Chemosphere 352 (2024) 141330

Contents lists available at ScienceDirect

Chemosphere

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

Risk assessment and predictors of the exposure to polycyclic aromatic hydrocarbons in Spanish adults by urinary human biomonitoring

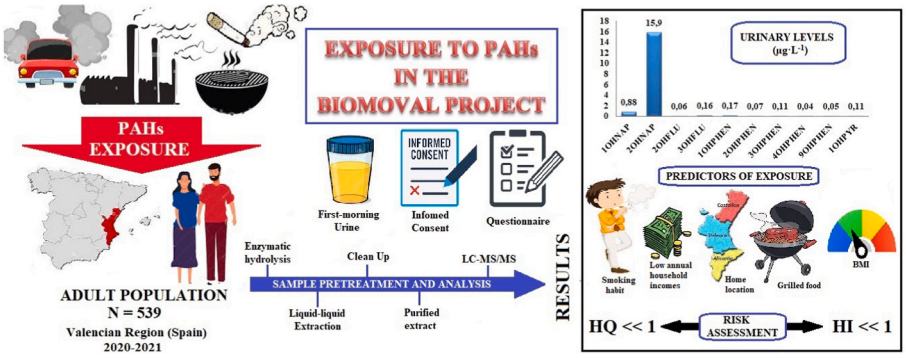
Borja Peris-Camarasa [a,b], Olga Pardo [b,*], Sandra F. Fernández [a,b], Pablo Dualde [a], Clara Coscollà [a]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Avda. Cataluña, 21, 46020, Valencia, Spain
[b] Department of Analytical Chemistry, University of Valencia, Doctor Moliner, 50, 46100, Burjassot, Spain

HIGHLIGHTS

- Overall, PAH biomarkers were quantified in more than 65 % of the urine samples.
- The highest biomarkers levels and EDIs were found for naphthalene.
- Smoking status was the predominant factor of PAH exposure.
- HQs of naphthalene, and consequently, HIs were above 1, especially for smokers.
- Risk assessment suggests that health risks could not be discarded.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: A. Gies

Keywords:
Polycyclic aromatic hydrocarbons
Human biomonitoring
Urine
Adult population
Risk assessment
Spain

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are produced primarily during incomplete combustion of organic matter and in various industrial processes. They are widespread environmental pollutants that are of significant interest due to their potential toxicity. Humans can be exposed to PAHs through ingestion, inhalation and dermal contact. In the present study, ten urinary PAH biomarkers were determined in first-morning urine samples (n = 504) from the adult population (aged 18–65 years) residing in the Valencian Region of Spain. These samples were analysed using liquid-liquid extraction followed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). All PAH biomarkers were quantified in more than 65 % of the urine samples. Naphthalene biomarkers, 1-hydroxynaphthalene (1OHNAP) and 2-hydroxynaphthalene (2OHNAP), exhibited the highest levels with geometric means (GMs) of 0.7 and 11.9 μg L^{-1}, respectively. The 95th percentile of all PAH biomarkers ranged from 0.22 to 64.8 μg L^{-1}. Estimated daily intakes (EDIs) for the analysed PAH families in the studied population ranged from 17 (pyrene) to 18581 (naphthalene) ng·kg-bw^{-1}·day^{-1} (GM). Significant associations were observed between the quantified urinary metabolites of PAHs and smoking status, home location, annual household incomes, BMI, and the consumption of grilled food in the last 24 h. Hazard quotients (HQs) of naphthalene and consequently, hazard indexes (HIs) were above 1, especially for smokers. Therefore, potential health risks associated with PAH exposure in the target population could not be discarded.

* Corresponding author.
E-mail address: olga.pardo@uv.es (O. Pardo).

https://doi.org/10.1016/j.chemosphere.2024.141330
Received 27 October 2023; Received in revised form 26 January 2024; Accepted 28 January 2024
Available online 31 January 2024

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), composed of two or more fused aromatic rings, are Persistent Organic Pollutants (POPs) produced during the incomplete combustion of organic matter, either naturally (e.g., bush fires and volcanoes) or anthropogenically (e.g., vehicular emissions, tobacco smoke, waste incineration, etc.), as well as in various industrial processes (tar distillation, charcoal burning, etc.).

The primary source of human exposure to PAHs is the ingestion of contaminated foodstuffs (EFSA, 2008). This exposure largely depends on food contaminations and cooking processes (Cao et al., 2020). PAHs are formed during culinary practises carried out at high temperatures, such as roasting, smoking and frying (EFSA, 2008; Health Canada, 2017). According to the European Food Safety Authority (EFSA), cereals and seafood are the highest contributors to dietary PAH exposure, but PAHs can also be present in meat, eggs, leafy vegetables, dairy products, etc. Inhalation of tobacco smoke is another significant source of PAH exposure. Rodgman and Perfetti (2013) and Vu et al. (2015) reported that cigarette smoke comprises several PAHs, predominantly consisting of lower-molecular-weight compounds such as naphthalene, fluorene and phenanthrene. Moreover, individuals living near refineries or waste incineration sites and those occupationally exposed (e.g., firefighters, asphalt workers, etc.) may be exposed to PAHs through inhalation of polluted air and by dermal uptake (EFSA, 2008).

Once PAHs enter the human body, they undergo biotransformation and metabolism in the liver through two phases. In phase I, PAHs are hydrolysed to hydroxy-PAHs (OHPAHs) by cytochrome P450 enzymes, resulting in the formation of reactive intermediate and more polar metabolites, which finally can be used as biomarkers. In phase II, the OHPAHs are conjugated to glucuronic acid or sulphate to increase their water solubility, enabling their excretion through urine (in the case of low molecular-weight PAHs with 2–3 aromatic rings) or faeces (in the case of high molecular-weight PAHs with 4 or more aromatic rings) (Cao et al., 2020). These metabolic processes help to eliminate PAHs from the body.

The toxicity of PAHs has been extensively explored due to their carcinogenic, mutagenic, genotoxic, and teratogenic properties (EFSA, 2008). The United States Environmental Protection Agency (USEPA) has identified a list of 16 PAHs for monitoring, considering their potential impact on human health and/or their ecological toxicity (USEPA, 2022). Furthermore, the International Agency for Research on Cancer (IARC) has classified certain PAHs into risk-prioritization groups. For example, benzo(a)pyrene (BaP) has been classified as a human carcinogen (Group 1), while some PAHs like naphthalene, anthracene and chrysene are classified as “possibly carcinogenic to humans” (Group 2B) (IARC, 2023; Cattley et al., 2023). Other compounds, such as fluorene, phenanthrene and pyrene have been deemed as unclassifiable (Group 3) due to the limited or insufficient experimental evidence (IARC, 2023). PAHs can have adverse effects on the immune system, development and reproduction in humans. They have been found to disrupt seminal DNA and impact male fertility. The U.S. Centres for Disease Control and Prevention (CDC) has indicated that several OHPAHs are significantly associated with self-reported cardiovascular diseases in humans (Guo et al., 2013). The potential effects of PAHs on Metabolic Syndromes (MetS) such as obesity, diabetes, high cholesterol, and hypertension have also been reported on different population groups (Shahsavani et al., 2021).

The European Commission included PAHs in the list of substances subject to emissions reduction provisions (European Commission, 2014). For this reason, several regulations and mitigation strategies have been taken in the European Union (EU) to reduce PAH exposure in the population: i) maximum levels (MLs) have been established for benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene in foods; ii) certain PAHs have been identified as priority substances to be controlled in water and ambient air quality; iii) and limits on PAH content in plastic and rubber articles have been set (European Commission, 2011, 2013; The European Parliament and the Council of the European Union, 2000; 2016). Nevertheless, PAHs continue to be a public health concern, leading the Agency for Toxic Substances and Disease Registry (ATSDR) and the Human Biomonitoring for Europe (HBM4EU) initiative to include PAHs in their respective lists of priority substances (ATSDR, 2022; HBM4EU, 2021). Therefore, further understanding of overall exposure to PAHs is still necessary.

Human biomonitoring (HBM) is useful for determining the exposure of the population to a wide range of PAHs by measuring the concentrations of their biomarkers in biological samples, as it takes into account all exposure pathways. HBM provides an accurate estimate of exposure levels (Bartolomé et al., 2015; Health Canada, 2017) and it may help to evaluate the effectiveness of mitigation measures adopted in Europe (HBM4EU, 2018). Different matrices can be examined, but due to the relatively short half-life of the low molecular-weight PAHs in the human body (a few hours), urine is the preferred matrix for HBM of these compounds. Urinary levels of OHPAHs are used as biomarkers of recent human exposure to PAHs (Guo et al., 2013; Shahsavani et al., 2021). Among the OHPAHs, 1-hydroxypyrene (1OHPYR) is the most commonly used biomarker to represent exposure to PAHs in most HBM studies. However, considering that PAHs are emitted from various sources, the biomonitoring of additional OHPAHs together with 1OHPYR, such as hydroxylated compounds of naphthalene, fluorene, phenanthrene and benzo(a)pyrene (Fernández et al., 2021b), would provide a more comprehensive assessment of PAH exposure (Dobraca et al., 2018).

The internal exposure to PAHs has been reported worldwide. In Spain, the BIOAMBIENT. ES project (2009) was the first study that reported urinary PAH metabolite levels on Spanish adults, specifically 1OHPYR and several phenanthrenes. In 2021, two studies on PAH exposure in children and lactating mothers, residing in the Valencian Region of Spain, provided more detailed information about this, revealing urinary levels of 11 metabolites of PAHs, including naphthalene, fluorene, phenanthrene, pyrene, and benzo(a)pyrene (Fernández et al., 2021a, 2021b). Nevertheless, there is still a need for updated and comprehensive data on PAH exposure in the adult Spanish population. Therefore, to better understand PAH exposure in this population group, in the present study, urinary levels of 10 biomarkers of naphthalene, fluorene, phenanthrene and pyrene were determined in urine samples from the adult population residing in the Valencian Region (Spain). Additionally, the relationship between urinary levels of each PAH family and the characteristics of the population was investigated and the risk assessment of PAH exposure was also performed. The results of this study were useful to establish reference values and to identify potential contributors to PAH exposure.

2. Materials and methods

2.1. Study population and recruitment of participants

The present study was part of the BIOMOVAL project, a three-year (2021–2023) study promoted by the Directorate General of Public Health and Addictions together with the Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (FISABIO). It focuses on determining the internal exposure to certain environmental and food contaminants on the adult population residing in the Valencian Region of Spain. The recruitment of participants took place during 2021 at various Prevention Health Centres affiliated with the Association of Occupational Risk Prevention Services of the Valencian Region (SERPRECOVA), Unimat Prevention and the University of Jaume I (UJI). The recruitment process has been fully described before in Peris-Camarasa et al. (2023).

2.2. Data and sample collection

A total of 539 out of 548 subjects, who were selected to participate in this study, completed the questionnaire and provided enough first-

morning urine samples. These spot samples were collected in 100-mL polypropylene containers and rapidly transported within 24 h using isothermal packaging at 4 °C to prevent compound degradation. Finally, the samples were stored in 10-mL aliquots at −80 °C in the Biobank for Biomedical Research and Public Health of the Valencian Region (IBSP-CV Biobank, Spain; PT13/0010/0064) to prevent successive cycles of freezing – thawing during the corresponding analysis.

Urinary levels of creatinine (Cre) and specific gravity (SG) were measured to address differences in dilution across the urine samples. Cre was determined using the Jaffé's reaction method in a KROMA biochemical parameter analyser from Linear Chemicals (Barcelona, Spain), while SG values were obtained by measuring the Brix grades of urine samples, prior to storage, using a refractometer from Lab Logistics Group GmbH (Meckenheim, Germany). Urine samples with Cre concentrations out of the range of 30–300 mg dL^{-1} (Barr et al., 2005) were excluded from the present research.

2.3. Ethical and confidentiality

The BIOMOVAL project was approved by the Ethics Committee for Research of the General Directorate of Public Health and Centre for Advanced Research in Public Health of the Valencian Government. Prior to data and sample collection, all participants provided a written informed consent. All personal data were handled, transferred and analysed in a secure environment and will be retained only for the necessary processing period. The processing of data adhered to all national and EU legal and ethical guidelines, specifically the General Data Protection Regulation.

2.4. Determination of biomarkers

In total, 10 urinary PAH biomarkers were determined using the methodology previously described by Fernández et al. (2021a). The 10 OHPAHs quantified in the present study, along with their corresponding internal standards (IS) are presented in Table S1.

In summary, 2.5 mL of urine was spiked with 25 μL of the IS working solution and in 0.5 M acetate buffer (pH 5.0). The mixture underwent enzymatic hydrolysis in the presence of 10 μL of β-glucuronidase/arylsulfatase (from Helix pomatia; Merck, Barcelona, Spain) for 18 h at 37 °C in darkness. Following that, the mixture was subjected to a liquid-liquid extraction (LLE), with 4 mL of ethyl acetate twice, and both organic extracts were transferred to a Supel™ QuE Z-Sep tube and shaken for 1 min. After centrifugation, the purified extract was evaporated to dryness under N_2 stream at 25 °C. Finally, the residue was dissolved in 100 μL of water:methanol (40:60) containing 0.1 % acetic acid and transferred into an injection vial for analysis into a Vanquish high-performance liquid chromatography system (Thermo Fisher Scientific, Germany) equipped with a KINETEX Core-shell technology F5 (Phenomenex, USA) column (150 × 2.1 mm, 2.6 μm), and coupled to a TSQ Altis™ detector (San José, CA, USA) tandem mass spectrometry system equipped with an electrospray ionization source (ESI) in the negative ion mode. The limits of quantification (LoQ) of the target metabolites are presented in Table 2. The procedure and criteria for quality assurance/quality control (QA/QC) and method validation are fully described in Fernández et al. (2021a).

2.5. Statistical analysis

IBM SPSS Statistics and R software were used to perform the statistical analysis. First, the maximum likelihood estimation (MLE) method proposed by EFSA (EFSA, 2010) was applied to estimate the concentration of unquantified OHPAHs (<LoQ). The following parameters of the OHPAH concentrations, unadjusted (μg·L^{-1}), and adjusted by SG (μg·L^{-1}) and urinary Cre levels (μg·g Cre^{-1}), were summarized: detection frequency (DF, %), arithmetic mean (AM), geometric mean (GM), 25th, 50th, 75th and 95th percentiles (P25, P50, P75, and P95),

Table 1
Socio-demographic characteristics, lifestyle and dietary habits of the studied population (n = 504).

Variable	N (%)	Median (range)
Socio-demographic characteristics		
Sex		
Male	266 (52.8)	–
Female	238 (47.2)	–
Age (years)	–	*43 (18–65)*
18–30	103 (20.4)	–
31–40	106 (21.0)	–
41–50	160 (31.8)	–
51–65	135 (26.8)	–
Province		
Castellon	60 (11.9)	–
Valencia	265 (52.6)	–
Alicante	179 (35.5)	–
BMI ($kg{\cdot}m^{-2}$)	–	*24.91 (16.94–56.30)*
<18.50	11 (2.2)	–
18.50–24.99	247 (49.0)	–
≥25.0	246 (48.8)	–
Annual household income (euros, €)		
<14499	70 (13.9)	–
14500-19999	111 (22.0)	–
20000-34999	170 (33.8)	–
35000-59999	104 (20.6)	–
≥60000	49 (9.7)	–
Residence style		
Chalet or detached house	61 (12.1)	–
Townhouse	44 (8.7)	–
Flat or apartment	383 (76.0)	–
Country house or others	16 (3.2)	–
Distance from trafficked roads		
Very Far	147 (29.2)	–
Far	100 (19.8)	–
Close	169 (33.5)	–
Very close	87 (17.3)	–
NS/NC	1 (0.2)	–
Distance from industries		
Very Far	327 (64.9)	–
Far	135 (26.8)	–
Close	35 (6.9)	–
Very close	5 (1.0)	–
NS/NC	2 (0.4)	–
Distance from agricultural activities		
Very Far	232 (46.0)	–
Far	164 (32.5)	–
Close	82 (16.3)	–
Very close	25 (5.0)	–
NS/NC	1 (0.2)	–
Lifestyle habits		
Smoking status		
Non-smoker	306 (60.7)	–
Passive smoker	68 (13.5)	–
Smoker	130 (25.8)	–
Reside with a smoker		
Yes	124 (24.6)	–
No	377 (74.8)	–
NS/NC	3 (0.6)	–
Dietary habits		
Food related to PAH exposure consumed last 24h		
Meat (grams)	–	150 (0–1350)
Seafood (grams)	–	0 (0–750)
Fish (grams)	–	0 (0–2250)
Bread (grams)	–	115 (0–800)
Rice (grams)	–	0 (0–375)
Consumption of fried food last 24h		
Yes	138 (27.4)	–
No	365 (72.4)	–
Missing data	1 (0.2)	–
Consumption of grilled food last 24h		
Yes	21 (4.2)	–
No	482 (95.6)	–
Missing data	1 (0.2)	–
Consumption of smoked food last 24h		
Yes	31 (6.2)	–

(continued on next page)

Table 1 (*continued*)

Variable	N (%)	Median (range)
No	471 (93.4)	–
Missing data	2 (0.4)	–
Consumption of baked food last 24h		
Yes	186 (36.9)	–
No	315 (62.5)	–
Missing data	3 (0.6)	–
Consumption of roasted food last 24h		
Yes	162 (32.1)	–
No	336 (66.7)	–
Missing data	6 (1.2)	–

minimum and maximum values, and standard deviation (SD). The adjustment by SG was performed using the formula reported by Kuiper et al. (2021). Next, OHPAHs with the same PAH parent were converted to nmol·L^{-1} and nmol·g Cre^{-1}, and added together as follows: the sum of hydroxynaphthalenes (∑OHNAPs) = 1OHNAP + 2OHNAP; the sum of hydroxyfluorenes (∑OHFLUs) = 2OHFLU + 3OHFLU; and the sum of hydroxyphenanthrenes (∑OHPHENs) = 1OHPHEN + 2OHPHEN + 3OHPHEN + 4OHPHEN + 9OHPHEN. Furthermore, the most relevant descriptors of the study population were extracted from the questionnaires. Qualitative variables were described using absolute frequencies and percentages; while quantitative variables were summarized using their median, minimum and maximum values (see Table 1). In relation to smoking status, based on the information available in the questionnaires, individuals who were not active smokers but lived with a smoker were classified as passive smokers. Additionally, data obtained from

Table 2
Descriptive statistics of the urinary OHPAHs concentrations from Spanish adult population (n = 504).

Biomarker	LoQ	DF (%)	P25	P50	AM	GM	P75	P95	Range[a]	SD	Units
Unadjusted											
1OHNAP	0.01	85	0.28	0.74	4.51	0.69	2.99	17.1	<0.01–513	23.6	μg·L^{-1}
2OHNAP	0.01	93	6.32	12.8	20.1	11.9	25.2	64.8	<0.01–222	22.6	
[b]∑OHNAPs	–	–	59	109	192	113	238	621	0.05–4056	263	nmol·L^{-1}
2OHFLU	0.01	66	0.01	0.08	0.27	0.05	0.24	1.35	<0.01–5.80	0.57	μg·L^{-1}
3OHFLU	0.05	73	0.05	0.11	0.40	0.13	0.30	2.15	<0.05–5.88	0.80	
[c]∑OHFLUs	–	–	0.6	1.2	4.0	1.5	3.2	17.6	0.04–58	7.0	nmol·L^{-1}
1OHPHEN	0.01	91	0.07	0.13	0.25	0.13	0.25	0.83	<0.01–10.9	0.58	μg·L^{-1}
2OHPHEN	0.01	88	0.03	0.05	0.09	0.05	0.09	0.31	<0.01–2.11	0.17	
3OHPHEN	0.01	92	0.04	0.08	0.17	0.09	0.16	0.57	<0.01–4.54	0.37	
4OHPHEN	0.01	78	0.01	0.03	0.07	0.03	0.06	0.22	<0.01–4.27	0.21	
9OHPHEN	0.01	84	0.02	0.04	0.09	0.04	0.08	0.37	<0.01–1.10	0.14	
[d]∑OHPHENs	–	–	1.2	2.0	3.8	2.1	3.8	12.3	0.2–119	7.4	nmol·L^{-1}
1OHPYR	0.01	88	0.04	0.08	0.18	0.08	0.17	0.61	<0.01–3.45	0.31	μg·L^{-1}
[e]∑OHPAHs	–	–	62	115	200	120	250	633	4–4061	269	nmol·L^{-1}
Adjusted by SG											
1OHNAP$_{SG}$	–	–	0.38	0.95	5.32	0.88	3.91	19.5	<0.01–587	27.1	μg·L^{-1}
2OHNAP$_{SG}$	–	–	8.52	16.1	25.2	15.9	29.3	72.6	0.01–464	34.5	
[b]∑OHNAPs$_{SG}$	–	–	73	144	238	143	288	717	0.04–4635	358	nmol·L^{-1}
2OHFLU$_{SG}$	–	–	0.01	0.11	0.33	0.06	0.27	1.58	0.01–15.7	0.91	μg·L^{-1}
3OHFLU$_{SG}$	–	–	0.07	0.13	0.46	0.16	0.35	2.41	0.05–5.43	0.84	
[c]∑OHFLUs$_{SG}$	–	–	0.8	1.5	4.8	1.9	4.1	21.0	0.08–107	8.6	nmol·L^{-1}
1OHPHEN$_{SG}$	–	–	0.09	0.17	0.29	0.17	0.29	0.94	<0.01–10.1	0.57	μg·L^{-1}
2OHPHEN$_{SG}$	–	–	0.04	0.06	0.11	0.07	0.11	0.36	<0.01–1.81	0.18	
3OHPHEN$_{SG}$	–	–	0.06	0.10	0.20	0.11	0.20	0.64	<0.01–4.19	0.39	
4OHPHEN$_{SG}$	–	–	0.02	0.04	0.08	0.04	0.07	0.26	<0.01–3.94	0.21	
9OHPHEN$_{SG}$	–	–	0.02	0.05	0.11	0.05	0.11	0.44	<0.01–2.48	0.18	
[d]∑OHPHENs$_{SG}$	–	–	1.5	2.4	4.5	2.7	4.6	14.2	0.16–110	7.5	nmol·L^{-1}
1OHPYR$_{SG}$	–	–	0.06	0.10	0.21	0.11	0.21	0.71	<0.01–3.97	0.33	μg·L^{-1}
[e]∑OHPAHs$_{SG}$	–	–	79	151	248	152	304	748	3–4641	366	nmol·L^{-1}
Adjusted by Cre											
1OHNAP$_{Cre}$	–	–	0.28	0.66	3.66	0.62	2.60	13.3	<0.01–417	19.1	μg·g Cre^{-1}
2OHNAP$_{Cre}$	–	–	5.70	11.4	17.0	11.1	20.7	50.9	<0.01–229	20.9	
[b]∑OHNAPs$_{Cre}$	–	–	50	101	161	100	195	480	0.04–3298	225	nmol· g Cre^{-1}
2OHFLU$_{Cre}$	–	–	0.01	0.07	0.21	0.04	0.19	1.03	<0.01–3.61	0.44	μg·g Cre^{-1}
3OHFLU$_{Cre}$	–	–	0.05	0.10	0.32	0.11	0.25	1.63	<0.05–3.53	0.56	
[c]∑OHFLUs$_{Cre}$	–	–	0.6	1.0	3.2	1.4	3.0	14.5	0.05–37.2	5.1	nmol· g Cre^{-1}
1OHPHEN$_{Cre}$	–	–	0.07	0.11	0.19	0.12	0.22	0.58	<0.01–7.13	0.38	μg·g Cre^{-1}
2OHPHEN$_{Cre}$	–	–	0.03	0.04	0.08	0.05	0.08	0.21	<0.01–1.30	0.11	
3OHPHEN$_{Cre}$	–	–	0.04	0.07	0.14	0.08	0.13	0.45	<0.01–2.97	0.25	
4OHPHEN$_{Cre}$	–	–	0.01	0.03	0.05	0.02	0.05	0.17	<0.01–2.79	0.14	
9OHPHEN$_{Cre}$	–	–	0.02	0.03	0.08	0.03	0.08	0.32	<0.01–1.03	0.11	
[d]∑OHPHENs$_{Cre}$	–	–	1.1	1.6	3.0	1.9	3.2	9.4	0.11–77.8	4.9	nmol· g Cre^{-1}
1OHPYR$_{Cre}$	–	–	0.04	0.07	0.14	0.07	0.15	0.49	<0.01–2.76	0.23	μg·g Cre^{-1}
[e]∑OHPAHs$_{Cre}$	–	–	54	106	168	106	202	508	3–3301	228	nmol· g Cre^{-1}

LoQ = Limit of quantification; DF (%) = Detection Frequency (% samples > LoQ); P = percentile; AM = Arithmetic mean; GM = geometric mean; SD = Standard deviation.
Suffix (Cre): Results of urinary PAHs metabolites corrected by urinary creatinine (Cre) levels.
Suffix (SG): Results of urinary PAHs metabolites corrected by specific gravity (SG) values.
[a] Minimum – Maximum.
[b] ∑OHNAPs = 1OHNAP + 2OHNAP.
[c] ∑OHFLUs = 2OHFLU + 3OHFLU.
[d] ∑OHPHENs = 1OHPHEN + 2OHPHEN + 3OHPHEN + 4OHPHEN + 9OHPHEN.
[e] ∑OHPAHs = ∑OHNAPs +∑OHFLUs +∑OHPHENs + 1OHPYR.

food intake in the last 24 h were clustered in food groups such as meat, fish, etc.

The Spearman's correlation test was performed to determine correlations among levels of PAH biomarkers individually. In addition, linear regression models were built to explore the relationship between the exposure variables (see Table 1) and each PAH family (naphthalene (∑OHNAPs), fluorene (∑OHFLUs), phenanthrene (∑OHPHENs) and pyrene (1OHPYR)). A forward stepwise selection procedure based on the corrected Akaike information criterion (AICc) (Konishi and Kitagawa, 2008) was employed to this end, considering the non-normal distribution of the raw data (nmol·L^{-1}). For this purpose, the logarithms of the data were used as dependent variables and statistical significance was set at 0.05. In these regression models, similar to Peris-Camarasa et al. (2023), SG was included as independent variable to account for the impact of hydration states on urinary levels. Following the construction of the regression models, several tests were conducted to verify the regression assumptions, including normality, non-autocorrelation, homoscedasticity, and non-multicollinearity. The Kolgomorov-Smirnov test, Durbin-Watson statistic, scatter plots, as well as tolerance and condition indexes, were employed for this purpose. Finally, while the risk assessment indicators are calculated using the three correction methods (unadjusted and adjusted by SG and Cre levels), our primary focus was on the correction by SG.

2.6. Risk assessment

Humans are exposed to PAHs in different ways, with dietary intake considered the main source of exposure in the general population (EFSA, 2008). However, inhalation exposure constitutes a significant pathway, occasionally surpassing the risks associated with ingestion, especially for smokers. In this study, considering all available sources of exposure, PAH exposure was assessed using an internal dose approach. For this purpose, the estimated daily intake (EDI), both unadjusted and adjusted by SG and Cre levels was calculated using the molar concentrations at GM of urinary PAH levels grouped for each parent compound, as described previously (Section 2.5). This calculation employed the formulas provided by Peng et al. (2020) and the parameters reported in Table S2.

$$EDI_{Vol}\left(\frac{ng}{kg-bw{\cdot}day}\right)=\frac{Conc_{vol}\ x\ V_{24h}\ x\ MW}{F_{UE}\ x\ BW} \quad (1)$$

$$EDI_{SG}\left(\frac{ng}{kg-bw{\cdot}day}\right)=\frac{Conc_{SG}\ x\ V_{24h}\ x\ MW}{F_{UE}\ x\ BW} \quad (2)$$

$$EDI_{Cre}\left(\frac{ng}{kg-bw{\cdot}day}\right)=\frac{Conc_{Cre}\ x\ Cre_{24h}\ x\ MW}{F_{UE}\ x\ BW} \quad (3)$$

where $Conc_{vol}$, $Conc_{SG}$, $Conc_{Cre}$ are the grouped molar concentrations for each parent compound (nmol·L^{-1} for EDI_{Vol} and EDI_{SG}, or nmol·gCre^{-1} for EDI_{Cre}) at GM; V_{24h} is the estimated total urinary volume excreted within 24h for adults (1.65 L·day^{-1}) obtained to the average between 1.7 L·day-1 for adult men and 1.6 L·day-1 for adult women (Aylward et al., 2011) due to equality in sex distribution; Cre_{24h} is the estimated daily urinary Cre excretion normalized by mean body weight (kg), age (years old) and height (cm) of the people under study (1.48 g Cre·day^{-1}), calculated as it is shown in Eq. 3b by Mage et al. (2004); MW is the molecular weight of the parent PAH compound (ng·nmol^{-1}); F_{UE} is the fraction of the administered dose of each PAH family eliminated as urinary OHPAHs, which values ranged from 1.76 to 11.3 % (Choi et al., 2023); BW is GM of the body weight of the participants.

The hazard quotient (HQ) was used to assess the non-cancer risk from the exposure of each PAH family separately. It is calculated as the ratio of EDI for each PAH to its reference limit for oral exposure (RfD), which serves as a toxicological reference value (Peng et al., 2020). The RfD values ranged from 20000 (NAP) to 40000 ng kg^{-1}·day^{-1} (FLU) (see Table S2). On the other hand, the hazard index (HI) was employed to estimate the cumulative risk of exposure to PAH mixtures. These factors were calculated using the following formulas (Peng et al., 2020):

$$HQ_i=\frac{EDI_i}{RfD_i} \quad (4)$$

$$HI_{PAHs}=\sum HQ_i \quad (5)$$

A HQ and a HI lower than 1 indicates that exposure to a specific PAH and cumulative exposure to certain PAH mixture, respectively, might not pose a health risk to the target population.

3. Results

3.1. Characteristics of the studied population and urinary PAH biomarkers levels

Finally, a total of 504 urine samples were included in this study, since thirty-five donors presented abnormal Cre concentrations outside the range of 30–300 mg dL^{-1} (Barr et al., 2005). A total of 10 PAH biomarkers of NAP, FLU, PHEN and PYR were analysed in the urine samples of the BIOMOVAL project. The information of socio-demographic characteristics, lifestyle, and dietary habits of studied population is shown in Table 1. The median age of participants was 43 years and the majority were non-smokers. The mean (±SD) concentration of urinary Cre was 127 ± 6 mg dL^{-1} and the mean SG value was 1.020, ranging between 1.002 and 1.035. Descriptive statistics for urinary OHPAH levels of the population under study are present in Table 2. The same information adjusted by Cre levels are exhibited in Table S3. All PAH biomarkers were quantified in more than 65 % of the urine samples.

Naphthalene was the most abundant PAH detected in these urine samples due to its urinary metabolites presented DFs above 85 %. Regarding the obtained concentrations, the highest levels were also found for naphthalene biomarkers, 1-hydroxynaphthalene (1OHNAP) and 2-hydroxynaphthalene (2OHNAP), with GMs of 0.7 and 11.9 μg L^{-1}, and 95th percentiles of 17.1 and 64.8 μg L^{-1}, respectively. By contrast, 2-hydroxyfluorene (2OHFLU) and 2-, 4- and 9-hydroxyphenanthrene (2OHPHEN, 4OHPHEN and 9OHPHEN) were found at the lowest levels with GMs of 0.05, 0.05, 0.03, and 0.04 μg L^{-1}, respectively. P95 of all PAH biomarkers ranged between 0.22 (4OHPHEN) and 64.8 μg L^{-1} (2OHNAP). The sum of OHPAHs (∑OHPAHs) were in the range of 4–4061 nmol L^{-1}, with GM and P95 of 120 and 634 nmol L^{-1}, respectively.

3.2. Correlations between urinary OHPAH levels and predictors of exposure to PAHs

The Spearman's correlation matrices obtained for the urinary levels of OHPAHs are shown in Tables S3, S4 and S5. These tables exhibit that all OHPAHs were highly and directly related to each other (p-values <0.01), with correlation coefficients ranging between 0.578 and 0.858 for unadjusted values, 0.493 and 0.787 for Cre-adjusted values, and 0.515 and 0.809 for SG-adjusted values. Strong associations exist among fluorene, phenanthrene and pyrene (Akoglu, 2018), suggesting common sources of exposure. However, the relationships with naphthalene are moderated (Akoglu, 2018). This moderation might be attributed to the diverse origins of naphthalene exposure, including potential exposure to the pesticide carbaryl (Fernández et al., 2021a).

Table 3 displays the results of the multiple linear regression models obtained for each PAH family. Overall, these models were weak but accounted for 27–57 % of the variance (R^2). According to them, the smoking status strongly influences urinary OHPAHs levels (β ranging from 0.301 to 0.885, $p < 0.001$), having smokers significantly higher

Table 3
Results of multiple linear regression model with influencing factors on the log-transformed OHPAH concentrations (n = 504).

Metabolite	Variable	Standardized coefficients (β) (95 % confidence interval)	p-value*	R^2
∑OHNAPs	Intercept	−19.510 (−25.356–−13.663)	< **0.001**	0.272
	Specific gravity	20.669 (14.925–26.413)	< **0.001**	
	Smoking status: Smoker	0.301 (0.214–0.387)	< **0.001**	
	Smoking status: Passive smoker	0.033 (−0.077–0.143)	0.557	
	Smoking status: Non-smoker	ref.		
	Home location: Castellon	−0.141 (−0.264–−0.018)	**0.024**	
	Home location: Valencia	0.118 (0.040–0.197)	**0.003**	
	BMI	0.014 (0.006–0.022)	**0.001**	
	Annual household income: <14499€	0.157 (0.002–0.312)	**0.048**	
	Annual household income: 14500-19999€	0.138 (−0.003–0.279)	0.054	
	Annual household income: 20000-349999€	0.142 (0.010–0.274)	**0.036**	
	Annual household income: 35000-599999€	0.031 (−0.108–0.169)	0.665	
	Annual household income: >60000€	ref.		
∑OHFLUs	Intercept	−25.990 (−31.569–−20.411)	< **0.001**	0.572
	Specific gravity	25.406 (19.928–30.883)	< **0.001**	
	Smoking status: Smoker	0.885 (0.774–0.936)	< **0.001**	
	Smoking status: Passive smoker	0.046 (−0.057–0.150)	0.379	
	Smoking status: Non-smoker	ref.		
	Home location: Castellon	−0.272 (−0.386–−0.157)	< **0.001**	
	Home location: Valencia	0.105 (0.030–0.179)	**0.006**	
	Home location: Alicante	ref.		
	Consumption of grilled food in the last 24 h	0.146 (−0.024–0.316)	**0.043**	
∑OHPHENs	Intercept	−24.387 (−29.250–−19.525)	< **0.001**	0.375
	Specific gravity	23.982 (19.200–28.764)	< **0.001**	
	Smoking status: Smoker	0.367 (0.294–0.439)	< **0.001**	
	Smoking status: Passive smoker	0.026 (−0.065–0.116)	0.581	
	Smoking status: Non-smoker	ref.		
	Home location: Castellon	−0.140 (−0.242–−0.038)	**0.007**	
	Home location: Valencia	0.075 (0.009–0.140)	**0.025**	
	Home location: Alicante	ref.		
	Consumption of grilled food in the last 24 h	0.169 (0.020–0.317)	**0.026**	
1OHPYR	Intercept	−27.960 (−34.127–−21.794)	< **0.001**	0.344
	Specific gravity	26.861 (20.807–32.916)	< **0.001**	
	Smoking status: Smoker	0.490 (0.401–0.580)	< **0.001**	
	Smoking status: Passive smoker	0.064 (−0.050–0.179)	0.271	
	Smoking status: Non-smoker	ref.		
	Home location: Castellon	−0.183 (−0.310–−0.056)	**0.005**	
	Home location: Valencia	0.113 (0.031–0.196)	**0.025**	
	Home location: Alicante	ref.		
	Consumption of grilled food in the last 24 h	0.196 (0.008–0.385)	**0.041**	

*p-values <0.05 are highlighted in bold.

OHPAHs levels than passive- and non-smokers. Furthermore, as expected, all PAH biomarkers were influenced by SG values (β ranging from 20.669 to 26.861, $p < 0.001$). Additionally, the home location seemed to have an influence on urinary levels of PAHs. Participants residing in the province of Valencia (β ranging from 0.075 to 26.860.1181, $p < 0.050$) had significantly higher levels of PAHs biomarkers in their urines than those living in Alicante. By contrast, living in the province of Castellon had a potential negative relationship on concentration of PAH metabolites (β ranging from −0.140 to −0.272, $p < 0.050$) comparing to living in Alicante. Moreover, low annual household incomes (β ranging from 0.142 to 0.157, $p < 0.050$) and BMI (0.014, $p < 0.050$) were also potentially related with higher ∑OHNAPs levels than participants with high annual household incomes and lower BMI.

Finally, regarding culinary practises of the participants, individuals who ate grilled food in the last 24 h (β ranging from 0.146 to 0.196, $p < 0.050$) had significantly higher concentrations of ∑OHFLUs, ∑OHPHENs and 1OHPYR than those who did not.

3.3. Risk assessment

EDIs for NAP, FLU, PHEN, and PYR were calculated for the entire analysed population based on their urinary metabolites levels using formulas (1), (2), and (3). NAP showed the highest EDIs, ranging from 14813 to 23653 ng·kg-bw^{-1}·day^{-1} at GM. Additionally, the EDIs for each PAH family were also stratified by smoking status of the participants. The obtained results are presented in Fig. 1 (for SG-adjusted values) and S1 (for unadjusted and Cre-adjusted values). There was no significant difference between non-smokers and passive smokers ($p > 0.050$) (see Table S7).

Concerning the non-cancer risk assessment, HQs and HIs were calculated and stratified by smoking status of the participants. These values are shown in Fig. 2 (for SG-adjusted values) and S2 (for volume and Cre-adjusted values). The obtained HQs were in the range of 0.00045 (pyrene) to 1.183 (naphthalene) at GM for the entire population. Regarding the cumulative risk calculations, NAP was the primary contributor to the risk of exposure to the assessed PAH mixture and its very high levels of EDI influenced a lot the HQs and HIs calculations. The HQs associated with NAP exceeded 1, and, consequently, the HIs were also greater than 1. In the context of smoking status, the HIs of smokers were approximately twice HIs of non-smokers. Given that certain HIs were above 1, a non-cancer health risk associated with exposure to PAHs

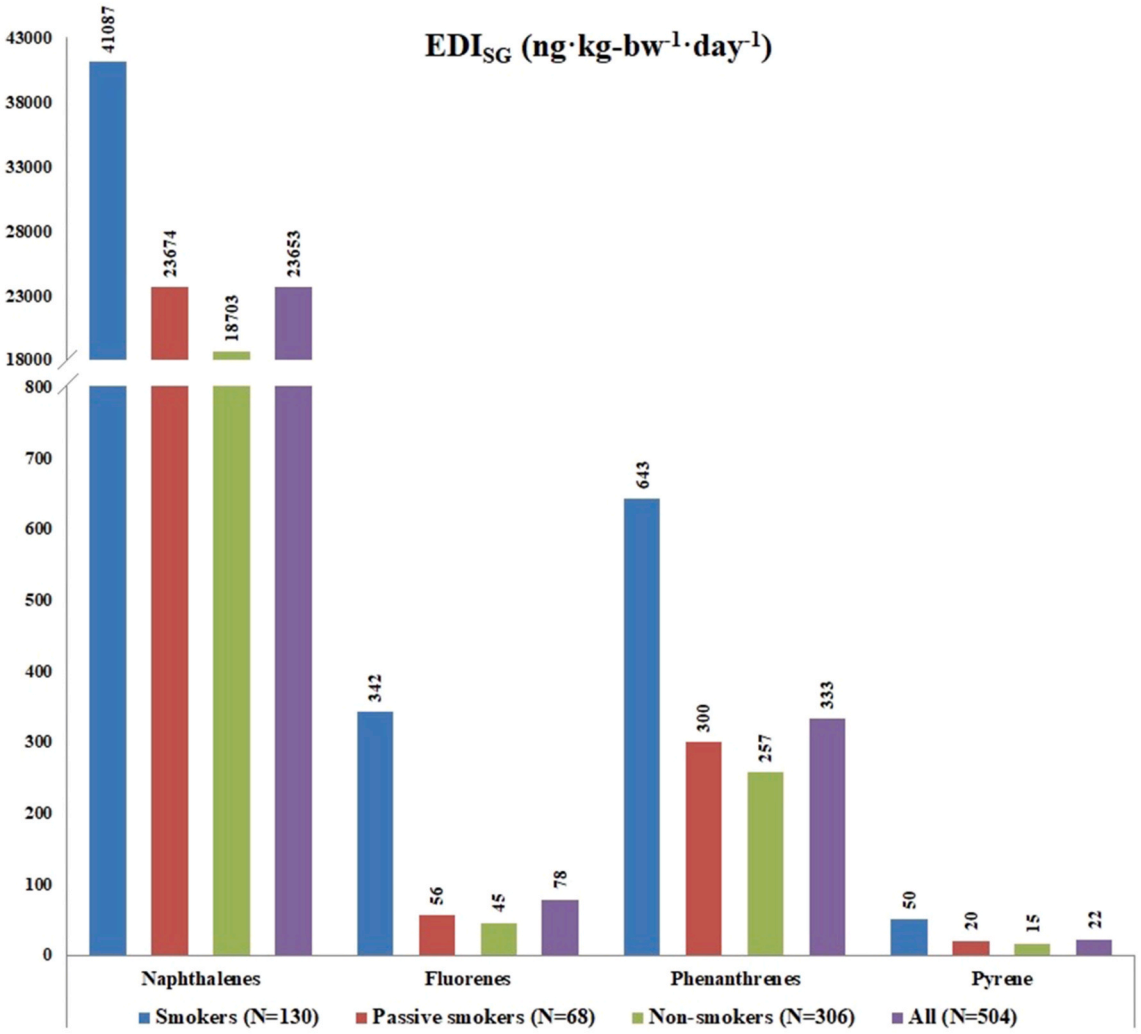


Fig. 1. Estimated daily intakes of individual PAHs adjusted by specific gravity and stratified by smoking status.

could not be discarded in the target population, especially for smokers.

4. Discussion

In the present study, ten urinary biomarkers of the PAH (NAP, FLU, PHEN and PYR) were quantified in the Spanish adult population to assess the risk of exposure to PAHs. The representativeness of the study sample allows using the data generated here as reference values for this population group. The P50 of urinary PAH biomarkers was used to compare our results with those previously reported in other HBM studies on adults worldwide (see Table S8).

NAP (sum of 2OHNAP and 1OHNAP, accounting for more than 90 % of the total internal dose of PAHs) was the predominant PAH in the present study. This finding is consistent with other HBM studies conducted in Spain (Fernández et al., 2021a, 2021b), China (Cao et al., 2020), and Canada (Ratelle et al., 2020). In comparison with other studies, concentrations of 1OHNAP and 2OHNAP were among the highest in our research. However, the observed 1OHNAP levels were lower than those observed in adults from Australia (Thai et al., 2020), China (Cao et al., 2020), Italy (Tombolini et al., 2018), India, Vietnam, and Kuwait (Guo et al., 2013), as well as in women from Spain (Fernández et al., 2021a) and the Czech Republic (Lankova et al., 2016). Only Spanish children (Fernández et al., 2021b) were reported to have lower levels of this metabolite than in the present study. In contrast, 2OHNAP concentrations presented here were similar to those reported in Spanish children (Fernández et al., 2021b) and adults from Vietnam (Guo et al., 2013). In terms of the smoking status, non-smokers from the USA (CDC, 2021b) and Italy (Tombolini et al., 2018) had higher 1OHNAP levels than the non-smoker population analysed here. Nevertheless, only North American smokers showed higher levels than smokers of the BIOMOVAL project. Regarding 2OHNAP, non-smokers and smokers evaluated here presented higher levels than similar populations in the USA (CDC, 2021b) and Italy (Tombolini et al., 2018).

By contrast, the concentrations of urinary FLU biomarkers in this study were the lowest documented so far, especially for 2OHFLU. Adult populations from several countries, such as the USA (CDC, 2021a), South Korea (Choi et al., 2017), Australia (Thai, et al., 2020), Canada (Ratelle et al., 2020), among others, presented higher concentrations of 2OHFLU compared to the participants of the BIOMOVAL project. However, the highest levels were reported in citizens of China (Cao et al., 2020) and India (Guo et al., 2013). On the other hand, the levels of 3OHFLU obtained in the studied population were in the same range as those reported for Spanish women (Fernández et al., 2021a), but lower than those presented in adult population from USA (CDC, 2021a) and Canada (Ratelle et al., 2020).

In relation to the urinary biomarkers of PHEN, Chinese adults (Cao et al., 2020) and Czech women (Lankova et al., 2016) had higher levels than the studied population. Similar concentrations reported in this study, except for 9OHPHEN, were found in Spanish women and children (Fernández et al., 2021a, 2021b) as well as the Canadian adult

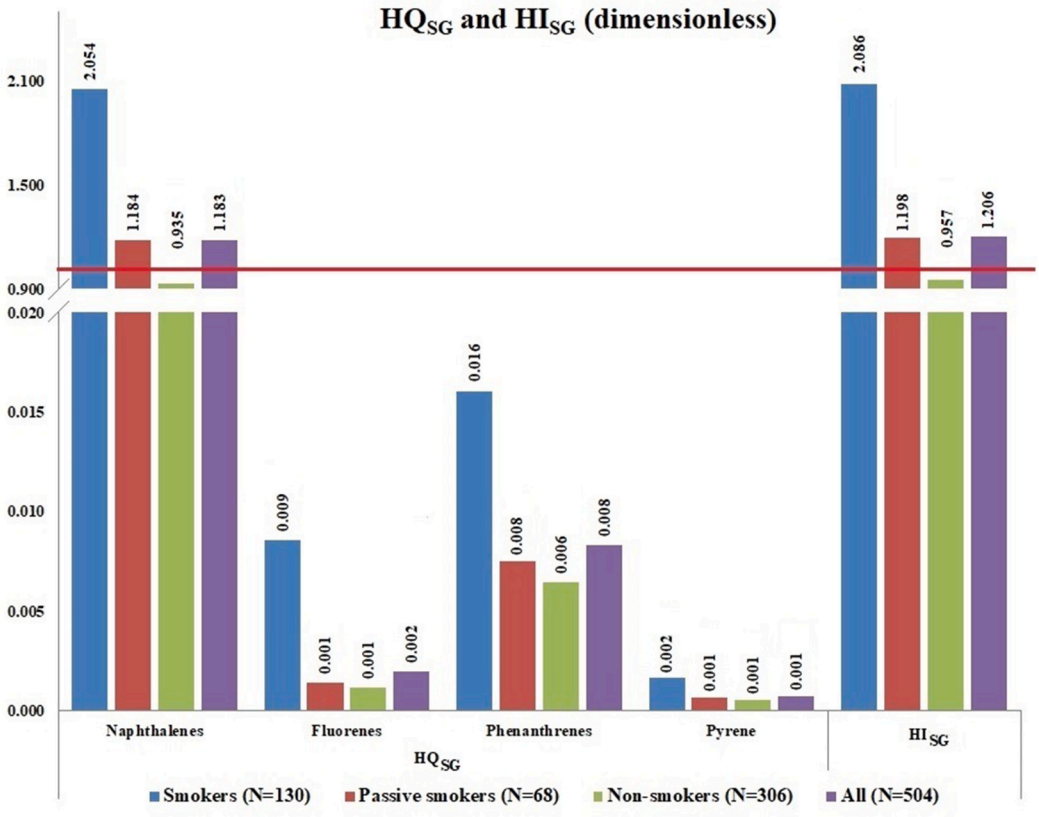


Fig. 2. Results of non-cancer risk assessment: HQs and HIs (dimensionless) adjusted by specific gravity and stratified by smoking status.

population (Ratelle et al., 2020). Within the framework of smoking behaviour smokers from our study seemed to be more exposed to PHEN than North American smokers (CDC, 2021b).

In the same way of 2OHFLU, urinary 1OHPYR levels found here were generally lower than in other countries, such as China (Cao et al., 2020), Iran (Shahsavani et al., 2021), India, Vietnam, Kuwait, Japan, Malaysia (Guo et al., 2013), Czech Republic (Lankova et al., 2016), South Korea (Choi et al., 2017), Canada (Ratelle et al., 2020), Spain (Fernández et al., 2021a, 2021b), Australia (Thai, et al., 2020) and the USA (CDC, 2021a). The only exception was Italy, where the levels reported by Tombolini et al. (2018) were lower than in the present research.

Identifying potential predictors of human exposure to PAHs is essential for the development of more accurate mitigation and control strategies. According to the findings reported in other studies (Tombolini et al., 2018; Fernández et al., 2021a), smoking had the predominant contribution to urinary PAH metabolite concentrations in our research, highlighting the significance of inhalation exposure. Second-hand cigarette smoking is among the largest contributor to personal exposures of naphthalene (Jia and Batterman, 2010). However, no significant differences in levels of urinary metabolites of NAP or other PAHs were observed between passive- and non-smokers (see Table S7). Moreover, statistical analysis also showed that some characteristics and dietary habits of the participants were potential contributors to the exposure to PAHs. Possible reasons for these findings are discussed in the following paragraphs.

Home location and annual household incomes were found to potentially influence OHPAH levels in the target population, consistent with findings reported in previous studies (Dobraca et al., 2018; Fernández et al., 2021b; Jain, 2020; Keir et al., 2020). Low annual household incomes may limit the ability to get a better residence, leading to residing in more densely populated areas, which might have higher traffic and, consequently, poorer air quality (Zhang and Batterman, 2013). This aligns with the results obtained here, where low annual household incomes were observed to be linked with higher levels of NAP metabolites compared to higher annual household incomes. Likewise, individuals in the province of Valencia appeared to be more exposed to PAHs compared to their counterparts in Alicante. Conversely, those residing in Castellon exhibited lower exposure levels than those living in Alicante. This aligns with the demographic distribution of the Valencian Region, with Valencia being the most densely populated area, followed by Alicante, and finally, Castellon. Although not all PAHs showed a correlation with both variables, particularly due to the low variability of urinary levels of their metabolites, these variables may suggest that PAH exposure is likely related to the air quality of the location. Exposure to PAHs can also occur from anthropogenic sources such as vehicle emmisions, industrial activities, burning of wood or crop residues, etc., as reported by Jia and Batterman (2010). However, exposure variables such as distance from trafficked roads, industries or agricultural activities did not show a significant relationship with PAH exposure in this study. Thus, drawing conclusive statements solely from this information is not possible.

In the present study, no associations between urinary OHPAHs and sex or age were found. In addition, as reported by other authors (Bartolomé et al., 2015; Jain, 2020), high BMIs were associated with increasing urinary PAH concentrations. A possible explanation for this observation was provided by Peng et al. (2020), who asserted that obesity may influence the urinary concentrations of PAH biomarkers, given that PAHs are fat-soluble substances. While some PAHs are more fat-soluble, we observed a relationship specifically with ∑OHNAPs. Similarly to the explanation provided earlier, the low variability of urinary metabolite levels of FLU, PHEN and PYR compared to NAP could explain this finding.

Regarding the food consumption habits of the participants, culinary practises, especially those carried out at high temperatures, are associated with PAH exposure (EFSA, 2008; Health Canada, 2017). As a result, in our study, individuals who consumed grilled food in the last 24 h

showed higher ∑OHFLUs, ∑OHPHENs and 1OHOYR concentrations, consistent with the findings reported by Hoseini et al. (2018) and Ratelle et al. (2020). However, the use of other food cooking processes did not present a significant correlation with PAH exposure. Similarly, despite seafood/seafood products and meat/meat products being considered significant contributors to dietary exposure to PAHs (EFSA, 2008; Domingo and Nadal, 2015), no significant associations were found here. This could be explained by the fact that PAH exposure might depend a lot on the other sources of exposure and cooking procedures.

In the context of risk assessment, EDIs for PYR reported from various countries were compared due to its major urinary metabolite (1OHPYR) is commonly measured in the majority of PAH-related HBM studies (HBM4EU, 2020). Adult populations from the USA (Zhu et al., 2021) and some Asian countries, such as Vietnam, India, China and Kuwait (Guo et al., 2013) presented EDIs for PYR exposure much higher than those observed in our study. Furthermore, for most of the European countries, the median daily intake of PYR exposure is around 50 ng·kg-bw^{-1}·day^{-1} (HBM4EU, 2020), based on the existing HBM data available now, which is also higher than the EDI reported here (22 ng·kg-bw^{-1}·day^{-1}). In addition, previous Spanish studies (Fernández et al., 2021a, 2021b) showed higher EDIs for PYR than our studied population. On the other hand, EDIs for FLU and PHEN exposure showed here were much lower than those reported in the USA (Zhu et al., 2021) and in several Asian countries (Guo et al., 2013). Regarding Spain, children and women residing in the same Spanish region as in the present study (Fernández et al., 2021a, 2021b) were reported to have lower EDIs than the target population. Furthermore, for NAP exposure, the EDIs obtained here were the highest values reported so far.

These findings may be attributed to variations in the excretion rates of PAHs, as the toxicological reference values employed in this study are consistent with those utilised in previous HBM studies (Fernández et al., 2021a, 2021b; Peng et al., 2020). We adopted the excretion rates reported by Choi et al. (2023). While the observed change is statistically significant only for NAP based on urinary levels, the decision to use these new excretion rates stems from limitations in the rates reported by Li et al. (2012) and Motorykin et al. (2015), which were used previously in HBM studies (Guo et al., 2013; Peng et al., 2020; Fernández et al., 2021a, 2021b; Zhu et al., 2021). These earlier rates were unable to distinguish between experimentally exposed levels and background levels due to the absence of isotope labelling in the administered doses. In contrast, Choi et al. (2023) employed deuterium-labelled PAHs in their administration, enabling a clear distinction between them.

Humans are exposed to PAHs through ingestion, inhalation and dermal uptake. However, no data are available regarding excretion rates after inhalation or dermal contact exposure. For this reason, given that these routes of exposure cannot be neglected, literature-derived excretion rates were used as an approximation of the fraction of OHPAHs excreted in urine after exposure to PAHs from various sources.

Finally, given that certain HIs, especially for smokers, were found to be above 1, the exposure to PAHs should not be discarded as a potential risk to the health of the Spanish target population. Furthermore, since taking into account that NAP has been classified as “possible carcinogenic to humans” (IARC, 2023), further HBM studies are necessary to monitor PAH exposure over time.

5. Limitations and strengths of the study

The representativeness of the study sample together with the accurately designed questionnaire used in the BIOMOVAL project and the high detection frequencies the urinary PAH biomarkers, allows an excellent approximation of the exposure to PAHs and to provide reference values for the adult population residing in the Valencian Region (Spain). The questionnaire relied on self-reported data, and the analysis of urinary PAH biomarkers may not adequately capture the long-term exposure of the target population. Despite passive smokers potentially being exposed to second-hand smoke in environments outside their homes, we chose the smoking status classification based on information available in the questionnaires, emphasizing the high relevance of indoor smoke exposure in our study. In future studies, we commit to modifying the questionnaire and incorporating the analysis of cotinine, to improve the smoking status classification. Additionally, the F_{UE} values used in the risk assessment approach are reported by only one study and solely based on ingestion exposure. Consequently, although not entirely accurate because multiple routes of exposure to PAHs are neglected, it can only be done using these literature-derived excretion rates, given that; unfortunately, there is no data on excretion after inhalation or dermal contact exposure. Hence, further kinetic studies on this aspect are necessary.

6. Conclusions

In the present research, the concentration profiles of 10 urinary PAH biomarkers in the Spanish adult population of the BIOMOVAL project is reported to increase and update the knowledge on exposure to PAHs. All analysed urinary biomarkers had high detection frequencies (>65 %). Two NAP biomarkers (1OHNAP and 2OHNAP) showed the highest concentration levels, being the responsibles for more than 90 % of the total exposure. Overall, the Spanish population exhibited urinary OHPAH levels within the same range as those found in similar studies worldwide.

Regarding the predictors of exposure, smoking status was the predominant factor, followed by home location, low annual household incomes, BMI and the consumption of grilled food in the last 24 h. According to the risk assessment results, the highest EDIs were observed for NAP, leading to HIs above 1, especially for smokers. Therefore, in contrast to what was observed in previous HBM studies, potential health risks associated with PAH exposure in the target population could not be discarded. For this reason, more mitigation and prevention measures, as well as further HBM studies on this exposure in the general population, especially focused on NAP, should be addressed in the future.

CRediT authorship contribution statement

Borja Peris-Camarasa: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Olga Pardo:** Writing – review & editing, Supervision, Conceptualization. **Sandra F. Fernández:** Writing – review & editing, Methodology. **Pablo Dualde:** Writing – review & editing, Methodology. **Clara Coscollà:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT – 3.5 in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no financial interests or personal relationship that could influence the work presented in this article.

Data availability

The data that has been used is confidential.

Acknowledgments

The present study was supported by the Public Health General Directorate of Valencia together with the Foundation for the Promotion

of Health and Biomedical Research in Valencian Region (FISABIO), which financed the BIOMOVAL project, and by the ACIF/2021/337 and ACIF/2019/183 projects funded by the Valencian Government. The European Commission financed the analytical instruments used here through the European Regional Development Funds (FEDER) Operation Program of the Valencian Region (2014–2020). Pablo Dualde acknowledges his "*Ayudas para la contratación PTA*" (PTA2018-016320-I) from Ministry of Science and Innovation (Spain). The authors would like to express their gratitude to the donors, SERPRECOVA, Unimat Prevention, and the UJI for their cooperation in the recruitment of participants and the sample collection, and the IBSPCV BioBank (PT13/0010/0064) for their collaboration in the processing and storage of the samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141330.

References

ATSDR, 2022. ATSDT's substance priority list [WWW document]. URL. https://www.atsdr.cdc.gov/spl/index.html. (Accessed 27 May 2023).

Aylward, L.L., Krishnan, K., Kirman, C.R., Nong, A., Hays, S.M., 2011. Biomonitoring equivalents for deltamethrin. Regul. Toxicol. Pharmacol. 60, 189–199. https://doi.org/10.1016/j.yrtph.2011.03.014.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192–200. https://doi.org/10.1289/ehp.7337.

Bartolomé, M., Ramos, J.J., Cutanda, F., Huetos, O., Esteban, M., Ruiz-Moraga, M., Calvo, E., Pérez-Gómez, B., González, O., Castaño, A., 2015. Urinary polycyclic aromatic hydrocarbon biomarkers levels in a representative sample of the Spanish adult population: the BIOAMBIENT.ES project. Chemosphere 135, 436–446. https://doi.org/10.1016/j.chemosphere.2014.12.008.

Cao, L., Wang, D., Wen, Y., He, H., Chen, A., Hu, D., Tan, A., Shi, T., Zhu, K., Ma, J., Zhou, Y., Chen, W., 2020. Effects of environmental and lifestyle exposures on urinary levels of polycyclic aromatic hydrocarbon biomarkers: a cross-sectional study of urban adults in China. Chemosphere 240, 124898. https://doi.org/10.1016/j.chemosphere.2019.124898.

Cattley, R.C., Kromhout, H., Sun, M., Tokar, E.J., Abdallah, M.A.E., Bauer, A.K., Broadwater, K.R., Campo, L., Corsini, E., Houck, K.A., Ichihara, G., Matsumoto, M., Morais, S., Mráz, J., Nomiyama, T., Ryan, K., Shen, H., Toyoda, T., Vähäkangas, K., Yakubovskaya, M.G., Yu, I.J., Debono, N.L., de Conti, A., El Ghissassi, F., Madia, F., Mattock, H., Pasqual, E., Suonio, E., Wedekind, R., Benbrahim-Tallaa, L., Schubauer-Berigan, M.K., 2023. Carcinogenicity of anthracene, 2-bromopropane, butyl methacrylate, and dimethyl hydrogen phosphite. Lancet Oncol. 24 (5), 431–432. https://doi.org/10.1016/S1470-2045(23)00141-9.

Centres for Disease Control and Prevention (CDC): U.S. National Centre for Environmental Health, 2021a. Fourth national report on human exposure to environmental chemicals updated tables, March 2021 volume two: NHANES 2011-2016 [WWW document]. URL. https://ecologycenter.org/wp-content/uploads/2021/04/FourthReport_UpdatedTables_Volume2_Mar2021-508.pdf.

Centres for Disease Control and Prevention (CDC): U.S. National Centre for Environmental Health, 2021b. Fourth national report on human exposure to environmental chemicals updated tables, March 2021 volume four: analysis of chemicals found in cigarette smoke in a special sample of U.S. Adults, NHANES 2011-2016 [WWW document]. URL. https://stacks.cdc.gov/view/cdc/105343.

Choi, W., Kim, S., Baek, Y., Choi, K., Lee, K., Kim, S., Yu, S.D., Choi, K., 2017. Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012–2014). Int. J. Hyg Environ. Health 220, 29–35. https://doi.org/10.1016/j.ijheh.2016.10.002.

Choi, J.W., Kim, M., Song, G., Kho, Y., Choi, K., Shin, M.Y., Kim, S., 2023. Toxicokinetic analyses of naphthalene, fluorene, phenanthrene, and pyrene in humans after single oral administration. Sci. Total Environ. 870, 161899 https://doi.org/10.1016/j.scitotenv.2023.161899.

Dobraca, D., Lum, R., Sjödin, A., Calafat, A.M., Laurent, C.A., Kushi, L.H., Windham, G. C., 2018. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peripubertal girls in Northern California: predictors of exposure and temporal variability. Environ. Res. 165, 46–54. https://doi.org/10.1016/j.envres.2017.11.011.

Domingo, J.L., Nadal, M., 2015. Human dietary exposure to polycyclic aromatic hydrocarbons: a review of the scientific literature. Food Chem. 86, 144–153. https://doi.org/10.1016/j.fct.2015.10.002.

European Food Safety Authority (EFSA), 2008. Scientific opinion of the Panel on contaminants in the food chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in food. EFSA J. 724, 1–114. https://doi.org/10.2903/j.efsa.2008.724.

European Food Safety Authority (EFSA), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA J. 8, 1–96. https://doi.org/10.2903/j.efsa.2010.1557.

European Commission, 2011. Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs [WWW document]. URL Off. J. Eur. Union. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:En:PDF.

European Commission, 2013. Commission regulation (EU) No 1272/2013 of 6 december 2013 amending annex XVII to regulation (EC) No 1907/2006 of the European parliament and of the Council on the registration, evaluation, authorization and restriction of chemicals (REACH) as regards polycyclic aromatic hydrocarbons [WWW document]. URL Off. J. Eur. Union. https://eur-lex.europa.eu/eli/reg/2013/1272/oj.

European Commission, 2014. Union's implementation plan for the Stockholm convention on persistent organic pollutants [WWW document]. URL. In: On the Review and Update of the First European Community Implementation Plan in Accordance with Article 8(4) of Regulation No 850/2004 on Persistent Organic Pollutants. Off. J. Eur. Union. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A52014SC0172.

Fernández, S.F., Pardo, O., Pastor, A., Yusà, V., 2021a. Biomonitoring of polycyclic aromatic hydrocarbons in the urine of lactating mothers: urinary levels, association with lifestyle factors, and risk assessment. Environ. Pollut. 268, 115646 https://doi.org/10.1016/j.envpol.2020.115646.

Fernández, S.F., Pardo, O., S Hernández, C., Garlito, B., Yusà, V., 2021b. Children's exposure to polycyclic aromatic hydrocarbons in the Valencian Region (Spain): urinary levels, predictors of exposure and risk assessment. Environ. Int. 153, 106535 https://doi.org/10.1016/j.envint.2021.106535.

Guo, Y., Senthilkumar, K., Alomirah, H., Moon, H., Minh, T.B., Mohd, M.A., Nakata, H., Kannan, K., 2013. Concentrations and profiles of urinary polycyclic aromatic hydrocarbon biomarkers (OH-PAHs) in several Asian countries. Environ. Sci. Technol. 47, 2932–2938. https://doi.org/10.1021/es3052262.

HBM4EU, 2018. D4.9 – scoping document. Prioritized substance group: PAHs and air pollutants – version 1.0 [WWW document]. URL. https://www.hbm4eu.eu/hbm4eu-substances/pahs/.

HBM4EU, 2020. Reporting for first and second set of substances. Deliverable Report AD5.4 – version 1.1 [WWW document]. URL. https://www.hbm4eu.eu/wp-content/uploads/2021/03/HBM4EU_AD5.4_Reporting_first_and_second_set_substances_v1.1.pdf.

HBM4EU, 2021. Prioritized list of biomarkers, matrices and analytical methods for the 1st prioritization round of substances. Deliverable Report D9.2 – Version 1.3 [WWW document] URL. https://www.hbm4eu.eu/work-packages/deliverable-9-2-prioritised-list-of-biomarkers-matrices-and-analytical-methods-for-the-1st-prioritisation-round-of-substances/.

Health Canada, 2017. Fourth report on human biomonitoring of environmental chemicals in Canada. Results of the Canadian Health Measures Survey Cycle 4, 2014–2015 [WWW document]. URL. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-biomonitoring-environmental-chemicals-canada.html.

Hoseini, M., Nabizadeh, R., Delgado-Saborit, J.M., Rafiee, A., Yaghmaeian, K., Parmy, S., Faridi, S., Hassanvand, M.S., Yunesian, M., Naddafi, K., 2018. Environmental and lifestyle factors affecting exposure to polycyclic aromatic hydrocarbons in the general population in a Middle Eastern area. Environ. Pollut. 240, 781–792. https://doi.org/10.1016/j.envpol.2018.04.077.

International Agency for Research on Cancer (IARC), 2023. List of Classifications – IARC Monographs on the Identification of Carcinogenic Hazard to Humans [WWW document]. URL. https://monographs.iarc.who.int/list-of-classifications/. (Accessed 23 October 2023).

Jain, R.B., 2020. Contributions of dietary, demographic, disease, lifestyle and other factors in explaining variabilities in concentrations of selected monohydroxylated polycyclic aromatic hydrocarbons in urine: data for US children, adolescents, and adults. Environ. Pollut. 266, 115178 https://doi.org/10.1016/j.envpol.2020.115178.

Jia, C., Batterman, S., 2010. A critical review of naphthalene sources and exposures relevant to indoor and outdoor air. Int. J. Environ. Res. Publ. Health 7, 2903–2939. https://doi:10.3390/ijerph7072903.

Keir, J.L.A., Cakmak, S., Blais, J.M., White, P.A., 2020. The influence of demographic and lifestyle factors on urinary levels of PAH biomarkers—empirical analyses of Cycle 2 (2009–2011) CHMS data. J. Expo. Sci. Environ. Epidemiol. 31, 386–397. https://doi.org/10.1038/s41370-020-0208-4.

Konishi, S., Kitagawa, G., 2008. Information Criteria and Statistical Modeling. Springer. https://doi.org/10.1007/978-0-387-71887-3.

Kuiper, J.R., O'brien, K.M., Ferguson, K.K., Buckley, J.P., 2021. Urinary specific gravity measures in the U.S. population: implications for the adjustment of non-persistent chemical urinary biomarker data. Environ. Int. 156, 106656 https://doi.org/10.1016/j.envint.2021.106656.

Lankova, D., Urbancova, K., Sram, R.J., Hajslova, J., Pulkrabova, J., 2016. A novel strategy for the determination of polycyclic aromatic hydrocarbon monohydroxylated biomarkers in urine using ultra-high-performance liquid chromatography with tandem mass spectrometry. Anal. Bioanal. Chem. 408, 2515–2525. https://doi.org/10.1007/s00216-016-9350-1.

Li, Z., Romanoff, L., Bartell, S., Pittman, E.N., Trinidad, D.A., McClean, M., Webster, M., Sjödin, A., 2012. Excretion Profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon biomarkers after dietary exposure. Chem. Res. Toxicol. 25, 1452–1461. https://doi.org/10.1021/tx300108e.

Mage, D.T., Allen, R.H., Gondy, G., Smith, W., Barr, D.B., Needham, L.L., 2004. Estimating pesticide dose from urinary pesticide concentration data by creatinine correction in the third national health and nutrition examination survey (NHANES-III). J. Expo. Anal. Environ. Epidemiol. 14, 457–465. https://doi.org/10.1038/sj.jea.7500343.

Motorykin, O., Santiago-Delgado, L., Rohlman, D., Schrlau, J.E., Harris, S., Harding, A., Kile, M.L., Simonich, S.L.M., 2015. Metabolism and excretion rates of parent and hydroxy-PAHs in urine collected after consumption of traditionally smoked salmon for native American volunteers. Sci. Total Environ. 514, 170–177. https://doi.org/10.1016/j.scitotenv.2015.01.083.

Peng, M., Lu, S., Yu, Y., Liu, S., Zhao, Y., Li, C., Ma, S., 2020. Urinary monohydroxylated polycyclic aromatic hydrocarbons in primiparas from Shenzhen, South China: levels, risk factors, and oxidative stress. Environ. Pollut. 259, 113854 https://doi.org/10.1016/j.envpol.2019.113854.

Peris-Camarasa, B., Pardo, O., F Fernández, S., Dualde, P., Coscollà, C., 2023. Assessment of acrylamide exposure in Spain by human biomonitoring: risk and predictors of exposure. Environ. Pollut. 331, 121896 https://doi.org/10.1016/j.envpol.2023.121896.

Ratelle, M., Khoury, C., Adlard, B., Laird, B., 2020. Polycyclic aromatic hydrocarbons (PAHs) levels in urine samples collected in a subarctic region of the Northwest Territories, Canada. Environ. Res. 182, 109112 https://doi.org/10.1016/j.envres.2020.109112.

Rodgman, A., Perfetti, T., 2013. The Chemical Components of Tobacco and Tobacco Smoke (Chapter 25) – Pyrolysis. CRC Press, Boca Raton. https://doi.org/10.1201/b13973.

Shahsavani, S., Fararouei, M., Soveid, M., Hoseini, M., Dehghani, M., 2021. The association between the urinary biomarkers of polycyclic aromatic hydrocarbons and risk of metabolic syndromes and blood cell levels in adults in a Middle Eastern Area. J. Environ. Health Sci. Eng. 19, 1667–1680. https://doi.org/10.1007/s40201-021-00722-w.

Thai, P.K., Banks, A.P.W., Toms, L.M.L., Choi, P.M., Wang, X., Hobson, P., Mueller, J.F., 2020. Analysis of urinary biomarkers of polycyclic aromatic hydrocarbons and cotinine in pooled urine samples to determine the exposure to PAHs in an Australian population. Environ. Res. 182, 109048 https://doi.org/10.1016/j.envres.2019.109048.

The European Parliament and the Council of the European Union, 2000. Directive 2000/06/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Communities [WWW document]. URL. https://eur-lex.europa.eu/eli/dir/2000/60/oj.

The European Parliament and the Council of the European Union, 2016. Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. Off. J. Eur. Union [WWW document]. URL. http://data.europa.eu/eli/dir/2016/2284/oj.

Tombolini, F., Pigini, D., Tranfo, G., Paci, E., Carosi, I., Marini, F., Bauleo, L., Ancona, C., Forastiere, F., 2018. Levels of urinary biomarkers of four PAHs and cotinine determined in 1016 volunteers living in Central Italy. Environ. Sci. Pollut. Res. 25, 28772–28779. https://doi.org/10.1007/s11356-018-1650-x.

US Environmental Protection Agency (USEPA), 2022. Integrated risk information system (IRIS) [WWW document]. URL. https://www.epa.gov/iris. (Accessed 1 June 2023).

Vu, A.T., Taylor, K.M., Holman, M.R., Ding, Y.S., Hearn, B., Watson, C.H., 2015. Polycyclic aromatic hydrocarbons in the mainstream smoke of popular U.S. cigarettes. Chem. Res. Toxicol. 28, 1616–1626. https://doi.org/10.1021/acs.chemrestox.5b00190.

Zhang, K., Batterman, S., 2013. Air pollution and health risks due to vehicle traffic. Sci. Total Environ. 450–451, 307–316. https://doi.org/10.1016/j.scitotenv.2013.01.074.

Zhu, H., Martinez-Moral, M., Kannan, K., 2021. Variability in urinary biomarkers of human exposure to polycyclic aromatic hydrocarbons and its association with oxidative stress. Environ. Int. 156, 106720 https://doi.org/10.1016/j.envint.2021.106720.