 0.08)*		
		Williams Creek		0.10 (0.09 – 0.10)*		
		Site 1 – La Brea		1.85 (1.79 – 1.90)*		
		Site 2 – La Brea		0.77 (0.74 – 0.81)*		

*number of particles expressed as number/cm³

Table 7.2. Review of firefighters' occupational exposure to PM (mean $\pm$ SD or range, expressed as mg/m³) during fire combat activities (continued).

Continent	Country; city	Note	PM _{2.5}	Other PM fractions	Total PM	Reference
Oceania	Australia; Victoria, Tasmania, Queensland, Northern, Western and Southern territory	Fuel reduction burns, daily maximum value			Respirable particles 0.07 – 16.00 (3.1 – 154)	Reisen et al. (2011)
		Experimental burns, daily maximum value			0.02 – 1.76 (4.5 – 86)	
		Slash or heap burns, daily maximum value			0.11 – 1.42 (3.5 – 26.5)	
		Prescribed burns, daily maximum value			0.02 – 16.00 (3.1 – 154)	
		Victorian wildfires, daily maximum value			0.02 – 1.46 (0.3 – 119)	
	Australia; Victoria, Tasmania, South and Northern territory	Prescribed and experimental burns			Respirable particles 0.09 – 4 3.5 – 110	Reisen and Brown (2009)
		daily maximum concentrations				
		Prescribed burns			Respirable particles 0.2 – > 9.0 8 – 280	
		daily maximum concentrations				
	Australia, Western	Smoke chamber burns			10.0 $\pm$ 4.10	De Vos et al. (2009)

*number of particles expressed as number/cm³

7.1.2 Firefighters occupational exposure to PAHs

Studies reported by Becker et al. (2006), Genualdi et al. (2009) and Lammel et al. (2013) enhanced the trans-boundary behaviour of PAHs released during forest fires. In addition, other studies focused their work in the estimation of PAH emissions due to the occurrence of forest fires (Alves et al., 2010; Andreae and Merlet 2001; Anttila et al., 2008; Galarneau et al., 2007; Garcia-Hurtado et al., 2014; Lee et al., 2005; Persson and Simonson 2007; Vicente et al., 2011; Vila-Escalé et al., 2007; Yuan et al., 2008). UNECE pointed that for the purpose of PAHs emission inventories, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene should be the four indicator compounds (UNECE 2015).

Occupational exposure to PAHs occurs via inhalation, and through dermal absorption as a result of either direct skin contamination or via clothing and undergarments. Since fire occurrences are usually unpredictable, assessment of firefighters' exposure to PAHs released during fire combat activities is very difficult.

The ACGIH and the Agency for Toxic Substances and Disease Registry (ATSDR) defined that occupational exposure to PAHs can be evaluated through the concentration of coal-tar pitch volatiles (which consists in an extractable benzene soluble fraction of total particulates that includes benzo[a]anthracene, benzo[b]fluoranthene, chrysene, anthracene, benzo[a]pyrene, phenanthrene, acridine and pyrene; ACGIH 2012, ATSDR 2015) (Table 7.1). NIOSH recognizes coal-tar pitch volatiles as potential occupational carcinogens as defined by OSHA carcinogen policy (CDC 2015). Also ACGIH states that, for any substance with no numerical threshold limit value (but designated as a suspected human carcinogen), worker exposure by all routes should be carefully controlled to levels as low as possible (ACGIH 2010).

7.1.2.1 Air exposure

Among the thousand studies concerning PAHs, only few concern firefighters' occupational exposure. Only two studies were found regarding exposure at fire stations (Baxter et al., 2014; Shen et al., 2015); on-duty firefighters work and live at fire stations when they are not actively suppressing fires. One study was focused on dust samples collected from different vacuum cleaner bags, which were used to routinely clean twenty Californian fire stations (Shen et al., 2015). Median total PAHs concentration in dust was 4124 ng/g. Pyrene was the predominant PAH measured in these dust samples (1040 ng/g), followed, in decreasing order, by benzo[ghi]perylene (688 ng/g),

fluoranthene (662 ng/g), and chrysene (547 ng/g) (Shen et al., 2015). The latter study (Baxter et al., 2014) was conducted in three different environments, including two metropolitan fire stations located in Cincinnati, USA. Air monitoring was performed in the fire truck bay, equipment storage area, the kitchen/common area, and in the sleeping quarters during a period of 8-hours on a single day. Among the seventeen screened PAHs, only naphthalene was found in one fire station, with mean concentrations of $9.22 \mu\text{g}/\text{m}^3$ and $9.24 \mu\text{g}/\text{m}^3$ at the kitchen and truck bay, respectively (Baxter et al., 2014). The detectable concentrations of naphthalene were well below the guideline of $50 \text{ mg}/\text{m}^3$ designated by the NIOSH (Table 7.1) but were in a close range to the guideline of $10 \mu\text{g}/\text{m}^3$ (annual mean) established by the WHO for an acceptable IAQ (WHO 2010).

Regarding indoor fires (i.e., in buildings), smoke divers are the first rescue force to go inside and try to find possible victims and the initial point of the fire. In addition, their work allows finding the more adequate fire extinction procedures (Laitinen et al. 2010, 2012). In many countries active firefighters need to update their smoke diving licence annually. To achieve that, firefighters need to participate in different and real fire episodes under controlled conditions, which include smoke diving simulators. IARC has estimated that during their training operations firefighters are exposed to mean concentrations of $10.68 \text{ mg}/\text{m}^3$ of total PAHs and to $2.19 \text{ mg}/\text{m}^3$ of naphthalene (IARC 2010). In 2012, Laitinen and colleagues (2012) performed an experimental assay to evaluate firefighters' exposures to 15 different PAHs in different smoke diving simulators; the authors found that conventional simulators presented much higher concentrations of PAHs than the modern ones. Globally, modern simulators caused higher concentrations of naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene while the levels of benz[a]anthracene, chrysene, benzo[b+k]fluoranthene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene were lower in comparison with the conventional simulators (Laitinen et al., 2012). In addition, based on the ratio between the concentrations of pyrene and benzo[a]pyrene, authors concluded that artificial smoke is not so dangerous as real smoke (1.9 in the fire house simulator *versus* 9 in the gas simulator) (Laitinen et al., 2010). Table 7.3 presents the available studies concerning firefighters' occupational exposure through the air during the firefighting combat activities; only studies that presented directly values for PAHs occupational exposure during fire combats were included. With the exception of the Australian study described by Kirk and Logan (2015a), the majority of other studies found in the literature were conducted in USA. Since the Southern European countries are strongly affected every year by intense and severe forest fires, studies regarding

this geographical area are needed. PAHs with low molecular weight, namely naphthalene, phenanthrene, acenaphthylene, and fluorene were the most abundant ones during firefighting activities (Table 7.3). Despite the high reported concentrations of naphthalene during firefighting (from 13 to 1300 $\mu\text{g}/\text{m}^3$ for live fire training scenarios (Kirk and Logan 2015a), between 3.2 to 8.1 $\mu\text{g}/\text{m}^3$ during prescribed burns (Robinson et al., 2008), and from 170 to 2100 $\mu\text{g}/\text{m}^3$ for vehicle fires (Fent and Evans 2011), its international occupational exposure limits were never reached, except for the IARC reported concentrations during fire scene investigations which exceeded the recommended and short-term exposure limits (Table 7.1).

In majority of the reported studies (Table 7.3) firefighters wore SCBA, which provides a complete respiratory and skin protection. In addition, the intensity of exposures to chemical vapours from outdoor fires depends on the direction and speed of the wind. Thus the measured PAH concentrations do not directly correspond to the air inhaled by firefighters during fire combat activities but describe the ambient concentrations outside firefighters' personal ensemble. However, it gives a clearly range of PAH concentrations for those fire scenarios when firefighters do not use that protection. To fill that gap, a recent study evaluated the concentrations of PAHs inside and outside the instructors' structural firefighting ensembles during training evolutions and found that PAH levels ranged from 430 to 2700 $\mu\text{g}/\text{m}^3$ outdoors while the concentrations inside ensembles varied between 32 and 355 $\mu\text{g}/\text{m}^3$ (Kirk and Logan 2015a). Also, a health hazard evaluation program was performed in order to determine if airborne PAHs generated during live fire training would contaminate and pass through the respiratory and ensembles protective devices of fifteen American firefighters during their intervention in structural fires (Fent et al., 2013). Air samples were collected before and after each burn. Personal air concentrations of total PAHs were higher during the first round than in the second one, being lower than those found in the area air concentrations and total particle-phase PAHs measured during the fire and knockdown phases of all burns (Fent et al., 2013). Authors observed that total PAH concentrations were well above the ACGIH excursion limit of 1000 $\mu\text{g}/\text{m}^3$ for coal-tar pitch volatiles (Table 7.1) during the overhaul in two of the three burns (2600 $\mu\text{g}/\text{m}^3$ at burn 2 and 6000 $\mu\text{g}/\text{m}^3$ at burn 3 of round 1) (Fent et al., 2013).

Table 7.3. Review of firefighters' occupational exposure to PAHs (mean $\pm$ SD or range, expressed as $\mu\text{g}/\text{m}^3$ unless indicated otherwise) during fire combat activities (continued).

Continent	Country; city	No firefighters	Notes	No PAHs	PAHs	Reference
North America	USA, Phoenix		Overhaul during structure fires (at least 20-minutes)		B[a]A: 24.9 ± 4.9 (19 – 28) B[b]FIn: 22.3 ± 10.6 (10 – 34) B[k]FIn: 23.8 ± 1.7 (23 – 25) B[a]P: 33.2 ± 13.6 (19 – 50) Chry: 12.9 DB[a,h]A: 45.5 ± 31.6 (23 – 68) Ind: 19.5 ± 8.4 (14 – 29)	Bolstad-Johnson et al. (2000)
		84	Wildfire suppression and prescribed burn management		Ant: 0.061 (0.005 – 0.152) B[a]A: 0.015 (nd – 0.034) B[a]P: 0.012 (nd – 0.034) B[b]FIn: 0.018 (nd – 0.120) B[k]FIn: 0.004 (nd – 0.014) B[ghi]P: 0.022 (nd – 0.032) Chry: 0.025 (nd – 0.080) Fin: 0.048 (nd – 0.101) Ind: 0.016 (nd – 0.042) Phen: 0.257 (0.013 – 0.869) Py: 0.075 (nd – 0.110)	
Oceania	Australia; Queensland		Live Fire Training Scenarios Personal air – outside ensembles With SCBA (20 to 44-minutes of exposure)	16	Naph: 73 – 1300 Ace: 83 – 390 Ace: 8 – 37 Flu: 27 – 120 Phen: 120 – 490 Ant: 24 – 110 Fin: 33 – 180 Py: 33 – 180	Kirk and Logan (2015a)

Naphthalene (Naph), acenaphthylene (Acen), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fin), pyrene (Py), benz[a]anthracene (B[a]A), chrysene (Chry), benzo[b]fluoranthene (B[b]FIn), benzo[k]fluoranthene (B[k]FIn), benzo[a]pyrene (B[a]P), benzo[ghi]perylene (B[ghi]P), dibenz[a,h]anthracene (DB[a,h]A), and indeno[1,2,3-cd]pyrene (Ind).
 a – median values
 nd – not detected.

Potentially carcinogenic PAHs accounted for more than 30% of the total PAHs measured in both rounds. Among those, naphthalene was the most variable, ranging from 1 to 51% of the total composition. Additionally, chrysene, indeno[1,2,3-cd]pyrene, benzo[b]fluoranthene, benz[a]anthracene, and benzo[a]pyrene presented relevant contributions, particularly at the first round. Benzo[a]pyrene contributed with less than 6% of the total PAHs.

The total concentrations of coal-tar pitch volatiles, namely benz[a]anthracene, benzo[b]fluoranthene, chrysene, anthracene, benzo[a]pyrene, phenanthrene, and pyrene reported by Kirk and Logan (2015a) as well as the total PAH concentrations by Fent et al. (2014) and Bolstad-Johnson et al. (2000) exceeded those reference levels (0.1 and 0.2 mg/m³, Table 7.1). The permitted (0.6 mg/m³) and maximum (1 mg/m³) excursion limits defined by ACGIH (Table 7.1) were also exceeded for total PAHs during suppression and overhaul of controlled structure burns (Fent et al., 2014), during a cabin and an engine fires for naphthalene (Fent and Evans 2011), and for naphthalene and the maximum range of total PAHs detected in personal firefighters air outside ensembles in live fire training scenarios (Kirk and Logan 2015a).

Only three studies reported concentrations of benzo[a]pyrene: mean of 0.012 µg/m³ (maximum of 0.034 µg/m³) for American firefighters in wildfire suppression and prescribed burn management (Booze et al., 2004); ranged between 1.8 and 47 µg/m³ and from 0.4 to 13 µg/m³ outside and inside personal ensembles, respectively, of Australian instructor firefighters (Kirk and Logan 2015a); and mean concentration of 33.2 µg/m³ (19 – 50 µg/m³) during overhaul operations of 25 structure fires (Bolstad-Johnson et al., 2000). Benzo[a]pyrene concentrations found at live fires, including inside the personal firefighters ensembles, were well above the ambient air guideline of 1 ng/m³ (Directive 2004/107/EC) (no specific occupational guideline is established for benzo[a]pyrene). Fent and co-workers (2014) characterized exposition of firefighters to PAHs during suppression and overhaul of controlled structure burns; benzo[a]pyrene and dibenzo[ah]anthracene accounted with 1.6 to 6.1%, and 1.2 to 3.3% of the total PAHs, respectively. The remaining possible carcinogenic PAHs, namely benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, indeno[1,2,3-cd]pyrene, and naphthalene, corresponded with to 21 to 43% of the total PAHs. Globally possible, probable and carcinogenic PAHs accounted for 26 to 50% of total PAHs (Fent et al., 2014).

Exposition of firefighters (and of the other people present in the proximity of fire fighting area, e.g., police forces, etc.) to high concentrations of PAHs, particularly to benzo[a]pyrene, may occur as a consequence of not wearing appropriate and/or in

good conditions breathing protection devices. Among all firemen, wildland firefighters are those exposed to more unpredictable and uncontrolled fire scenarios and usually they do not use SCBA. Also, firefighters commonly do not wear SCBA when responding to vehicle fires because those fires tend to be suppressed within minutes and inhalation exposures are assumed to be minimal, donning a SCBA takes time and is cumbersome to wear, and also due to the belief that breathing air apparatus should be saved for more intense fires when it is really needed (Fent and Evans 2011). Furthermore, SCBA is also frequently removed during the overhaul phase of all fire combat activities and, since PAHs are products of incomplete combustion, this phase may be the most important one causing high PAHs exposure.

Firefighters' exposures include not only gases, but also smoke particles that may be inhaled or contaminate the skin or clothing, with subsequent absorption of chemicals through the skin at the fire scene or at later times through the direct contact with contaminated and uncleaned firefighting ensembles (Fabian et al., 2014). Structural firefighting ensembles are designed to provide a limited degree of protection from thermal radiation, hot gas convection, and direct contact with hot surfaces, as well as protection against minor cuts and abrasions. However, those ensembles are not specifically designed to provide protection against chemical and/or biological agents. So far it is unknown how well turnout gear protects from a dermal exposure to PAHs and related compounds. Regarding this topic, limited studies were found (Alexander and Baxter 2014; Fabian et al., 2014; Fent et al., 2013; Laitinen et al., 2010; Kirk and Logan 2015a, 2015b). The ranking of the median post exposure levels by each anatomical site considered by the study conducted by Fent et al. (2013) were, in descending order: neck > face > hand $\approx$ arm. Authors observed that during exercises hoods became untucked from the turnout gear jacket, exposing firefighter's neck to fire contaminants. Additionally, Fent et al. (2013) measured much higher post-exposure PAH levels on the exterior of the helmets, respirators, and pants as compared to the interior of the sleeves. Authors also found much higher pre-exposure PAH levels on the exterior of the helmets, which were not required to be cleaned before the next burn. Additionally, firefighter's gloves and hoods were collected by Fabian and co-workers (2014) as part of their standard turnout ensemble at regular intervals for analysis of accumulated combustion residues. Concentrations of organic compounds, including PAHs, were approximately 100 times greater for gloves than for the hoods. Benz[a]anthracene, benzo[a]pyrene, chrysene (confirmed animal carcinogen with unknown relevance to humans; USEPA-group A3), and dibenz[a,h]anthracene (IARC group 2A-probably carcinogenic to humans) were found in at least three of eight used

gloves, still none of the concentrations exceeded 0.02 µg/g glove (Fabian et al., 2014). Without much detailed information regarding the protection, a total of 20 face and neck wipes were collected from 10 American firefighters following five different fire events (Baxter et al., 2014). Benzo[b]k]fluoranthenes and pyrene were the most abundant PAHs, being present in 65% and 30% of the wipe samples, respectively. However benz[a]anthracene, chrysene, fluoranthene, phenanthrene, benzo[a]pyrene, and benzo[e]pyrene were also found (Baxter et al., 2014).

Globally studies suggest that contaminants accumulate in firefighter's protective clothing over time. Little information is available about firefighters clothing and respective ensembles cleaning. It is believed that some fire departments centrally maintain protective clothing; still many other firefighters are probably responsible for the maintenance of their own cloths and equipment. Many volunteer firefighters often keep their clothes into their personnel vehicles until the use and wash them from times to times at home with the rest of the family laundry. These practices strongly contribute to long-term exposure to hazardous chemicals and allow the spread of contamination outside the fire ground (Stull et al., 1996). The study performed by Kirk and Logan (2015b) assessed the off-gassing of PAHs from ensembles post-fire and after laundering of those ensembles according to manufacturer specifications, in order to quantify potential firefighter inhalation exposures after storage of contaminated structural firefighting ensembles. Authors found that laundering returns ensembles with off-gassing concentrations to pre-exposure levels. Thus storage of unlaundered structural firefighting ensembles, in small kit bags and/or unventilated storage lockers immediately after use, may create a source of future exposure (by inhalation and dermal absorption) to toxic combustion products for firefighters and other individuals that come into direct contact with the firefighter personal protection garments and equipment (Fabian et al., 2014; Kirk and Logan 2015b).

7.1.2.2 Biomonitoring

Biomonitoring of firefighters is an effective assessment tool that can be used to further understand exposures and evaluate the effectiveness of worker protection strategies. Exposure biomarkers provide a key tool to relate health outcomes to individual personal exposures. Biomonitoring comprise a wide range of measurements of metabolites in different body fluids such as exhaled breath, plasma, blood, and urine. Exhaled breath and urine are excellent matrices for determining internal PAHs exposure due to the non-invasiveness and ease of collecting samples.

The most relevant studies concerning firefighter's biomonitoring through the assessment of OH-PAHs contents in urine samples are presented in Table 7.4. 1OHPy followed by 1OHNaph are the more investigated compounds; only one study reported concentrations of other metabolites, namely several OHPhen compounds in firefighter's urine (Edelman et al., 2003). No study concerning firefighters occupational exposure included the analysis of 3OHB[a]P. The comparison of the reported levels is extremely difficult since, in the majority of the studies, concentrations are not adequately normalized with the personal urinary creatinine levels. The most complete study concerns the New York City Firefighters responding to the World Trade Center (WTC) fire and collapse (Edelman et al., 2003). Special operations command firefighters presented 1OHPy (159 ng/L *versus* 77.9 ng/L *versus* 62.5 ng/L), 1-, 2-, and 3-OHPhen (248 ng/L *versus* 164 ng/L *versus* 158 ng/L; 211 ng/L *versus* 147 ng/L *versus* 119 ng/L; 214 ng/L *versus* 145 ng/L *versus* 127 ng/L, respectively) in greater amounts than in other responding firefighters and controls, respectively (Table 7.4). Other two field studies were published concerning firefighters from Canada, Ontario (Caux et al., 2002) and USA, Arizona (Robinson et al., 2008). Firefighters participating at both knockdown and overhaul fire activities presented 1OHPy concentrations up to ten times higher (80 – 3630 nmol/mol creatinine) than firefighters participating exclusively at knockdown or overhaul (50 – 340 nmol/mol creatinine) (Caux et al., 2002). The levels of 1OHPy and 1OHNaph were also characterized in trainees' urine samples during diving and gas simulator sessions (Laitinen et al., 2010). The highest 1OHPy levels (0.2 – 7.3 nmol/L and 1.7 – 4.4 nmol/L) were measured when conifer plywood and chipboard was burned at smoke diving simulators, respectively. Those authors (Laitinen et al., 2010) recommended a limit value of 16 nmol/L of 1OHPy in order to reduce risks in the fire house and container simulator training sessions (Bouchard and Viau 1999; Jongeneelen 2004; Laitinen et al., 2010).

The British Health and Safety Executive, based on many measurements in different British companies in various industries with good industrial hygiene practices, defined an hygiene based Biological Monitoring Guidance Value for 1OHPy of 4.0 µmol/mol creatinine in post-shift samples (HSE EH40 2011). Whenever 1OHPy biological guideline is exceeded, it indicates that investigation of current occupational hygiene measures and work practices is necessary. The Biological Exposure Index Committee of the ACGIH stated that the presence of urinary 1OHPy above a benchmark level of about 1 µg/L (0.5 µmol/mol) indicates occupational exposure to PAHs, since very few non-occupationally exposed persons, smokers or non-smokers, will excrete this amount of 1OHPy (ACGIH 2010).

Table 7.4. PAHs firefighters' biomonitoring, before and after fire combat activities, through assessment of hydroxyl-PAHs contents in urine samples (mean $\pm$ SD or range, expressed as nmol/L unless indicated otherwise).

Continent	Country; city	No firefighters	Note	Hydroxyl-PAHs	Reference
Europe	Finland; Kuopio		Diving simulators		Laitinen et al. (2010)
			Fire house, chipboard		
			Before exposure	1OHPy: 1.7	
			immediately after exposure	1OHPy: 4.4	
North America	Canada, Ontario	43	6-hour after the end of exposure	1OHPy: 4.3	Caux et al. (2002)
			Next morning	1OHPy: 2.1	
			Fire house, conifer plywood board		
			Before exposure	1OHPy: 0.8	
	USA, Arizona	18	immediately after exposure	1OHPy: 5.1	Robinson et al. (2008)
			6-hour after the end of exposure	1OHPy: 0.2	
			Next morning	1OHPy: 7.3	
			Fire house, pure spruce and pine wood		
	USA, New York	318	Before exposure	1OHPy: 0.6; 1OHNaph: 43	Edelman et al. (2003); IARC (2010)
			immediately after exposure	1OHPy: 0.8; 1OHNaph: 135	
			6-hour after the end of exposure	1OHPy: 1.5; 1OHNaph: 135	
			Next morning	1OHPy: 1.2; 1OHNaph: 48	
North America	France; Paris		Gas simulator		Laitinen et al. (2010)
			Before exposure	1OHPy: 0.6; 1OHNaph: 30	
			immediately after exposure	1OHPy: 0.9; 1OHNaph: 45	
			6-hour after the end of exposure	1OHPy: 1.2; 1OHNaph: 65	
	Canada, Ontario	43	Next morning	1OHPy: 1; 1OHNaph: 40	Caux et al. (2002)
			Participation at knockdown and overhaul	1OHPy: 80 – 3630 nmol/mol creatinine	
			Participation at knockdown	1OHPy: 50 – 190 nmol/mol creatinine	
			Participation at overhaul	1OHPy: 110 – 340 nmol/mol creatinine	
	USA, Arizona	18	Baseline	1OHPy: 0.14 $\pm$ 0.19 (<0.01 – 0.56) μ g/L	Robinson et al. (2008)
			End-of-shift	1OHPy: 0.09 $\pm$ 0.12 (<0.01 – 0.50) μ g/L	
			Next morning	1OHPy: 0.05 $\pm$ 0.12 (<0.01 – 0.53) μ g/L	
			Present at World Trade Center collapse	1OHPy: 110 ng/L	
1-hydroxypyrene (1OHPy), 1-hydroxynaphthalene (1OHNaph), 1-, 2-, 3-hydroxyphenanthrene (1, 2, 3OHPen)	USA, New York	318		1OHPen: 197 ng/L	Edelman et al. (2003); IARC (2010)
				2OHPen: 163 ng/L	
				3OHPen: 168 ng/L	

Table 7.4. PAHs firefighters' biomonitoring, before and after fire combat activities, through assessment of hydroxyl-PAHs contents in urine samples (mean $\pm$ SD or range, expressed as nmol/L unless indicated otherwise) (continued).

Continent	Country; city	No firefighters	Note	Hydroxyl-PAHs	Reference
North America	USA, New York	318	Present after World Trade Center collapse	10HPy: 113 ng/L	Edelman et al. (2003); IARC (2010)
				1OHPhen: 206 ng/L	
				2OHPhen: 191 ng/L	
				3OHPhen: 185 ng/L	
			Special operation command	10HPy: 159 ng/L	
				1OHPhen: 248 ng/L	
				2OHPhen: 211 ng/L	
				3OHPhen: 214 ng/L	
			Other firefighters	10HPy: 77.9 ng/L	
				1OHPhen: 164 ng/L	
				2OHPhen: 147 ng/L	
				3OHPhen: 145 ng/L	
			Control	10HPy: 62.5 ng/L	
				1OHPhen: 158 ng/L	
				2OHPhen: 119 ng/L	
				3OHPhen: 127 ng/L	

1-hydroxypyrene (10HPy), 1-hydroxynaphthalene (1OHNaph), 1-, 2-, 3-hydroxyphenanthrene (1, 2, 3OHPhen)

ACGIH also recommends that the benchmark should be considered as a post-shift level. Recently, Jongeneelen (2014) proposed an occupational level of 1.0 $\mu\text{mol/mol}$ creatinine in urine as state-of-the-art guidance which is valid when the PAH-profile in the work environment is similar to that of coke oven with a typical pyrene/benzo[a]pyrene ratio of 2.5 (Jongeneelen 2014). Whenever a deviating-PAH profile is observed, an adjustment procedure is recommended (Jongeneelen 2014). However, a risk based limit level of urinary 1OHPy has not yet been recommended since the results of epidemiological studies and/or animal studies with dose–response data of cancer are still lacking.

7.1.3 Firefighters' health risks

In general, firefighters' occupational exposure has been associated with excess morbidity and mortality. Cardiovascular disease is considered the leading cause of death in approximately 45% of firefighters and a major cause of their morbidity (Gaughan et al., 2014a, 2014b; Soteriades et al., 2011).

Weiden et al. (2013) performed a case–cohort study with never–smoker WTC exposed firefighters that presented a normal lung function before 9th September 2011. Within six months after the attack, cardiovascular disease (CVD) biomarker distribution was assessed; it was observed that WTC lung injury cases exhibited higher levels of all apolipoproteins, C-reactive protein and macrophage inflammatory protein-4. C-reactive protein constitutes one of the most studied biomarkers of inflammation in relation to CVD and events (Black et al., 2004). Gaughan et al. (2014b) evaluated serum cholesterol, biomarkers of systemic inflammation (high sensitivity C-reactive protein and fibrinogen), oxidative stress through the urinary 8-iso-prostaglandin F₂ α (8-isoprostane) and 8-hydroxy-2-deoxyguanosine oxidative DNA damage. Their results suggested that exposure to welding fume particulates is associated with acute adverse vascular responses. Gaughan et al. (2014b) also observed an association between cross-shift differences in oxidative DNA damage and recent firefighters occupational exposure to PM. PM also promotes effects on coagulation properties that lead to increased risk of stroke or myocardial infarction (WHO 2006). PM exposure may also result in endothelial and general vascular dysfunction; chronic exposure may increase the progression of atherosclerosis (WHO 2006). Exposure to PM is also known to increase emergency department attendances, exacerbation of symptoms, and increase use of therapy in asthma; ultimately PM also increases mortality in patients (Tham et al., 2009; WHO 2006).

In addition, it is well established that electrophilic compounds such as PAHs, and related compounds formed during combustion emissions, play a key role in environmental cancer; recent evidences associate their role in cardiovascular disease and adverse reproductive outcomes (Lewtas 2007). As a consequence high exposures to PAHs during intense and/or severe fire episodes may promote the development or aggravation of firefighters' cardiovascular illnesses. Some studies have indicated that exposure to high concentrations of naphthalene (possible human carcinogenic) may lead to health risks such as haemolytic anaemia, nephrotoxicity, ophthalmologic and dermal changes (Kamal et al., 2011). The US Department of Health and Human Services considers that naphthalene contributes considerably to human cancer risk (NTP 2000). This information is highly relevant since naphthalene (and its respective 1OHNaph) is one of the most reported and abundant PAHs during firefighting activities (Tables 7.3 and 7.4), including in the air inside firefighters' ensembles (Kirk and Logan 2015a).

PAHs exposure has been associated with epigenetic alterations, including genomic cytosine methylation. Both global hypomethylation and hypermethylation of specific genes have been associated with cancer and other diseases in humans (Herbstman et al., 2012). Alshaarawy et al. (2014) observed a positive association between urinary biomarkers of 1OHNaph, 2OHNaph, 2OHPhen and summed low molecular weight PAH biomarkers, with diabetes mellitus in US adults aging 20 to 65 years old. Based on mortality studies, reports on exposure and cancer incidence, Golka and Weistenhofer (2008) concluded that an elevated risk of urothelial cancer in firefighters is in general not confirmed. Only in professional firefighters who were severely exposed for decades, occupational exposure might be discussed as causative for urothelial cancer. Within its meta-analysis of cancer studies among firefighters, LeMasters et al. (2006) found a probable or possible increased risk for twelve types of cancers, including leukemia, skin, and testicular cancer, but not lung or bladder cancer. More recently and in the largest firefighter cancer study to this date, Daniels et al. (2013) found an increased incidence of eight types of cancers including lung and kidney cancer, but not leukemia, skin, or testicular cancer. More research is needed to clarify these contradictory results, and to better understand and quantify these cancer risks, as well as the role that chemical exposures may play. Thus, in this study, the levels of 18 PAHs (16 USEPA priority PAHs, dibenzo[a,l]pyrene, and benzo[j]fluoranthene recommended by EU (Directive 2004/107/EC)) were assessed in the breathing air zone of firefighters' during their work shift at Portuguese fire stations, and the firefighters' total internal dose by six biomarkers of exposure (1OHNaph,

1OHAce, 2OHFlu, 1OHPhen, 1OHPy, and 3OHB[a]P) in urine samples. The contribution of personal airborne individual (naphthalene, acenaphthene, naphthalene + acenaphthene, fluorene, phenanthrene, pyrene) and PAHs exposure to respective individual (1OHNaph + 1OHAce, 2OHFlu, 1OHPhen, and 1OHPy) and OH-PAHs excretion in urine was explored for the first time in firefighters.

7.2 Material and Methods

7.2.1. Characterization of the sampling sites

Portugal is one of the five Southern European countries that are the most affected by forest fires every year (JRC 2015), being the North and Centre regions the most disturbed areas (ICNF 2014). In 2014 the district of Bragança, in the sub-region of Alto Trás-os-Montes, (north of Portugal), was the third Portuguese district with a higher incidence of burnt area (ICNF 2014). This region is characterized by typically hot and very dry summers with absolute maximum temperatures exceeding the 30 °C; winters are usually long and cold with absolute minimum temperatures reaching values below 0 °C. Sampling was performed at eight different fire stations: Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC) (Figure 7.1). Two pairs of fire stations, namely MDL and TDC, as well as MRD and SDM were 22-23 km away from each other. The fire stations FEC, MDL, and MRD were located in the urban center of the city, near commercial shops and busy roads, while the firehouses TMC and TDC were situated in villages. FEC, MDL, and MRD were constructed before 1984 while the other fire stations were constructed and/or restored after 2007. At FEC, MDL and MRD, the truck bay where firefighters' and emergency vehicles were parked on had direct access to the operational control center and social room where firefighters spend most of their time (Figure 7.2a). The door that separated these two microenvironments was frequently opened. At fire stations TDC, SDM, TMC, VNH, and BRG, the control center and social room were indirectly (through corridors and with some divisions; Figure 7.2b) connected with the truck bay.

Information on outdoor meteorological conditions, namely T, RH, wind speed, precipitation, and solar radiation during the sampling campaigns were retrieved from the local meteorological station (Table 7.5). The concentrations of 24-h ambient PM₁₀ and PM_{2.5}, NO, NO₂ and nitrogen oxides (NO_x), sulphur dioxide (SO₂), and O₃ were

also monitored from the meteorological station during the sampling campaign (Table 7.5).

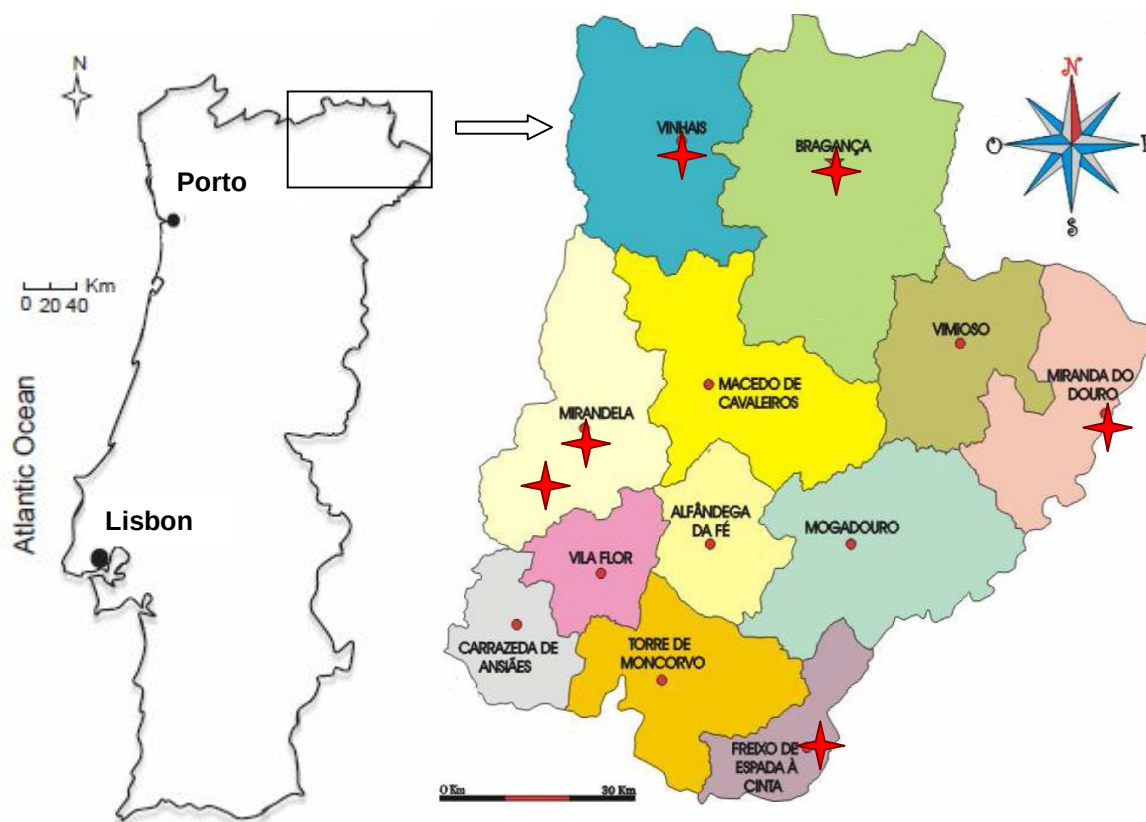
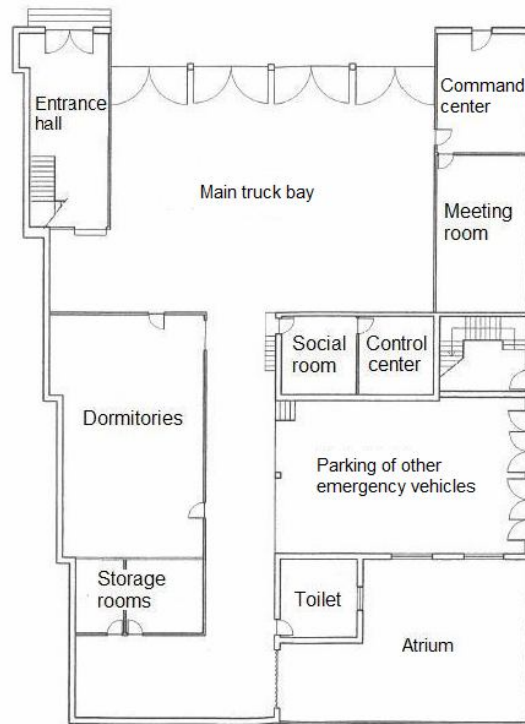


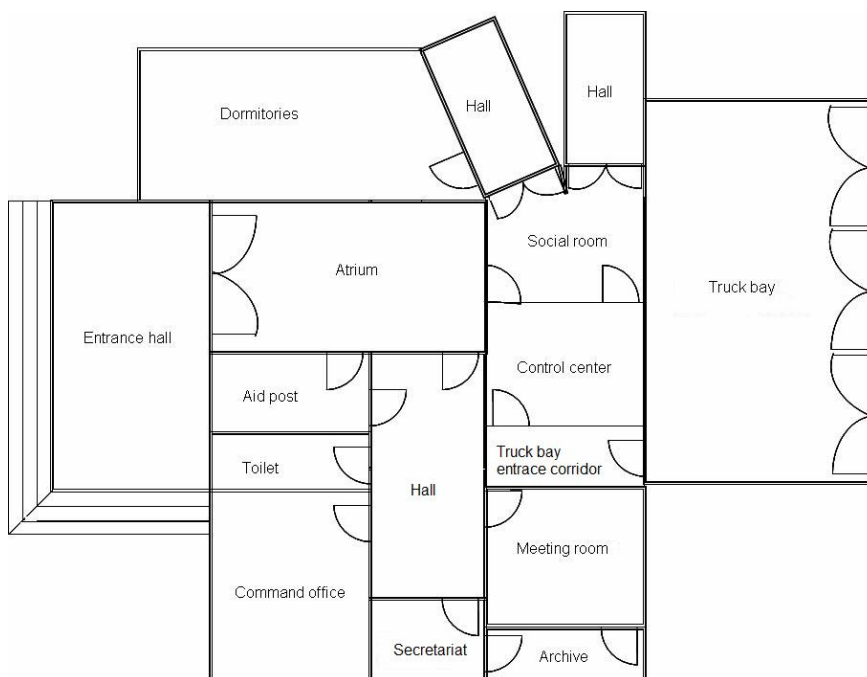
Figure 7.1. Geographical location of the monitored fire stations in the district of Bragança.

7.2.2. Personal air and urine sampling

The study population consisted of eight units of professional firefighters serving at six municipal cities (Figure 7.1). A structured questionnaire adapted from validated questionnaire (WHO 2002) was filled by each firefighter. The questionnaire collected information on gender, age, number of years as firefighter (Table 7.6), and factors reported to be associated with PAH exposures, namely smoking habits, and the most consumed meals (boiled, roasted, and grilled) during the five days before urine collection. Subjects were excluded from the study if they participated in firefighting activities within five days prior the sampling campaigns and if they were exposed to tobacco smoke.



(a)



(b)

Figure 7.2. Representative layout of: (a) Freixo de Espada à Cinta (FEC), Mirandela (MDL), and Miranda do Douro (MRD) fire stations; (b) Torre Dona Chama (TDC), Sendim (SDM), Torre de Moncorvo (TMC), Vinhais (VNH), and Bragança (BRG) fire stations.

Table 7.5. Characterization of the meteorological and outdoor air pollution conditions during the sampling campaigns.

Outdoor meteorological parameters	
Mean $\pm$ SD; (Min - Max)	Temperature: 20.1 ± 5.87 °C; (5.74 – 35.8 °C) Relative humidity: $58.1 \pm 17.7\%$ (16.8 – 91.5%) Wind speed: 1.91 ± 1.30 m s ⁻¹ (0.01 – 7.72 m s ⁻¹) Precipitation: 0.01 ± 0.11 mm (0 – 4.60 mm) Solar radiation: 243 ± 159 W m ⁻² (5 – 571 W m ⁻²)
Outdoor pollution parameters	
Mean (Min – Max)	PM ₁₀ : 17 (1.0–49) $\mu\text{g m}^{-3}$ PM _{2.5} : 3.9 (1–11) $\mu\text{g m}^{-3}$ SO ₂ : 3.0 (1.0–7.0) $\mu\text{g m}^{-3}$ NO: 1.5 (1.1–6.6) $\mu\text{g m}^{-3}$ NO ₂ : 4.2 (1.4–17) $\mu\text{g m}^{-3}$ NO _x : 6.5 (3.4–28) $\mu\text{g m}^{-3}$ O ₃ : 76 (18–175) $\mu\text{g m}^{-3}$

Table 7.6. Characteristics of firefighters that participated in the study.

Characteristic	
n	75
Gender	
Male (%)	84
Female (%)	16
Age (mean $\pm$ SD; min – max; years)	32.2 $\pm$ 6.9; 22 – 48
Man	31.8 $\pm$ 6.5; 22 – 48
Woman	34.8 $\pm$ 9.2; 22 – 44
Weight (mean $\pm$ SD; min – max; kg)	80.6 $\pm$ 11.8; 57 – 100
Man	82.8 $\pm$ 10.7; 67 – 100
Woman	65.7 $\pm$ 7.5; 57 – 70
Number of years as firefighter	
≤ 10 years (%)	40
10 – 20 years (%)	44
≥ 20 (%)	16

Based on the information collected through the individual questionnaires, PAHs intake through food ingestion was considered not significant. All participants read and signed informed consent forms approved by the Ethic Committee of University of Porto.

Personal exposures were monitored during a period of fifty-four days (June and July 2014) in firefighters that were not directly involved in firefighting activities. At each fire station, air sampling campaigns of 18 selected PAHs in PM_{2.5} were performed on a single day (6 individuals at SDM and FEC; 9 at MRD, MDL and TMC; 12 at TDC, VNH and BRG) and over a continuous 4-h period of a regular work shift inside the fire

station; duplicate samples per individual were collected. During the sampling campaign firefighters were encouraged to move freely at the fire station as in a normal working day. Air sampling was done by personal constant flow samplers (Gilian, models GilAir3 and ProValue3; Sensidyne, USA) that were placed at the waist of each firefighter in a way that could not disturb the regular activities; an air flow rate of 2 L/min was used. The inlets were positioned at the breathing zone of firefighters. Air samples were collected on PTFE membrane filters (2 µm porosity, Ø47 mm, SKC Ltd., United Kingdom). PM_{2.5} masses were determined gravimetrically according to the procedure described in section 3.2.3. After the sampling, filters were stored in a freezer (– 20 °C) before chemical analysis.

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

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

7.2.3. PAHs and OH-PAHs chromatographic analysis

Extraction and quantification of PAHs from PM_{2.5} filters, and OH-PAHs from urine samples were performed by previously validated analytical procedures (Castro et al., 2009, 2011; Chetianukornkul et al., 2006) and as described in detail in Chapter 4 for PAHs (sections 4.2.3-4.2.4) and in Chapter 5 for OH-PAHs (sections 5.2.2.2 and 5.2.3). All OH-PAH concentrations were normalized by urinary creatinine (mol/mol). Urinary creatinine measurements were performed according to the Jaffe colorimetric method (Kanagasabapathy and Kumari 2000). Each analysis was performed at least in triplicate.

7.2.4 Statistical analysis

Statistical analysis was performed using SPSS (IBM SPSS Statistics 20). PAHs and OH-PAHs median values were compared through the nonparametric Mann–Whitney U test, since normal distribution was not observed by Kolmogorov–Smirnov with Lilliefors correction and Shapiro–Wilk's tests. When the concentration

was below the LOD, the value of the respective LOD/ $\sqrt{2}$ was used (Hornung and Reed 1990). Statistical significance was defined as $p \leq 0.05$. Spearman correlation coefficients (r) were calculated to examine the relation between airborne individual and total PAH concentrations with firefighters' urinary individual and total OH-PAHs for each fire station.

7.3 Results and Discussion

7.3.1 Personal exposure monitoring

The concentrations of individual and Σ PAHs in the breathing air zone of firefighters working at each fire station are presented in Table 7.7. Among the 18 PAHs considered, acenaphthylene was the most abundant compound (except in the environmental exposures of the rural sites of TMC and TDC where it was not detected) with concentrations representing 46.4% (at FEC) to 61.5% (at SDM) of Σ PAHs. Acenaphthylene is a component of crude oil, coal tar, tobacco smoke and a product of combustion which may be produced and released to the environment during fires (Kirk and Logan 2015a, 2015b; Lin et al., 2002); emissions from wood combustion contain more acenaphthylene than other PAHs (Perwak et al., 1982). For workers at MRD, SDM, MDL, TMC, and BRG firehouses, the other most abundant PAHs were, by descending order, acenaphthene, naphthalene, and phenanthrene with contributions ranging from 15.5 (BRG) to 38.6% (MRD), 4.48 (MDL) to 16.8% (BRG), and from 2.65 (MRD) to 7.82% (TMC), respectively. Together with acenaphthylene, these four $PM_{2.5}$ -bound PAHs represented 84.6-95.0% of Σ PAHs for those fire stations. In the breathing air zone of firefighters from FEC and VNH, dibenz[a,h]anthracene was respectively the second and third predominant PAH, accounting with 11.9% and 7.84% of Σ PAHs. Dibenz[a,h]anthracene concentrations in workers from the remaining six fire stations represented only 0.30-1.73% of Σ PAHs. The prevalence of this compound indicates a higher influence from light-duty gasoline vehicle emissions (Ravindra et al., 2008) in the surrounding areas of FEC and VNH fire stations. Globally $PM_{2.5}$ -bound PAHs with 2-3 aromatic rings (naphthalene, acenaphthylene, and acenaphthene) represented 63.9% (FEC) to 95.7% (MDL) of Σ PAHs; 4 aromatic ring compounds (anthracene, fluoranthene, and pyrene) accounted with 0.79% (MRD) to 2.05% (TMC) and 5 or more aromatic ring PAHs corresponded to 2.74% (MDL) to 34.7% (FEC) of Σ PAHs (Figure 7.3).

Table 7.7. Concentrations of PM_{2.5}-bound PAHs (median^a; min-max; ng/m³) in the breathing air zone of firefighters at the fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

Compound	Fire station							
	MRD	TDC	SDM	MDL	TMC	VNH	BRG	FEC
Naphthalene	12.2 (8.51 – 15.1)	6.67 (5.61 – 6.99)	7.95 (6.04 – 10.3)	11.5 (10.9 – 13.5)	4.95 (4.27 – 8.74)	7.19 (5.95 – 8.53)	9.21 (4.23 – 13.0)	35.5 (33.4 – 38.6)
Acenaphthylene	110 (75.6 – 208)	24.0 ^a	56.4 (27.8 – 86.9)	125 ^{**} (24.0 – 198)	24.0 ^a	43.9 ^{***} (24.0 – 89.1)	27.2 ^{***} (24.0 – 111)	199 (60.3 – 352)
Acenaphthene	88.5 (56.1 – 111)	2.48 ^{**} (1.62 – 4.26)	18.7 (18.4 – 20.8)	97.3 ^{**} (1.62 – 121)	10.3 (9.11 – 16.2)	1.62 ^a	8.54 ^{***} (1.62 – 18.2)	4.33 ^{***} (1.62 – 8.02)
Fluorene	1.05 (0.540 – 1.49)	0.272 ^{***} (0.272 – 0.588)	0.272 ^a	1.86 (1.62 – 3.53)	0.446 ^{**} (0.272 – 0.528)	0.272 ^a	0.272 ^{***} (0.272 – 0.450)	6.73 (3.13 – 9.94)
Phenanthrene	6.07 (3.74 – 7.27)	4.08 (3.71 – 5.97)	2.88 (2.76 – 3.00)	9.45 (6.83 – 9.88)	3.99 (3.39 – 4.42)	4.32 (3.42 – 5.01)	3.63 (2.97 – 4.84)	28.5 (21.0 – 35.9)
Anthracene	0.223 ^a	0.223 ^{***} (0.223 – 0.364)	0.223 ^a	0.223 ^{***} (0.223 – 1.59)	0.223 ^a	0.223 ^{***} (0.223 – 0.657)	0.223 ^a	0.636 (0.601 – 0.675)
Fluoranthene	0.351 ^{***} (0.351 – 0.603)	0.351 ^{***} (0.351 – 0.592)	0.351 ^a	1.50 (0.968 – 2.16)	0.351 ^{***} (0.351 – 0.589)	0.596 ^{**} (0.351 – 1.02)	0.351 ^{***} (0.351 – 0.631)	1.88 ^{***} (0.351 – 3.88)
Pyrene	1.24 ^{**} (0.292 – 1.34)	0.292 ^a	0.292 ^a	2.28 (1.76 – 2.77)	0.473 ^{**} (0.292 – 0.755)	0.582 ^{**} (0.292 – 1.09)	0.292 ^a	3.41 (2.01 – 4.92)

^aWhen the concentration was below the LOD, the value of the respective LOD/√2 was used (Hornung and Reed 1990).

Note: Detection frequency of each compound was 100% unless otherwise indicated. *80% ≤ detection frequency < 100%; **60% ≤ detection frequency < 80%; ***15% ≤ detection frequency < 60%.

Table 7.7. Concentrations of PM_{2.5}-bound PAHs (median^a; min-max; ng/m³) in the breathing air zone of firefighters at the fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)) (continued).

Compound	Fire station							
	MRD	TDC	SDM	MDL	TMC	VNH	BRG	FEC
Benzo[a]anthracene	0.205 ^a	0.205 ^a	0.205 ^a	0.205*** (0.205 – 0.866)	0.205 ^a	0.205*** (0.205 – 0.452)	0.205*** (0.205 – 0.324)	0.446*** (0.205 – 0.824)
Chrysene	0.145*** (0.145 – 0.358)	0.145 ^a	0.145 ^a	0.145*** (0.145 – 2.01)	0.365** (0.145 – 0.475)	0.196*** (0.145 – 0.743)	0.145 ^a	2.41 (2.01 – 3.08)
Benzo[b]fluoranthene	0.844 ^a	0.844 ^a	0.844 ^a	0.844*** (0.844 – 3.55)	0.844 ^a	2.31** (0.844 – 3.51)	0.844 ^a	24.5 (15.5 – 33.9)
Benzo[k]fluoranthene	0.238** (0.134 – 0.642)	0.134 ^a	0.134 ^a	0.134*** (0.134 – 0.412)	0.134 ^a	0.399** (0.134 – 0.594)	0.134 ^a	3.84 (2.50 – 5.15)
Benzo[a]pyrene	0.277*** (0.277 – 1.02)	0.277*** (0.277 – 0.398)	0.277 ^a	0.277*** (0.277 – 1.24)	0.277 ^a	1.22** (0.277 – 2.45)	0.277 ^a	15.1 (9.76 – 20.5)
Dibenzo[a,h]pyrene	0.671 ^a	0.671 ^a	0.671 ^a	0.671 ^a	0.671 ^a	0.671 ^a	0.671 ^a	0.671 ^a
Dibenzo[a,h]anthracene	3.97** (0.499 – 13.9)	0.499*** (0.499 – 1.66)	0.499 ^a	0.767** (0.499 – 3.63)	0.499 ^a	5.85** (0.499 – 9.96)	0.499 ^a	51.1 (38.1 – 65.5)
Benzo[ghi]perylene	3.09** (0.355 – 4.48)	5.08 (2.66 – 6.78)	1.66 (1.44 – 1.88)	3.80 (2.86 – 11.8)	3.17 (1.98 – 4.00)	4.83 (3.06 – 8.53)	2.29 (1.55 – 4.18)	32.9 (27.9 – 40.8)

^aWhen the concentration was below the LOD, the value of the respective LOD/2 was used (Hornung and Reed 1990).
Note: Detection frequency of each compound was 100% unless otherwise indicated: *80% ≤ detection frequency < 100%; **60% ≤ detection frequency < 80%; ***15% ≤ detection frequency < 60%.

Table 7.7. Concentrations of PM_{2.5}-bound PAHs (median^a; min-max; ng/m³) in the breathing air zone of firefighters at the fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)) (continued).

Compound	Fire station						
	MRD	TDC	SDM	MDL	TMC	VNH	BRG
Indeno[1,2,3-cd]pyrene	0.185*** (0.185 – 1.18)	0.185 ^a	0.185 ^a	0.185 ^a	0.185 ^a	0.185 ^a	0.185 ^a
ΣPAHs	229 (200 – 296)	46.4 (44.0 – 49.4)	91.7 (64.5 – 124)	256 (77.6 – 352)	51.1 (48.8 – 57.5)	74.6 (44.4 – 125)	55.0 (49.8 – 137)
ΣPAHs _{carc}	20.8 (15.8 – 26.0)	9.82 (8.57 – 11.1)	10.9 (9.00 – 13.3)	16.8 (13.9 – 23.9)	8.24 (7.45 – 11.7)	19.6 (9.43 – 23.6)	12.2 (7.19 – 16.1)

^aWhen the concentration was below the LOD, the value of the respective LOD/2 was used (Hornung and Reed 1990).
Note: Detection frequency of each compound was 100% unless otherwise indicated: *80% ≤ detection frequency < 100%; **60% ≤ detection frequency < 80%; ***15% ≤ detection frequency < 60%.

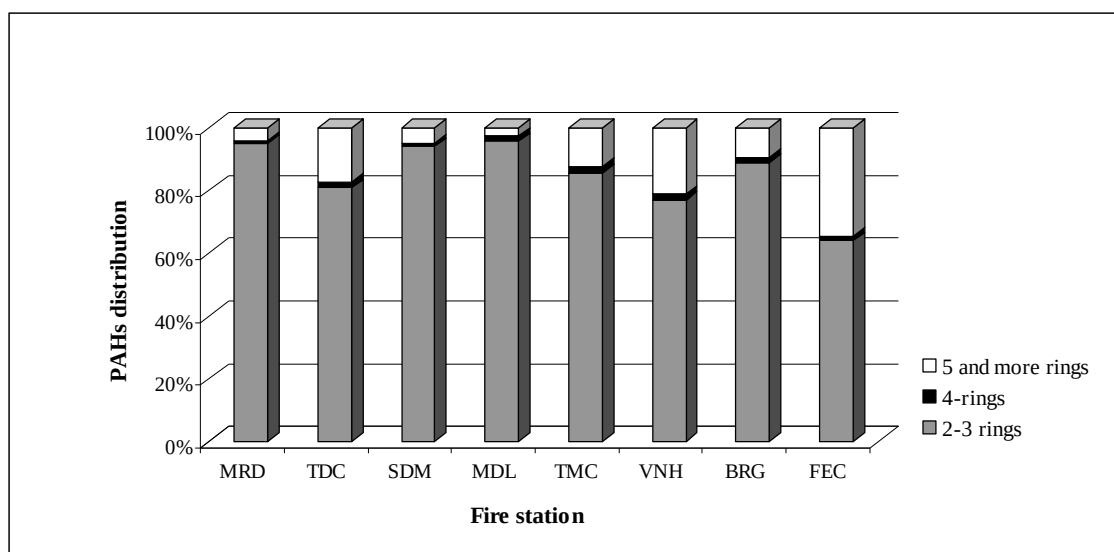


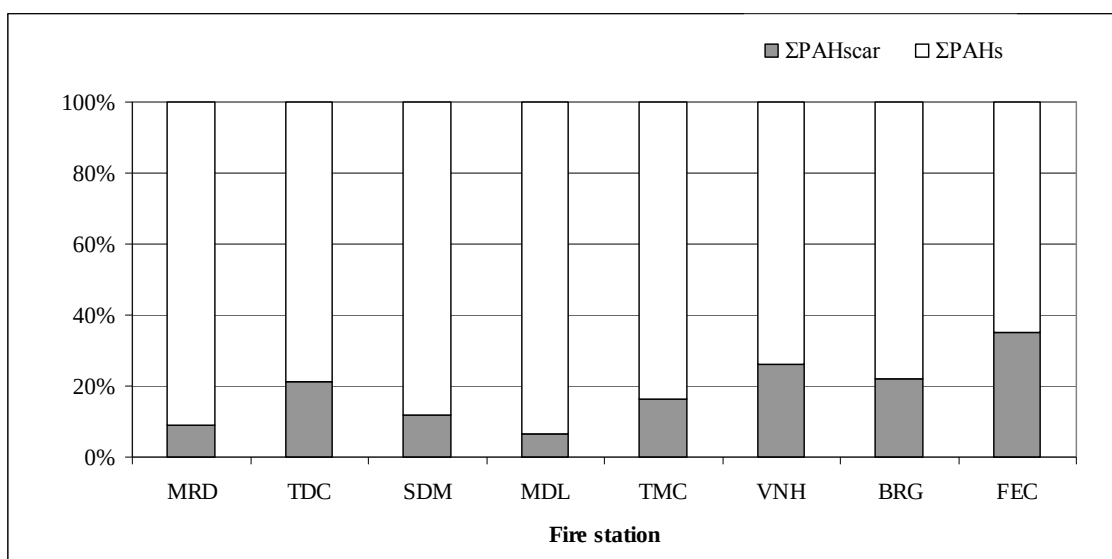
Figure 7.3. PAHs distribution according to the number of aromatic rings at different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

It is worth to mention that lighter PAHs usually predominate in the gas phase (Oliveira et al., 2015a, 2015b) suggesting that the determined personal exposures may be underestimated. Overall airborne PAH profiles obtained at FEC and VNH exhibited the highest contributions of the heavier molecular weight compounds (the most hazardous), while those at MRD, SDM and MDL presented the lowest. As a general rule, PAHs toxicity increases as the number of rings increases (with the exception of naphthalene, 2-rings compound that is classified as possible human carcinogen; (IARC 2002)).

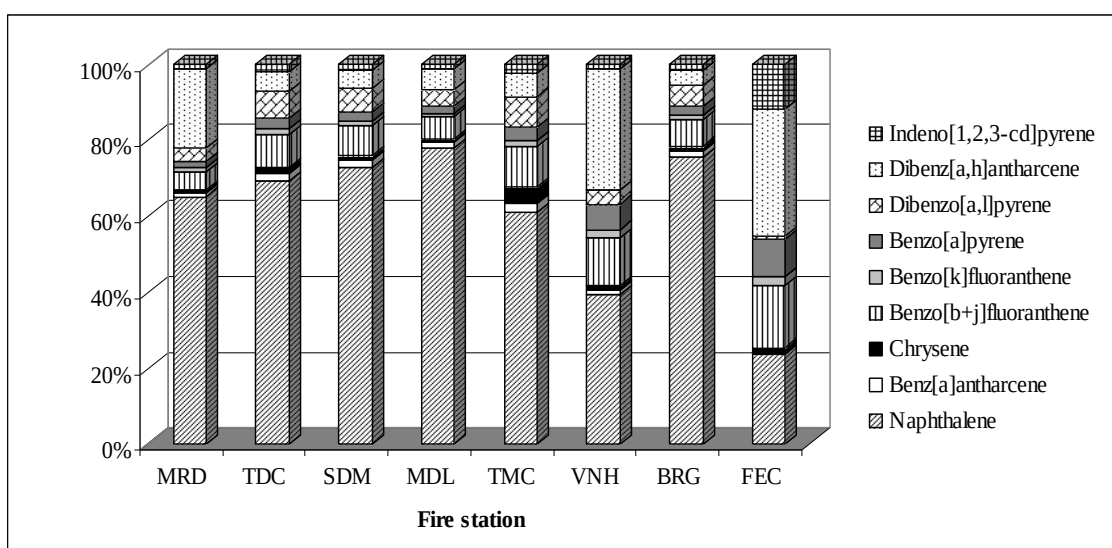
Global mean Σ PAHs varied widely. The highest total median content of PAHs was found, by descending order, in the breathing air zone of firefighters from FEC >> MDL > MRD >> SDM > VNH > BRG > TMC $\approx$ TDC, with levels at FEC (428 ng/m³) 2 (256 ng/m³, MDL) to 9 (46.4 ng/m³, TDC) times significantly higher ($p \leq 0.036$) than at the other fire houses. Indoor PAH levels, and personal exposure, depend on activities conducted, occupancy rates, physical characteristics of buildings (permeability, particle size-specific difference), site specificity, ventilation habits, season and meteorology. The observed differences may be mainly attributed to site specificity (urban/rural) and characteristics of buildings. The two firehouses located at rural areas with low traffic influences (TMC and TDC) presented the lowest levels of PAHs in the breathing air zone of firefighters (Table 7.7), followed by those situated in urban sites but

constructed and/or restored more recently (BGR, VNH and SDM). Vehicular traffic emissions are one of the major sources of PAHs in urban areas (Slezakova et al., 2013). In addition, the age of a building reflects its condition and affects penetration of outdoor PAH concentrations to indoor air. The older a building, the greater will be the impact of outdoor sources owing to higher air exchange through such routes as poorly fitting windows and doors (WHO 2010). The urban location (near busy roads), building age (constructed before 1984) and lay-out of FEC, MDL, and MRD contributed significantly to the elevated firefighters' environmental exposure at these firehouses. The truck bay at FEC, MDL and MRD was physically separated from the operational control center and social room (where firefighters spend most of their time) only by a door (that was frequently opened). Moreover, the determined levels at these three stations were higher than those reported (30.5-126.5 ng/m³) for two sites located in the outskirts of the city of Porto that is a more urbanized area and has much higher traffic density than Bragança district (Metropolitan Area of Porto has 2042 km² with 1.7 million citizens, while Bragança district has an area of 6 608 km² with 139 344 citizens); Porto district corresponds to the nearest geographical region (Figure 7.1) that was previously characterized in terms of atmospheric PAH levels (Albuquerque et al., 2016).

More detailed analysis was performed concerning the $\Sigma\text{PAHs}_{\text{car}}$ (Table 7.7). The inter-fire stations comparison of the content of $\Sigma\text{PAHs}_{\text{car}}$ in the breathing air zone of firefighters was $\text{FEC} \gg \text{MRD} \approx \text{VNH} > \text{MDL} > \text{BRG} \approx \text{SDM} \approx \text{TDC} \approx \text{TMC}$. In accordance with the ΣPAH levels, workers from FEC exhibited significantly higher ($p \leq 0.002$) $\Sigma\text{PAHs}_{\text{car}}$ (Figure 7.4a). Globally the compound that contributed the most to $\Sigma\text{PAHs}_{\text{car}}$ was naphthalene (39.4% at VNH to 78.1% at MDL), except for firefighters from FEC firehouse. For that fire station, dibenz[a,h]anthracene (with a toxicity equivalent factor 5 times higher than benzo[a]pyrene; (Okona-Mensah et al., 2005) was the predominant PAH accounting with 33.8% of $\Sigma\text{PAHs}_{\text{car}}$, followed by naphthalene (23.5%) (Figure 7.4b). In addition, firefighters working at VNH firehouse also presented a relevant contribution of dibenz[a,h]anthracene (32.1% of $\Sigma\text{PAHs}_{\text{car}}$) in their breathing air zone; for the other sites, values varied from 4.1% (BRG) to 21.1% (MRD) of $\Sigma\text{PAHs}_{\text{car}}$. The maximum concentrations of benzo[a]pyrene, the PAHs marker of carcinogenicity, accounted with 10% at FEC, 6.7% at VNH, 1.5-3.4% of $\Sigma\text{PAHs}_{\text{car}}$ for the other firehouses (Figure 7.4b). Furthermore, it is important to mention that the median concentrations of benzo[a]pyrene of FEC firefighters exceeded 10 to 20 times the existent limit value of 1 ng/m³ (annual mean total content in PM₁₀ fraction) for ambient air (Directive 2004/107/EC).



(a)



(b)

Figure 7.4. PAHs distribution of a) total carcinogenic PAHs ($\Sigma\text{PAHs}_{\text{car}}$) among the 18 PAHs under study (ΣPAHs) at each fire station; b) individual carcinogenic PAHs among the different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

Dibenzo[a,l]pyrene, which carcinogenic potency has been estimated to be approximately 100 times that of benzo[a]pyrene (Okona-Mensah et al., 2005), presented concentrations lower than 0.671 ng/m^3 (0.44-8.2% of $\Sigma\text{PAHs}_{\text{car}}$) in the breathing air zone of firefighters.

Firefighters personal exposure to PAHs at Portuguese fire stations were well below the existent PAHs occupational exposure limits proposed by ACGIH, NIOSH and the US OSHA (Table 7.1).

Studies regarding firefighters' occupational exposure to PAHs at fire stations are very scarce (Baxter et al., 2014; Shen et al., 2015); none in European facilities. Moreover, comparison between various studies is hampered by different designs (analyzed particulate fraction or/and number of determined PAHs). Shen and coworkers (Shen et al., 2015) collected dust samples from vacuum cleaner bags used to routinely clean twenty American firehouses. Median total PAHs concentration in dust was 4124 ng/g. Among the 10 compounds under study, pyrene was the predominant PAH measured in these dust samples, followed, in decreasing order, by benzo[ghi]perylene, fluoranthene and chrysene. Baxter and colleagues (2014) conducted an air monitoring study in three different environments, including in the fire truck bay and equipment storage area, the kitchen/common area, and the sleeping quarters of two American fire stations (Baxter et al., 2014). Surprisingly, out of 16 quantified PAHs, only naphthalene (at 9.22-9.24 $\mu\text{g}/\text{m}^3$) was detected in $\text{PM}_{2.5}$ -bound PAHs samples collected in the kitchen and truck bay of one firehouse. Median concentrations of naphthalene (4.95 at TMC to 12.2 ng/m^3 at MRD, Table 7.7) in the breathing air zone of Portuguese firefighters were lower than those reported levels (Baxter et al., 2014). Furthermore, some recent studies (Kirk and Logan 2015a,b) have reported that a quantifiable deposition of PAHs outside and inside firefighters' ensembles (helmets, gloves, hoods, and turnout gear) occurs which may significantly contribute to total PAHs body burden. Further research is required to investigate this aspect.

7.3.2. Biomonitoring

Median concentrations of individual and $\Sigma\text{OH-PAHs}$ in the urine samples of firefighters are presented in Table 7.8. Globally creatinine concentrations in the urine of firefighters ranged between 0.82 to 2.74 g/L, being within the accepted creatinine range for healthy people (> 0.3 and < 3 g/L) (WHO 1996). Since PAHs are ubiquitous pollutants, each participating firefighter was requested to fill a questionnaire regarding the existence of possible exposure to PAHs outside the occupational environment. Only non-smoking firefighters with dietary exclusive of barbecue and deep-fried foods within five days before urine samples collection were considered.

Table 7.8. Monohydroxyl-PAH concentrations (median[#]; min-max; $\mu\text{mol/mol}$ creatinine) measured in firefighters urine samples from different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

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

*1OHNaph+1OHAce: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene
[#] When the urine concentration was below the LOD, the value of the respective LOD/2 was used (Hornung and Reed 1990).

Thus, it was assumed that firefighters had similar (non-significant) levels of PAHs exposure through food consumption and that tobacco smoke contribution was negligible. Still, the assessment of tobacco biomarker data, and the use of pre-exposure urine samples, would be precious to validate these assumptions. Knowledge of the elimination kinetics of the urinary OH-PAHs is essential to define the urine sampling strategy. Limited studies regarding elimination kinetics of OH-PAHs in humans are available: half-life of urinary 1OHPy excretion rates varies between 6 to 35 h after inhalation exposure (Brzeznicki et al., 1997; Jongeneelen et al., 1990) and 4.4 (Buckley and Lioy 1992) to 12 h (Viau et al., 1995) after ingestion exposure; half-life ranging from 3.3 to 6.2 h for 1OHNaph, 2.3 to 4.0 h for 2OHFlu, and 4.3 to 6.1 h for 1-OHPhen were also reported for ingestion exposure (Li et al., 2012). 1OHNaph+1OHAc were detected in more than 87% of samples, 2-OHFlu and 1OHPy presented detection rates above 96% and 1OHPhen was detected in all urine samples. In agreement with some previous studies, urinary 3OHB[a]P presented null or very low detection rates (Gündel et al., 2000; Li et al., 2012; Yamano et al., 2014). Some studies with animals revealed that urinary concentrations of 3OHB[a]P represent only 0.1–0.2% of the benzo[a]pyrene dose, due to a complex metabolism that produces several different metabolites which are mainly excreted in feces (Marie et al., 2010).

Overall the inter-comparison of urinary Σ OH-PAHs of firefighters from fire stations: FEC >> VNH > MRD $\approx$ SDM > MDL > BRG > TMC >> TDC, followed the same trend as the content of airborne Σ PAHs with the exception of workers from VNH and MDL that exchanged position. Accordingly, urinary Σ OH-PAHs in individuals from FEC were significantly elevated ($p \leq 0.002$) (2 (MRD) to 14 (TDC) times higher) than at other firehouses. The concentrations of urinary OH-PAHs in firefighters were inversely related to the size of compounds, i.e., the higher the molecular weight the lower the detected OH-PAH concentrations (Table 7.8). Urinary 1OHNaph+1OHAc were by far the predominant compounds accounting with 66% (TDC) to 96% of Σ OH-PAHs (FEC, SDM), being followed by 2OHFlu (1% at SDM to 16% at TDC), 1OHPhen (1% at SDM and FEC to 11% at TDC), and 1OHPy (1% at FEC to 7% at TDC) (Figure 7.5). This distribution profile follows the same pattern as the PAHs distribution in the breathing air zone of firefighters while working at fire stations (Table 7.7); airborne PAHs with two and three aromatic rings were the most abundant ones in all fire stations (63.9% at FEC to 95.7% of Σ PAHs at MDL, Figure 7.3). Urinary 1OHNaph+1OHAc concentrations were significantly different ($p \leq 0.002$) from the other metabolites for all groups of individuals (Table 7.8).

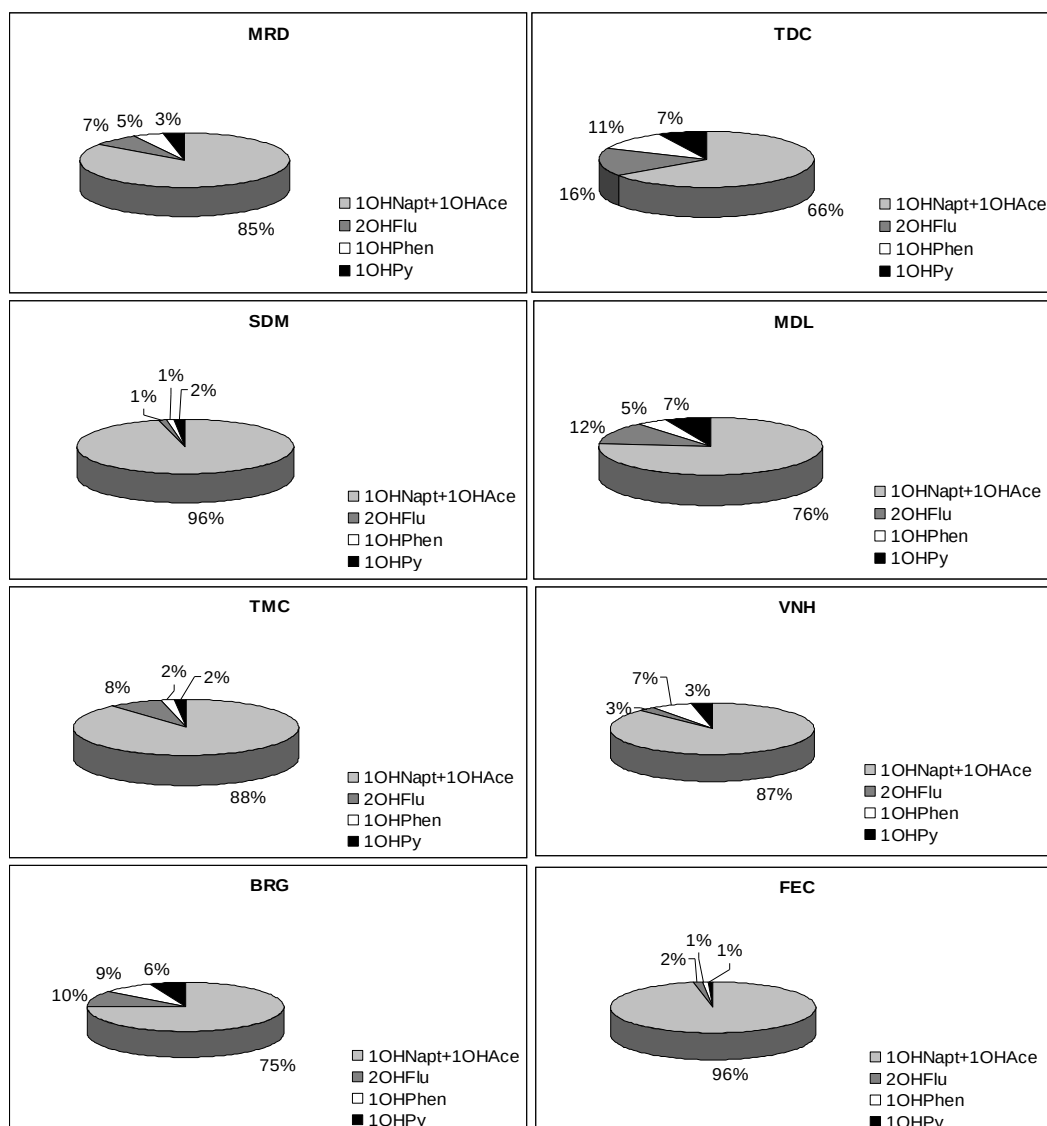


Figure 7.5. Distribution of monohydroxyl-PAHs (1OHNapt+1OHAc: 1-hydroxybaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) in the urine samples of firefighters at different fire stations (Miranda do Douro (MRD), Torre Dona Chama (TDC), Sendim (SDM), Mirandela (MDL), Torre de Moncorvo (TMC), Vinhais (VNH), Bragança (BRG), and Freixo de Espada à Cinta (FEC)).

The median concentrations of urinary 1OHPy in Portuguese firefighters ranged between 1.36×10^{-2} (SDM) to 0.146 (MDL) $\mu\text{mol/mol}$ creatinine (Table 7.8). Nowadays, measurements of urinary 1OHPy are routinely applied to control industrial exposure to PAHs in coke ovens and primary aluminium production and to control exposure of professionals when handling coal tar derived products (Jongeneelen 2014). Although no reference standard guidelines are established for urinary OH-PAHs, Jongeneelen (2001, 2014) proposed a no-biological effect level of 1.4 $\mu\text{mol/mol}$ creatinine of 1OHPy

in urine of exposed workers, i.e. the lowest reported level at which no genotoxic effects were found. In addition the Biological Exposure Index Committee of ACGIH stated that the presence of urinary 1OHPy above a benchmark level of about 1 µg/L (0.5 µmol/mol) indicates occupational exposure to PAHs, since very few non-occupationally exposed persons, smokers or non-smokers, will excrete this amount of 1OHPy (ACGIH 2010). ACGIH also recommends that the benchmark should be considered as a post-shift level. The quantified concentrations of post-shift urinary 1OHPy in firefighters from all fire stations were well below those recommended values. This observation is important since firefighters participating at firefighting activities are heavily exposed to PAHs (Fent et al., 2013, 2014; Kirk and Logan 2015a) and thus may present high background levels of 1OHPy.

Regarding characterization of firefighters' exposure, 1OHPy and 1OHNaph are the more investigated metabolites (Caux et al., 2012; Edelman et al., 2003; Laitinen et al., 2010; Robinson et al., 2008). The comparison of the detected levels with previous reports is extremely difficult since, in the majority of the studies, concentrations are not adequately normalized with the personal urinary creatinine levels. Creatinine is eliminated from the human body at a constant rate, and thus is widely used to minimize the variability of parameters such as individual fluid intake, body temperature, physical exercise and ambient temperature, which changes from person to person. Still, urinary 1OHPy concentrations in Portuguese firefighters (overall range: 0.078-3.28 nmol/L) were slightly higher than levels found in firefighters before their participation in gas simulators (0.6-1.2 nmol/L) but lower than concentrations at diving simulator fires (0.6-9.2 nmol/L) (Shen et al., 2015). In addition 1OHPy levels were similar with the levels reported in firefighters before their participation in prescribed pile burns (<0.01-0.56 µg/L (Robinson et al., 2008)) but slightly higher than the concentrations observed in the control group of firefighters that were present at the WTC Collapse in 2001 (157 ng/L in this study *versus* 62.5 ng/L; Edelman et al., 2003). Only two studies reported concentrations of other metabolites, namely several OHPhen (Edelman et al., 2003) and 1OHNaph (Laitinen et al., 2010) compounds among firefighter's urine. Urinary 1OHPhen levels were similar with those observed in the control group of firefighters (170 ng/L in this study *versus* 158 ng/L; Edelman et al., 2003). In firefighters, urinary 1OHAc, 2OHFlu, and 3OHB[a]P were never assessed before. Urinary OH-PAHs excretion in firefighters during their regular work at fire stations was lower than levels reported for other industrial workers with known PAHs exposure (Barbeau et al., 2014, 2015; Forster et al., 2008; Gendre et al., 2002; Rossbach et al., 2007; Yamano et al., 2014).

7.3.3 Correlations between airborne PAHs and urinary OH-PAHs

The possible contribution of airborne individual (naphthalene, acenaphthene, naphthalene+acenaphthene, fluorene, phenanthrene, and pyrene) and Σ PAHs to respective individual (1OHNaph+1OHAc, 2OHFlu, 1OHPhen, and 1OHPy) and Σ OH-PAHs excretion in the post-shift urine was estimated. Moderate to strong correlations were observed between Σ PAHs and urinary Σ OH-PAHs (ranging from $r=0.367$, $p=0.332$ at TMC to $r=0.886$, $p=0.019$ at FEC) for firefighters from six fire stations (FEC, BRG, MDL, MRD, TDC, TMC); statistical significance was reached for workers from MRD ($r=0.733$, $p=0.025$), MDL ($r=0.786$, $p=0.021$), BRG ($r=0.839$, $p=0.001$) and FEC ($r=0.886$, $p=0.019$) which were three of the firehouses where the highest environmental exposure were detected (Table 7.7).

These findings are in agreement with those described for coke oven workers ($r=0.680$, $p\leq 0.01$ between Σ PAHs of 15 compounds and Σ OH-PAHs of 10 metabolites) (Vlau et al., 1995). The positive correlations confirm that OH-PAH excretion increases with an increasing exposure of PAHs at fire stations. It seems that, at these sites, firefighters had fire station indoor air as their major exposure source of PAHs. Also, Σ PAHs correlated well with 1OHPy, the biomarker of PAHs exposure, for individuals from MRD ($r=0.703$, $p=0.035$), SDM ($r=0.941$, $p=0.005$), MDL ($r=0.262$, $p=0.531$) and VNH ($r=0.944$, $p\leq 0.001$). These urban sites are in the list of the 5 firehouses where environmental PAHs exposure were the highest (FEC >> MDL > MRD >> SDM > VNH > BRG > TMC $\approx$ TDC; Table 7.7); the only observed exception was FEC. These results suggest that 1OHPy may be a suitable biological indicator of internal dose of exposure to those PAHs that are emitted by the predominant source. These correlations are higher than those reported in coke oven workers ($r=0.456$, $p<0.01$; Yamano et al., 2014) but similar with the relationships described in workers employed in coke production, production of graphite electrodes, special carbon products, and production of refractory materials ($r=0.717$, $p<0.01$; Rossbach et al., 2007). Other significant relationships were reached between naphthalene + acenaphthene with urinary 1OHNaph+1OHAc for firefighters at MDL ($r=0.833$, $p=0.010$), fluorene and 2OHFlu at FEC ($r=0.771$, $p=0.072$) and BRG ($r=0.736$, $p=0.006$), phenanthrene and 1OHPhen at FEC ($r=0.971$, $p=0.001$); and pyrene with 1OHPy at TMC ($r=0.878$, $p=0.002$) and VNH ($r=0.745$, $p=0.005$). Yamano et al. (2014) and Rossbach et al. (2007) also reported moderate to strong correlations between urinary metabolite concentration and personal exposure to the respective PAH.

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

7.4 Conclusions

This work characterized firefighters' exposure to PAHs during their work shift at eight Portuguese fire stations by personal exposure monitoring and biomonitoring. Overall, PM_{2.5}-bound Σ PAH concentrations ranged from 46.4–428 ng/m³, with 2–3 aromatic rings accounting with 63.9–95.7% of Σ PAHs. PAHs with 4–5 or more aromatic rings corresponded to 0.789–2.05% and 2.54–34.7% of Σ PAHs, respectively. The obtained data highlighted the importance of site specificity and poor building construction/conservation, as well as inappropriate building layout on environmental PAH levels at the studied fire stations. Still, firefighter's personal exposure to PAHs at Portuguese fire stations was well below the existent occupational exposure limits.

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

7.5 Ethical statement

The study proposal was reviewed and approved by Ethic Committee of University of Porto. Full information about the measurements conducted in this work was provided to all firefighters from all fire stations considered in the study.

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**CHAPTER 8 – FIREFIGHTERS’ EXPOSURE BIOMONITORING:
IMPACT OF FIREFIGHTING ACTIVITIES ON LEVELS OF
URINARY MONOHYDROXYL METABOLITES**

Chapter 8. Firefighters' exposure biomonitoring: impact of firefighting activities on levels of urinary monohydroxyl metabolites

The concentrations of six urinary OH-PAHs, namely 1OHNaph, 1OHAce, 2OHFlu, 1OHPhen, 1OHPy, and 3OHB[a]P, 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 levels of urinary individual and Σ OH-PAHs suggest an increased exposure to PAHs during firefighting activities with Σ OH-PAH levels in exposed firefighters 1.7 to 35 times higher than in non-exposed ones. Urinary 1OHNaph and 1OHAce were the predominant compounds, representing 63-98% of Σ OH-PAHs, followed by 2OHFlu (1-17%), 1OHPhen (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} - $0.152 \mu\text{mol/mol creatinine}$ in exposed subjects *versus* 1.21×10^{-2} - $5.44 \times 10^{-2} \mu\text{mol/mol creatinine}$ in non-exposed individuals) were lower than the benchmark level ($0.5 \mu\text{mol/mol creatinine}$) 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 Σ OH-PAHs corroborating the prevalence of an emission source.

8.1 Introduction

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), and workers involved in the use and application of pesticides in the agriculture (MacFarlane et al., 2013). 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, 2010a).

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., 2016b; 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). Despite firefighters' occupational exposure be classified as possible carcinogen to humans (IARC 2010b; NIOSH 2007), they are among the less characterized occupational groups mostly due to difficulties in the study design. 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 2010b). 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, the total PAHs internal dose of Portuguese wildland firefighters' by six urinary OH-PAHs, namely 1OHNaph, 1OHAce, 2OHFlu, 1OHPhen, 1OHPy, and 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.

8.2 Material and Methods

8.2.1 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 8.1) 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 8.1). All firefighting corporations belong to cities of the district of Bragança (north of Portugal; total area of 6 608 km² 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. 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 (that was previously approved by the Ethic Committee of University of Porto, Portugal). 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 8.1).

Table 8.1. Characteristics of firefighters that participated in the study.

Characteristic	Non exposed	Exposed
n	96	57
Gender		
Male (%)	78	79
Female (%)	22	21
Age (mean $\pm$ SD; min – max; years)	36.2 $\pm$ 9.6; 22.0 – 55.0	36.3 $\pm$ 8.5; 21.0 – 52.0
Man	37.7 $\pm$ 9.4; 23.0 – 55.0	36.7 $\pm$ 8.4; 22.0 – 52.0
Woman	30.7 $\pm$ 8.7; 22.0 – 45.0	34.8 $\pm$ 10.0; 21.0 – 45.0
Weight (mean $\pm$ SD; min – max; kg)	81.5 $\pm$ 15.5; 54.0 – 118	79.7 $\pm$ 9.9; 59.0 – 98.0
Man	86.5 $\pm$ 5.1; 67.0 – 118	82.1 $\pm$ 8.5; 73.0 – 98.0
Woman	61.5 $\pm$ 5.1; 54.0 – 68.0	67.7 $\pm$ 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

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.

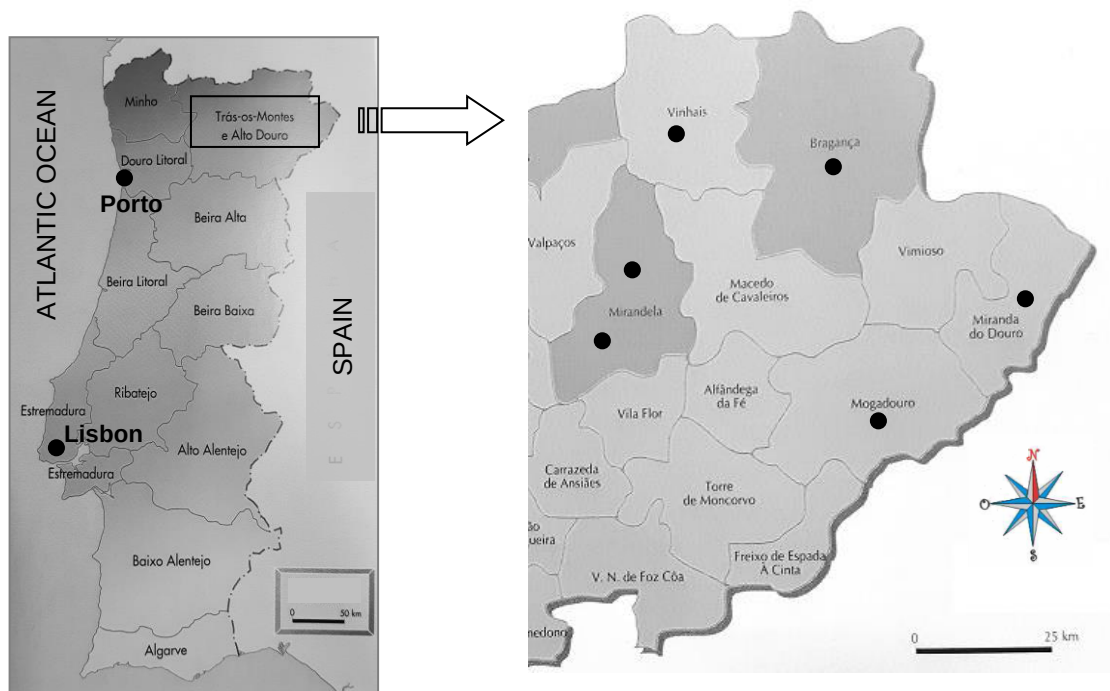


Figure 8.1. Geographical location of the selected fire corporations.

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 hours prior the urine collection, and exposed firefighters who were actively involved in fires combat and extinction.

8.2.2 Urinary OH-PAHs extraction and chromatographic analysis

Extraction and quantification of OH-PAHs from urine samples were performed according to Chetianukornkul and colleagues (Chetianukornkul et al., 2006) and according with the detailed description in sections 5.2.2.2 and 5.2.3. Urinary concentrations of all OH-PAHs were normalized with the urinary creatinine levels (mol/mol) of each subject (Kanagasabapathy and Kumari 2000).

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

8.2.3. 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 $p \leq 0.05$. When the urinary OH-PAH concentration was below the LOD, the respective $\text{LOD}/\sqrt{2}$ 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.

8.3 Results and Discussion

8.3.1 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 ($0.3 \text{ g/L} < \text{creatinine} < 3.0 \text{ g/L}$) proposed by WHO (1996) for healthy people assuring sample validity.

Overall, 1OHNaph+1OHAc, 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+1OHAc), 92% (2OHFlu) and 100% (1OHPhen) in non-exposed male subjects and between 87% (1OHNaph+1OHAc) to 100% (2OHFlu, 1OHPhen, and 1OHPy) for exposed male firefighters; 1OHNaph+1OHAc, 2OHFlu, 1OHPhen and 1OHPy were detected in 100% of non-exposed and exposed women. 3OHB[a]P was never detected, which 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). As previously described (Adetona et al.

2015; Edelman et al., 2003; Laitinen et al., 2010; Li et al., 2016), 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 8.2-8.3). 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). However 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. 2009; 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.

Median concentrations and ranges of Σ OH-PAHs measured in non-exposed and exposed firefighters working at the selected fire stations are presented in Table 8.2. Values varied from 0.249 (TDC) to 1.57 (VNH) $\mu\text{mol/mol}$ creatinine for non-exposed individuals, and between 0.973 (BRG) to 8.75 (TDC) $\mu\text{mol/mol}$ creatinine for firefighters involved in firefighting activities (Table 8.2). Figure 8.2 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 8.3).

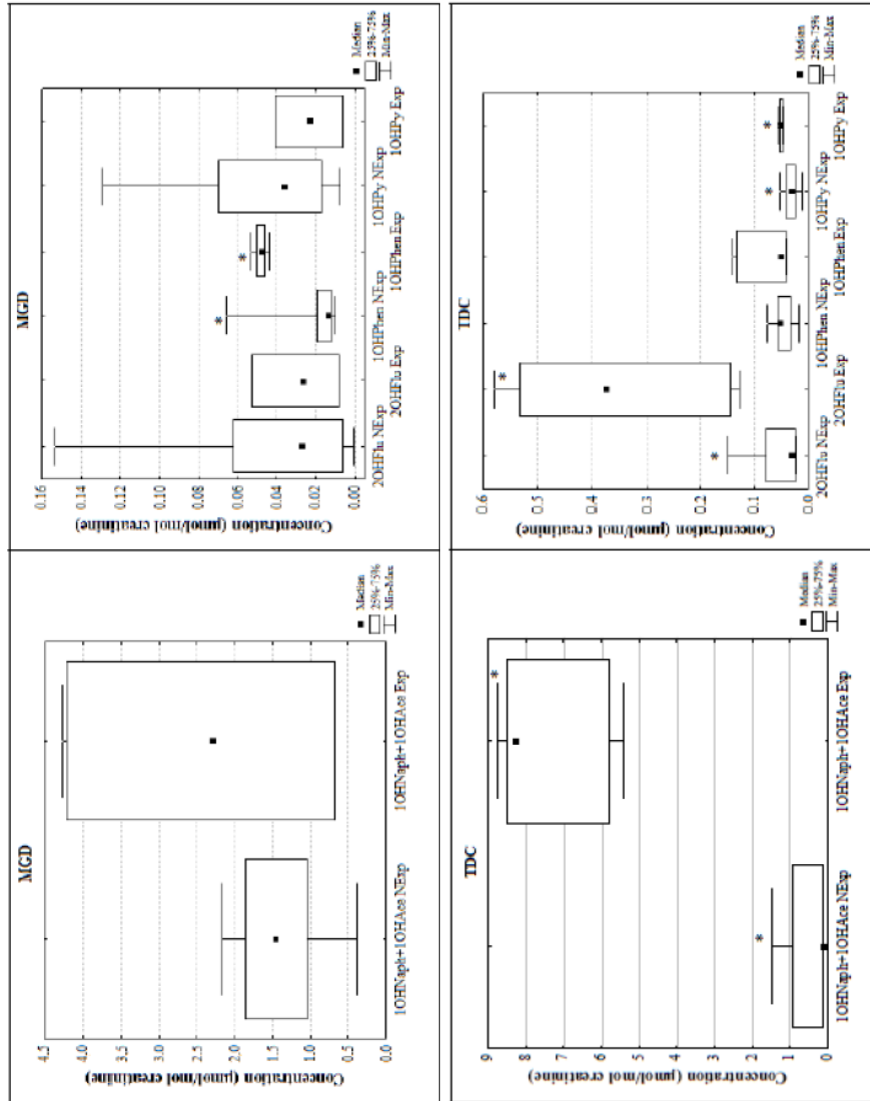


Figure 8.2. Concentrations of monohydroxyl-PAHs (median; 25-75% and range; µmol/mol creatinine) measured in non-exposed (NExp) and exposed (Exp) firefighters' urine from different fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL)). *Statistically significant differences at $p \leq 0.05$ between non-exposed and exposed firefighters from the same corporation.

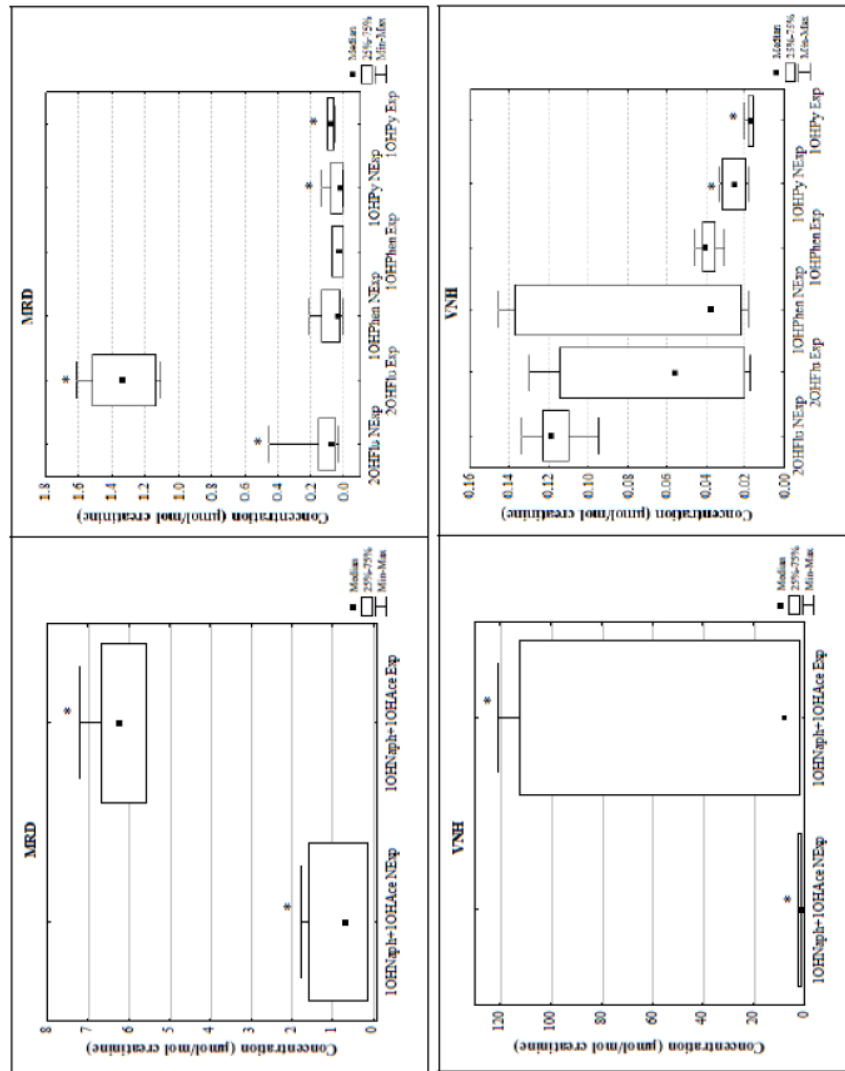


Figure 8.2. Concentrations of monohydroxyl-PAHs (median; 25-75% and range; μmol/mol creatinine) measured in non-exposed (NExp) and exposed (Exp) firefighters' urine from different fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL)). *Statistically significant differences at $p < 0.05$ between non-exposed and exposed firefighters from the same corporation (continued).

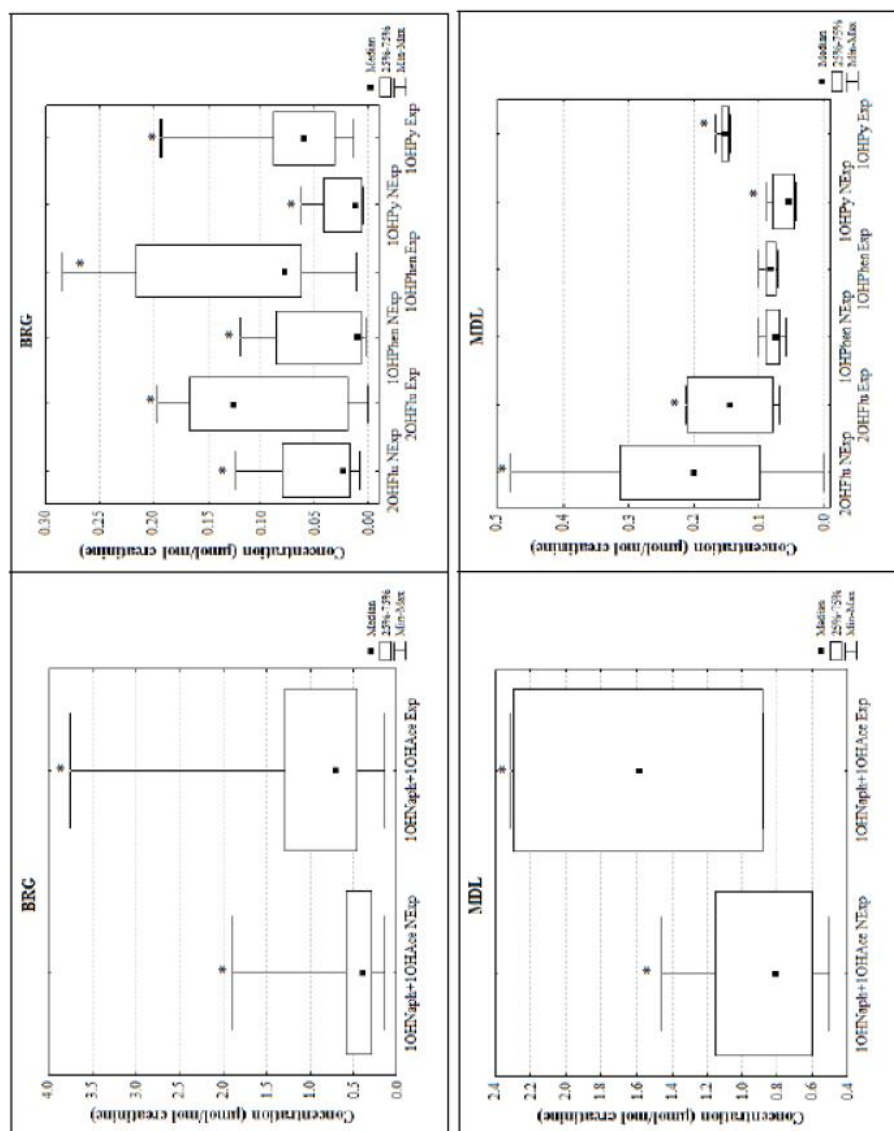


Figure 8.2. Concentrations of monohydroxyl-PAHs (median; 25-75% and range; $\mu\text{mol/mol}$ creatinine) measured in non-exposed (NExp) and exposed (Exp) firefighters' urine from different fire stations (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), and Mirandela (MDL). *Statistically significant differences at $p \leq 0.05$ between non-exposed and exposed firefighters from the same corporation (continued).

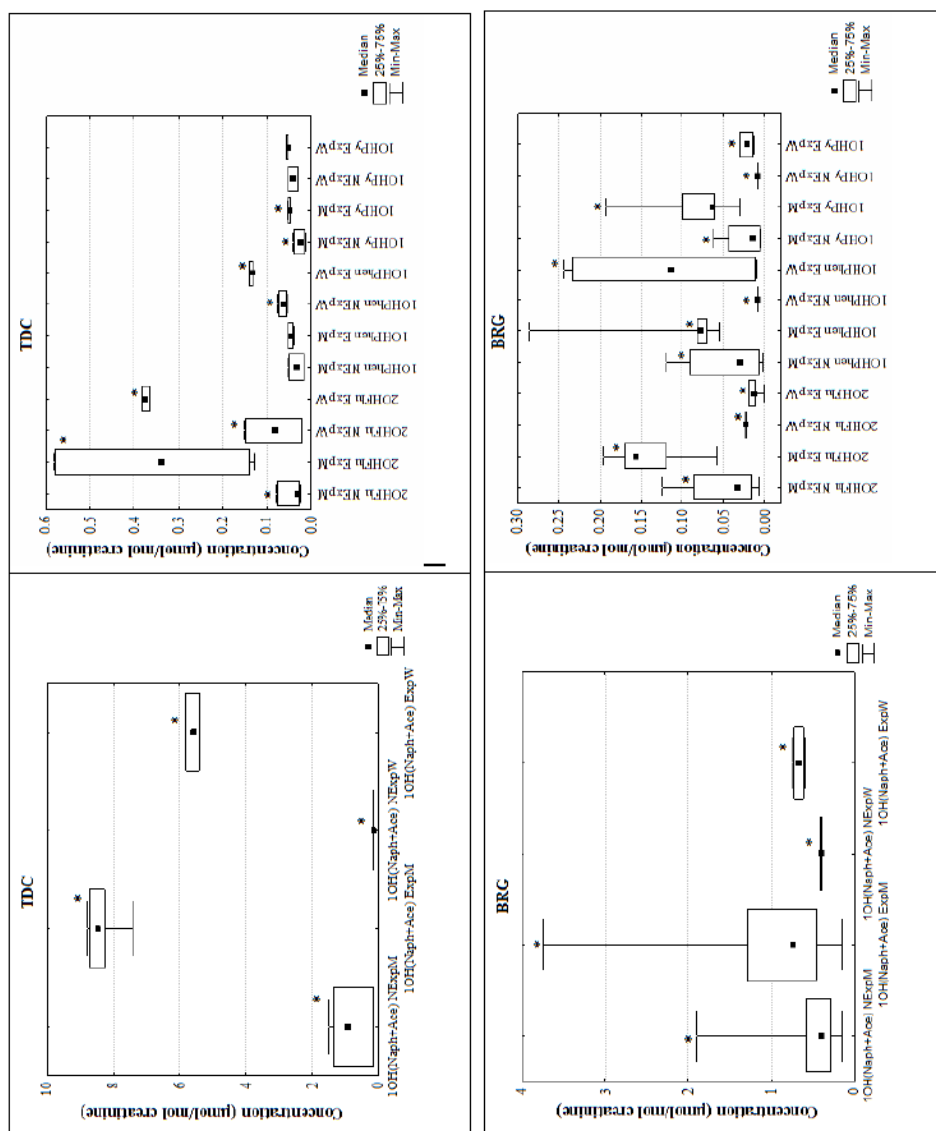


Figure 8.3. Concentrations of monohydroxyl-PAHs (median; 25-75% and range; $\mu\text{mol/mol}$ creatinine) measured in men (non-exposed (NExpM) and women (non-exposed (NExpW) and exposed (ExpW) firefighters' urine from different fire stations (Torre Dona Chama (TDC), and Bragança (BRG)). *Statistically significant differences at $p \leq 0.05$ between non-exposed and exposed subjects from the same corporation.

Table 8.2. Total urinary monohydroxyl-PAH (Σ OH-PAHs) concentrations (median; min-max; μ mol/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	MDL
Non-exposed subjects					
Σ OH-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)
Exposed subjects					
Σ OH-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)

*Statistically significant ($p \leq 0.05$) between non-exposed and exposed firefighters total monohydroxyl-PAH concentrations for each firefighting corporation.

Overall it was observed that exposed wildland firefighters presented higher urinary concentrations of Σ OH-PAHs (ca. 1.7 (MDL) to 35 (TDC) times higher ($p < 0.05$); Table 8.2, except in subjects from MGD) and individual OH-PAH (mainly 1OHNaph+1OHAc, 2OHFlu, and 1OHPy) than non-exposed firefighters (Figure 8.2) 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). Edelman and coworkers (2003) analyzed the urine of firefighters that intervened in the WTC 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 MGD 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 Σ OH-PAHs in non-exposed firefighters from this corporation (due to elevated levels of 1OHNaph+1OHAc when compared with the other workers).

Among the six fire corporations considered, firefighters from BRG had all OH-PAH levels (and Σ OH-PAHs) statistically different after occupational exposure. Urinary 1OHNaph+1OHAc concentrations were significantly elevated ($p < 0.05$) 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. Statistically significant differences for 1OHPhen levels were only identified in individuals from MGD (253% increase; Figure 8.2), BRG (634% increase (Figure 8.2); 159% for men and 1153% for women, (Figure 8.3)), and in women from TDC (113%; Figure 8.3) seeming to be the compound which excretion is less affected by fire combat activities.

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 Σ OH-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 8.3). Due to the very limited amount of data for women, no other conclusion was drawn based on gender separation.

Table 8.3. Total urinary monohydroxyl-PAH (Σ OH-PAHs) concentrations (median; min-max; $\mu\text{mol/mol}$ creatinine) separated by gender and measured in non-exposed and exposed firefighters (men and women) from the fire stations: Torre Dona Chama (TDC), and Bragança (BRG).

	Fire Station	
	TDC	BRG
Non-exposed subjects		
Men	1.01* (0.252 – 1.55)	0.484* (0.208 – 2.20)
Women	0.321* (0.264 – 0.389)	0.437* (0.429 – 0.445)
Exposed subjects		
Men	8.94* (8.05 – 9.06)	0.971* (0.402 – 4.39)
Women	6.14* (5.99 – 6.35)	0.817* (0.742 – 0.913)

*Statistically significant ($p \leq 0.05$) between non-exposed and exposed men and women firefighters total monohydroxyl-PAH concentrations for each firefighting corporation.

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.

1OHNaph+1OHAce were the metabolites that contributed the most for Σ OH-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 8.4). Overall (with the exception of BRG values and 1OHPy at MDL), participation in fires suppression promoted an increase of the distribution (%) of 1OHNaph+1OHAce while those of 1OHPhen and 1OHPy decreased.

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). Urinary 1OHPy contributed the less for Σ OH-PAHs (1.21×10^{-2} (BRG) - 5.44×10^{-2} $\mu\text{mol/mol}$ creatinine (MDL) for non-exposed firefighters, and 1.73×10^{-2} (VNH) - 0.152 $\mu\text{mol/mol}$ creatinine (MDL) for exposed firefighters, Figure 8.2). Within its pioneer study Jongeneelen (Jongeneelen 2001, 2014) proposed a no-biological effect level of 1.4 $\mu\text{mol/mol}$ creatinine of urinary 1OHPy in exposed workers. ACGIH proposed a post-shift 1OHPy benchmark level of about 0.5 $\mu\text{mol/mol}$ 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 contributed the less for Σ OH-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. However, if comparison with guidelines is made based on the total internal dose (Σ OH-PAHs) determined, urinary levels of exposed firefighters exceeded 2 (BRG) to 17 (TDC) times the ACGIH benchmark (0.5 $\mu\text{mol/mol}$ creatinine) and about 1.4 (MDL) to 6 (TDC) times the no-biological effect level of proposed by Jongeneelen (Jongeneelen 2001, 2014). Since PAHs exist always as a mixture of compounds, it is believed that 1OHPy by itself may not be a suitable biomarker of exposure to PAHs, as also previously concluded by other authors (Li et al., 2016).

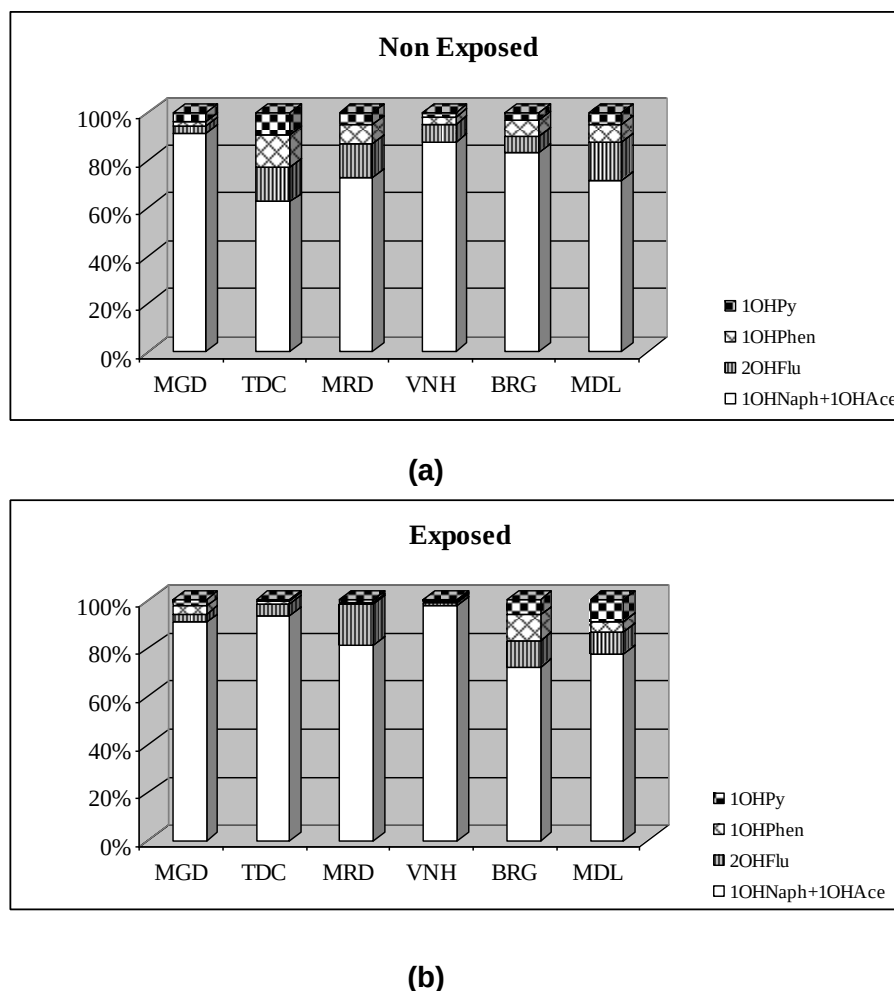


Figure 8.4. Distribution (%) of urinary monohydroxyl-PAHs (1OHNaph+1OHAce: 1-hydroxynaphthalene + 1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhen: 1-hydroxyphenanthrene; 1OHPy: 1-hydroxypyrene) in a) non-exposed and b) exposed firefighters at the studied fire stations (Mogadouro (MGD), Torre Dona Chama (TDC), Miranda do Douro (MRD), Vinhais (VNH), Bragança (BRG), Mirandela (MDL)).

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 2010b; 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 1OHAc 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} $\mu\text{mol/mol}$ creatinine (1.73×10^{-2} to 0.152 $\mu\text{mol/mol}$ 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 $\mu\text{mol/mol}$ 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 $\mu\text{g/L}$; <0.01 to 0.50 $\mu\text{g/L}$) after their active participation in prescribed pile burns (Robinson et al. 2008). 1OHPhen levels (186 ng/L) found in WTC 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+1OHAc 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+1OHAc, 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).

8.3.2 Correlations between urinary OH-PAHs

Spearman correlation coefficients (r) were determined to explore the relation between urinary individual and $\Sigma\text{OH-PAHs}$. Overall moderate to strong correlations between individual OH-PAH and $\Sigma\text{OH-PAHs}$ were found for the majority of firefighters corroborating the prevalence of an emission source in the exposed and non-exposed

groups. The strongest correlations were found between urinary 1OHNaph+1OHAc and Σ OH-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). Good correlations were also found between urinary 1OHPy and Σ OH-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, moderate to good correlations between urinary 1OHPhen and 1OHPy ($0.390 < r < 0.851$; $p \leq 0.222$) were found for all participants (non-exposed and exposed) involved in this study, with the exception of those working at MDL fire station. Thus based on questionnaires data and on a previous background study, which involved personal air monitoring (Oliveira et al., 2016a, 2016b), 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 Σ OH-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). Additionally there are a number of factors (besides the use of personal protective equipment) that influence the impact of smoke on firefighters' health: the levels of air pollutants, the duration of exposure to fires, exertion levels and subject predisposition to lung or heart diseases (Fent et al., 2013; Laitinen et al., 2010; Miranda et al., 2012; Oliveira et al., 2016a). 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.

8.4 Conclusions

In this study, consistent evidences were 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+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+1OHAce concentrations probably reflect the contribution of air. 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.

8.5 Ethical statement

The study proposal was reviewed and approved by Ethic Committee of University of Porto. Full information about the measurements conducted in this work was provided to all firefighters from all fire stations considered in the study.

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CHAPTER 9 – PART II: GLOBAL CONCLUSIONS

9.1 Global conclusions

The experiments performed in the second part of this thesis contributed with relevant information on firefighters' occupational exposure to PM and PAHs by both external (air inhalation) and internal dose (bio)monitoring (through six urinary biomarkers of exposure to PAHs) at fire stations (without participation in fire combat) and immediately after firefighting activities. Although some information exists, there is a great gap of knowledge regarding firefighters' occupational exposure in the severely affected South-eastern European countries such as Portugal, Spain, Italy, Greece and France.

Occupational exposure to PM and PAHs at fire stations were firstly assessed. Personal PM_{2.5} exposure levels at fire stations were well below the established limit for occupational exposure (5 mg/m³; NIOSH 2007). ΣPAHs concentrations varied widely, mainly due to site specificity (urban/rural) and characteristics (age and layout) of buildings. All firefighters' occupational exposure to PAHs were below the available regulations proposed by ACGIH, the NIOSH and the US OSHA (ACGIH 2012; IARC 2010; NIOSH 2007). However, the main limitations of this study were the lack of data concerning the gaseous phase, and the use of 4 h personal sampling campaigns instead of the total period of consecutive working hours. In addition, the inclusion of finest particulate fractions (e.g. PM₁) should also be considered.

Firefighters' total exposure to PAHs was assessed through the determination of urinary OH-PAHs. As in schoolchildren, the most predominant urinary metabolites detected in firemen were 1OHNaph and 1OHAce, followed by 2OHFlu. Urinary OH-PAHs distribution presented a similar profile as the airborne PAH detected in the breathing air of firefighters during a regular work shift at fire stations, revealing the important contribution of inhalation to the total exposure of firefighters. After firefighting activities (knockdown and overhaul) the concentrations of urinary OH-PAHs were substantially higher than the background levels in non-exposed firefighters. 3OHB[a]Py, the PAH biomarker of carcinogenicity, was not detected in the urine of Portuguese firefighters even in those firefighters that were more time involved in fire suppression. 1OHPy, the biomarker of exposure, was the less abundant OH-PAHs detected in firefighters which revealed the inadequacy of this biomarker to solely characterize the occupational exposure of firefighters to PAHs.

In this work, it was assumed that complete personal protective equipment (helmet with eye protection, flash hood, gloves, boots, and clothes with flame-retardant characteristics) was properly fitted, worn, and maintained by firefighters during the

entire period of firefighting activities, as described by each subject in its individual questionnaire. In these conditions firefighters have the highest assigned protection against the smoke and particles released during fires. However, it is known that the complete protection devices are not always used by wildland firefighters, particularly during the overhaul phase of controlled fires. Thus, future research should clearly control the length of use by each subject.

Since limited information regarding firefighters' occupational exposure exists (at fire stations and during fire combat activities), it is important that more studies characterizing fire stations at other geographical regions and containing a higher number of subjects should be performed be developed in order to support the evidences found in these preliminary studies.

9.2 References

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**CHAPTER 10 – FINAL REMARKS AND SUGGESTIONS FOR
FUTURE WORK**

The work conducted in this thesis contributed with relevant information related with the assessment of children environmental exposure at pre- and primary schools environments (outdoor courtyards and classrooms) and on firefighters' occupational exposure (at fire stations i.e. background exposure and during firefighting activities) to air pollutants with particular emphasis to PM and PAHs (air inhalation and urinary biomarkers). However some challenges remained unaddressed and should be explored in future works.

The quantitative contributions of sources of indoor-generated PM/metals/PAHs are not well characterized and thus should be explored. Also, speciation studies for the more toxic elements (mainly As, Cr and Hg), although being very complex to perform in particulate samples, are essential to refine the assessment of potential health risks. The determined levels of airborne PAHs enhanced the need to use other markers of exposure to carcinogenic PAHs rather than only benzo[a]pyrene (for example naphthalene for the gas phase; dibenzo[a,h]anthracene and benzo[b+j]fluoranthene for PM). In addition, in order to obtain a correct representation of human overall exposure, future studies need to measure the concentrations and the time spent in all the relevant indoor microenvironments (such as canteens, gyms, library at schools; truck bay, kitchen, and dormitories in fire stations). Specifically concerning children, limited information on personal exposure is available, mainly due to difficulties in the experimental design, and thus this approach may be included in future works to clarify the relationship between school ambient concentrations (indoor and outdoor concentrations of inorganic/organic chemical substances and biological pollutants) and overall children personal exposure.

The quantification of urinary OH-PAHs proved to be an important tool to assess the total burden of exposure to PAHs. Still the challenge to biomonitor the total exposure to probable/possible carcinogenic PAHs remains. Scientific information reveals that PAHs with five or more aromatic rings present a more complex metabolism. It is expected that low molecular weight PAHs would be almost completely metabolized (glutathione, glucuronide or sulphate conjugates) while the heavier PAHs may persist (at least in some extent) and may also be excreted through urine but as free compounds (unmetabolized). Thus this issue should also be explored giving especial emphasis to the possible/probable carcinogenic PAHs.

Since dermal exposure may assume a relevant route of exposure in firefighters, the assessment of PAHs deposited in the contaminated and uncleaned firefighting ensembles, as well as in firefighters most exposed skin (neck, face, hands and arms) should be also determined. Additional research is needed to estimate the contribution

of dermal exposure to the total internal dose of OH-PAHs in order to evaluate the extension of the protection given by the personal protective devices. The evaluation of exposure to other relevant air pollutants (CO, NO₂, toxic elements including Pb, Cd, As, and other VOCs) released during wildland fires should be considered. The use of biomarkers of effect (micronucleous in lymphocytes and buccal cells) and susceptibility (polymorphisms of genes involved in xenobiotic metabolism and DNA repair) combined with clinical tests (such as anthropometry, vital signs and pulmonary function tests) would be precious to attain complementary information about the potential health risks.

Finally, results for a larger number of sampling sites, participants, type and number of samples (air, food and urine) are also crucial to draw more meaningful and statistically more significant conclusions. Altogether this information will allow a more precise estimation of the potential health risks associated with environmental and/or occupational exposure to air pollutants, and will support the development of mitigation strategies. Solutions must be found that involve technological development, structural changes, including the optimisation of infrastructures and urban planning, and behavioural changes. Furthermore there is a clear need to establish air quality international guidelines or limits, particularly in (pre)schools (micro)environments.