Chemosphere xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Urinary polycyclic aromatic hydrocarbon metabolites levels in a representative sample of the Spanish adult population: The BIOAMBIENT.ES project

Mónica Bartolomé [a,1], Juan José Ramos [a,1], Francisco Cutanda [a], Olga Huetos [a], Marta Esteban [a], Montserrat Ruiz-Moraga [b], Eva Calvo [b], Beatriz Pérez-Gómez [c,d], Oscar González [a], Argelia Castaño [a,*], BIOAMBIENT.ES [2]

[a] Environmental Toxicology, National Center for Environmental Health (CNSA), Institute of Health Carlos III, 28220 Madrid, Spain
[b] Ibermutuamur, Ramírez de Arellano 27, Madrid, Spain
[c] National Centre for Epidemiology, Institute of Health Carlos III, 28029 Madrid, Spain
[d] CIBER in Epidemiology and Public Health (CIBERESP), Spain

HIGHLIGHTS

- A nationwide Human Biomonitoring survey on PAH exposure performed in Spanish adults.
- 1-Hydroxypyrene and hydroxyphenanthrenes were used as biomarkers of PAH exposure.
- The influence of lifestyle and personal features on PAH body burden were assessed.
- For the first time, reference values of these biomarkers were established in Spain.
- Levels were in the same range or lower than those reported in neighboring countries.

ARTICLE INFO

Article history:
Received 4 May 2014
Received in revised form 18 November 2014
Accepted 2 December 2014
Available online xxxx

Handling Editor: A. Gies

Keywords:
Human Biomonitoring
PAH urinary metabolites
1-Hydroxypyrene
Hydroxyphenanthrene
Spain

ABSTRACT

In 2009, the Spanish Ministry of Agriculture, Food and Environment promoted the BIOAMBIENT.ES project, a Human Biomonitoring program on the national scale to estimate reference levels of environmental pollutants on a representative sample of the Spanish adults. The present study focuses on polycyclic aromatic hydrocarbons (PAHs). The urinary metabolites 1-hydroxypyrene, 1-,2-,3-,4- and 9-hydroxyphenanthrene and 3-hydroxybenzo[a]pyrene were selected as indicators of PAH exposure. Urine samples from 957 subjects (16–65 years old) were collected during year 2009–2010. Geometric mean and 95th percentile for 1-hydroxypyrene in $\mu g\ g^{-1}$ creatinine were 0.117 (non-smoker: 0.079, smokers: 0.184) and 0.67 $\mu g\ g^{-1}$ creatinine (non-smokers: 0.31, smokers: 0.69) respectively. GM and 95th percentile for sum of hydroxyphenanthrenes in $\mu g\ g^{-1}$ creatinine were 0.130 (non-smokers: 0.089, smokers: 0.317) and 1.29 (non-smokers: 0.71, smokers: 1.51) respectively. 3-Hydroxybenzo[a]pyrene was below the limit of quantitation (0.05 $\mu g\ L^{-1}$) in all cases. Significant differences ($p < 0.05$) regarding smokers and non-smokers, coal and wood heating, body mass index and second hand smoke were found, while other variables like gender, age, or diet showed no significant association. The geographical distribution of the metabolites showed higher levels in people who lived in the north and northwest of Spain. The PAH metabolites levels found were in the same range or lower than those reported from other European countries and they were higher than those found in the U.S. This study represents the first nationwide survey of exposure to PAHs in Spain and provides a background reference range for exposure to PAHs in the Spanish adult occupied population.

Abbreviations: PAH, polycyclic aromatic hydrocarbon; 1-HP, 1-hydroxypyrene; PHh, hydroxyphenanthrene; SPE, solid phased extraction; HPLC-FD, high resolution liquid chromatography with fluorimetric detection; LOQ, limit of quantification; LOD, limit of detection; GM, geometric mean; CI, confidence intervals; SHS, second hand smoke; BMI, body mass index..

* Corresponding author. Tel.: +34 918223540.
E-mail address: castano@isciii.es (A. Castaño).

[1] The first two authors contributed equally to the article and both should be considered as first authors.

[2] BIOAMBIENT.ES: Aleixandre, J.L.; Aragonés, N.; Cañas, A.I.; Cervantes-Amat, M.; Cortés, M.V.; Jimenez, J.A.; López-Abente, G.; López-Herranz, A.; Mayor, J.; Méndez, C.F.; Molina, M.A.; Navarro, C.; Pollán, M.; Pastor, R. and Román, J.

http://dx.doi.org/10.1016/j.chemosphere.2014.12.008
0045-6535/

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemicals with two or more fused benzene rings in their structure. The sources of PAHs are classificated into two origins, petrogenic (unburned petroleum and its product) and pyrolytic (natural or synthetic organic materials undergo incomplete combustion). Depending on the pyrolytic process and the material, a complex mixture of several compounds is released to the environment. Therefore, PAHs are ubiquitous in rural and urban environments and can give rise to human exposure from a variety of sources, such as dietary, air pollution from automobile, industrial or incinerator emissions, as well as incomplete combustion of organic compounds such as coal, gas, oil, wood and tobacco (Mastral and Callén, 2000). The main reason for concern after human exposure is that several PAHs are considered by International Agency of Research on Cancer (IARC) as carcinogenic (group 1) or potentially carcinogenic in humans (group 2a and 2b) (IARC, 1983). The European Union has listed these pollutants in the Annex III of the Regulation 850/2004/EC on Persistent Organic Pollutants (POPs), as substances subjected to release reduction provisions.

Diet constitutes the main source to PAH exposure for the general non-smoking population (Phillips, 1999; Martí-Cid et al., 2008) whereas, exposure by inhalation is comparatively low in non-smoker population but an important source of PAH exposure in smokers (Grainger et al., 2006). PAHs are metabolized to their monohydroxylated PAH metabolites and finally conjugated to glucuronides and sulfates, which are excreted in urine and bile (Chetiyanukornkul et al., 2006).

1-Hydroxypyrene (1-HP) is the principal metabolite of the four-ring PAH pyrene, representing 90% of pyrene urinary excretion in humans (Brzeznicki et al., 1997). 1-HP is the preferred biomarker to assess PAH exposure both in general population and professionals (Jongeneelen, 1997; Freire et al., 2009; Li et al., 2010). 1-HP has been used to assess PAH exposure from tobacco smoke (Siwinska et al., 1998), air pollution from traffic (Tsai et al., 2004), area of residence (Schroijen et al., 2008) and the dietary exposure (Ibáñez et al., 2005). Several studies have shown widespread exposure to phenanthrene in the general population, which finds expression in urinary hydroxyphenanthrene (HPh) excretion that is detected by urine analysis. Thus, the 5 isomers of HPh, 1-, 2-, 3-, 4- and 9-HPh, either individually or as a sum, have been used as biomarkers (Hecht, 2002; Chetiyanukornkul et al., 2006). Recently, the main urinary metabolite of benzo(a)pyrene, 3-hydroxybenzo[a]pyrene, has also been considered as a potential biomarker of exposure to carcinogenic polycyclic aromatic hydrocarbons (Barbeau et al., 2009).

Human Biomonitoring (HBM) provides an accurate estimate of the exposure level to chemicals by measuring the concentrations of these compounds or its metabolites in humans. HBM takes into account different exposure sources and routes, individual variability or lifestyle differences. Several countries have systematically established HBM programs in their general population. The first of these initiatives was carried out by the National Health and Nutrition Examination Surveys in the mid-70s in the U.S. (CDC, 2011). The HBM program was pioneered in Europe by the German Environmental Survey (GerES) at the beginning of the 90s (Seifert et al., 2000). Afterward similar projects have also been implemented in other countries (IVL, 2010; Sul et al., 2012; Berman et al., 2013).

In 2009, the Ministry of Agriculture, Food and Environment funded the BIOAMBIENT.ES project, the first national level HBM survey on environmental pollutants carried out in Spain to estimate the levels of selected pollutants in a representative sample of Spanish adults. In the present work, the Spanish occupied population has been studied, which represented 63% of adult population in Spain in 2009, with a survey designed to cover all geographical areas, gender, and activity sectors. The results provided by this study will be useful to establish reference values, to identify highly exposed populations or geographical areas in the country, to compare the PAH levels obtained with other countries, and eventually to serve as a base in the future to evaluate temporary trends and effectiveness of environmental and health policies.

2. Materials and methods

2.1. Study design

The detailed design of the nationwide cross-sectional epidemiological study BIOAMBIENT.ES, as well as the ethical approval, has been described previously (Pérez-Gómez et al., 2013). In summary, participants were recruited among employed people older than 16 years, living in Spain at least for the 5 years previous to the study, which underwent their annual occupational medical check-up in health facilities run by Societies for Prevention of IBERMUTUAMUR, MUTUALIA, MC-PREVENCIÓN, MUGATRA, UNIMAT PREVENCIÓN, and PREVIMAC from March 2009 to July 2010. Participants were selected through a stratified cluster sampling which covered all geographical areas, gender, and activity sectors, with the aim of obtain a representative sample of Spanish occupied population.

The sample obtained had a total number of 1892 people (Pérez-Gómez et al., 2013); 1860 of them supplied a urine specimen, although 90 were discarded because of insufficient amount of sample. Of the 1770 urine specimens, a subset of 993 was randomly selected, keeping ratios of genders, geographical areas, occupational sector and age.

Individual information on sociodemographic data, lifestyle and environmental conditions—including specific questions on tobacco active and passive exposure, was registered in a self-administered epidemiological questionnaire. A food frequency questionnaire to register usual diet in the last year of the participants was designed including the following food groups: eggs, fruits, meat, cold meat, bread and cereals, legumes, potatoes-rice and pasta, dairy, vegetables, fish and sweets. Data from the medical check-up were also provided.

2.2. Analysis of urine samples

Participants collected their first morning urine samples in a provided prewashed containers (10% nitric acid and Milli-Q). Samples were stored at 4 °C and shipped to the laboratory within the first 96 h after collection. Urine aliquots were stored at −20 °C until analysis (Esteban et al., 2013).

The analysis of PAH metabolites in urine was an adaptation of methods described previously (Gündel et al., 2000; Jongeneelen, 2001). Briefly, 1 mL of sodium acetate buffer (0.5 M, pH 4.8), and 50 μL of β-glucuronidase arylsulfatase enzyme were added to 10 mL of urine. The pH was controlled and corrected with hydrochloric acid 1 N. After 18 h at 37 °C, the samples were centrifuged 10 min at 4000 rpm. PAH metabolites were extracted by solid phased extraction (SPE) using C_{18} 500 mg cartridges and methyl terc-butyl ether as solvent extraction. Once evaporated to dryness and re-dissolved in 200 μL of acetonitrile, the extract was analyzed by high resolution liquid chromatography with fluorimetric detection (HPLC-FD). Quantification of 1-HP and HPhs were done at 242 nm and 388 nm excitation and emission wavelength respectively, whereas 262 nm and 462 nm wavelength were selected for 3 hydroxybenzo[a]pyrene. A RP-8 250 × 4 mmID column and a mixture of acetonitrile/sodium acetate buffer 10 mM pH 5.0 (54%/46%) as mobile phase were used.

Please cite this article in press as: Bartolomé, M., et al. Urinary polycyclic aromatic hydrocarbon metabolites levels in a representative sample of the Spanish adult population: The BIOAMBIENT.ES project. Chemosphere (2015), http://dx.doi.org/10.1016/j.chemosphere.2014.12.008