Environment International 153 (2021) 106535

Contents lists available at ScienceDirect

Environment International

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

# Children's exposure to polycyclic aromatic hydrocarbons in the Valencian Region (Spain): Urinary levels, predictors of exposure and risk assessment

Sandra F. Fernández [a,b], Olga Pardo [a,c,*], Cristina S. Hernández [a,b], Borja Garlito [a], Vicent Yusà [a,b,d], on behalf of the BIOVAL task force

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Av. Catalunya, 21, 46020 València, Spain
[b] Department of Analytical Chemistry, University of València Doctor, Moliner 50, 46100 Burjassot, Spain
[c] Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine Department, University of València Doctor, Moliner 50, 46100 Burjassot, Spain
[d] Public Health Laboratory of València Av. Catalunya, 21, 46020 València, Spain

ARTICLE INFO

Handling Editor: Lesa Aylward

*Keywords:*
Human biomonitoring
Polycyclic aromatic hydrocarbons
Urine
Children
Reference values

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are pollutants that are released into the environment during incomplete combustion of organic matter and which can have a negative effect on human health. PAHs enter the human body mostly through ingestion of food or inhalation of tobacco smoke. The purpose of the present study is to evaluate the internal levels of PAHs that children living in the Valencian Region (Spain) are exposed to.

In total, we measured eleven biomarkers of exposure to naphthalene, fluorene, phenanthrene, pyrene, and benzo(a)pyrene in the urine of 566 children aged 5–12. The analytical method was based on a liquid-liquid extraction of the PAH metabolites from the urine samples, followed by their determination by liquid chromatography coupled to tandem mass spectrometry. In addition, we used a questionnaire to collect the sociodemographic characteristics and 72 h dietary recall information of the participants in our study.

Overall, we detected PAH metabolites in more than 78% of the children, with the exception of 3-hydroxyfluorene and 3-hydroxybenzo(a)pyrene, which were found in less than 37% of the analyzed samples. The most abundant biomarker found was 2-hydroxynaphthalene, with a geometric mean of 10 $ng{\cdot}ml^{-1}$. Reference values ($RV_{95}$) ranging from 0.11 (4-hydroxyphenanthrene) to 53 $ng{\cdot}ml^{-1}$ (2-hydroxynaphthalene) in urine of Spanish children were derived from the present study. According to the statistical analysis, the factors that were significantly associated with the internal exposure to PAHs were province of residence, body mass index (BMI), children's age, consumption of plastic-wrapped food, and dietary habits. The estimated daily intakes in geometric mean terms ranged from 5 (fluorene) to 204 $ng{\cdot}kg\text{-}bw^{-1}{\cdot}day^{-1}$ (naphthalene). Risk assessment calculations showed higher hazard quotients and hazard indexes for children aged 5–8 than those aged 9–12, but all were below 1.

In conclusion, no potential non-cancer health risk due to PAH exposure was observed in children living in Spain.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a complex mixture of over 100 different organic compounds composed of two or more aromatic rings (EFSA, 2008). These substances are released into the environment during incomplete combustion of organic matter originating from natural sources (e.g., volcanic eruptions, forest fires), and anthropogenic activities, such as tobacco smoke, vehicle exhausts, processing of fuels, forest fires, and some culinary practices (drying, grilling, roasting, frying, and smoking food) (EFSA, 2008; Liu et al., 2016; Martínez-Salinas et al., 2010; Miri et al., 2018). Additionally, they are used in the manufacture of plasticizers, pigments, drying agents, and pesticides (Martínez-Salinas et al., 2010).

In general, the main pathway of human exposure to PAHs is the ingestion of contaminated foodstuffs (EFSA, 2008). According to the European Food Safety Authority (EFSA), the two highest contributors to the PAH dietary exposure are cereals and seafood (EFSA, 2008), although they have been also detected in meat, eggs and dairy products,

* Corresponding author. Av. Catalunya 21, 46021 València, Spain.
*E-mail address:* pardo_olg@gva.es (O. Pardo).

https://doi.org/10.1016/j.envint.2021.106535
Received 22 December 2020; Received in revised form 9 March 2021; Accepted 17 March 2021
Available online 5 April 2021

among others (Domingo and Nadal, 2015; Luzardo et al., 2013). However, inhalation of tobacco smoke, passive smoking and dermal contact are also significant contributors to PAH exposure in smokers and the occupationally exposed population (e.g., firefighters, asphalt workers, etc.), respectively (EFSA, 2008; Fan et al., 2012). With regard to inhalation of polluted air, this is a marginal contributor to total PAH exposure (<10%), except for people living in areas close to refineries, certain industries or waste incinerators (HBM4EU, 2019).

PAHs have been associated with a wide range of toxicological effects, such as reproductive and developmental toxicity, immunotoxicity, and carcinogenic and genotoxic potencies (Huang et al., 2019a). More specifically, human exposure to PAHs has been linked to many diseases including cancer, diabetes, cardiovascular diseases, and hypertension (HBM4EU, 2018; Oliveira et al., 2019). Indeed, the United States Environmental Protection Agency (USEPA) has classified 16 PAH compounds as priority pollutants (US EPA, 2014), and the International Agency for Research on Cancer (IARC) has classified benzo(a)pyrene as a human carcinogen (Group 1). Other PAHs, such as naphthalene and chrysene, have been deemed as "possibly carcinogenic to humans" (Group 2B), while other PAH compounds (e.g., fluorene, phenanthrene, pyrene, etc.) have been considered as unclassifiable with regard to their carcinogenicity risk to humans (Group 3), because of limited or inadequate experimental evidence (IARC, 2021). In this context, children are considered one of the most vulnerable population groups, since they have a less efficient detoxification system, lower body weight and higher inhalation rates compared to adults, resulting in higher exposure to PAHs (Oliveira et al., 2019), which may disrupt their growth and development process (Sochacka-Tatara et al., 2018).

To control and mitigate PAH exposure in the general population, the European Union (EU) has implemented several regulatory measures for PAHs in food and environment. In 2006, maximum levels (MLs) for certain PAHs in foods were established by Commission Regulation No 1881/2006 (European Commission, 2006). Furthermore, several PAHs are now deemed as priority hazardous substances under the Directives 2000/60/EC and 2016/2284, which aim to improve the quality of water and ambient air, respectively (The European Parliament and the Council of the European Union, 2016, 2000). The Commission Regulation No 1272/2013 also limited the PAH content in rubbers and plastic components of consumer articles (European Commission, 2013).

Human biomonitoring (HBM), defined as the measurement of concentrations of chemicals or their metabolites in biological samples such as blood or urine, has been considered as a relevant tool to evaluate the PAH exposure of different population groups, since the internal dose of these pollutants accounts for all sources and pathways of exposure (Ganzleben et al., 2017). In the EU, the Human Biomonitoring for Europe (HBM4EU) initiative included PAHs in its first round of priority substances and is currently working on determining the internal exposure of European citizens to these pollutants and their adverse effects with a view to supporting policy making (Ganzleben et al., 2017; HBM4EU, 2018).

For the HBM of PAHs, it should be taken into account that these compounds are absorbed and rapidly metabolized in the human body (Li et al., 2012). After exposure, they are biotransformed in the liver into reactive epoxides by the cytochrome P450, and reduced or hydrolyzed to hydroxylated PAHs (OHPAHs) (Hoseini et al., 2018). After that, these OHPAHs are conjugated to glucuronic acid or sulphate compounds to increase their water solubility and, finally, excreted through the bile, urine, or feces (HBM4EU, 2018). Specifically, because low-molecular-weight PAHs have relatively short elimination half-lives and are primarily excreted in urine (Li et al., 2012; Sochacka-Tatara et al., 2018), the presence of OHPAHs in urine reflects recent exposure to PAHs (Sochacka-Tatara et al., 2018).

Among all OHPAHs, the metabolite of pyrene, 1-hydroxypyrene (1OHPYR), is the most common biomarker used as indicator of exposure to PAHs (Huang et al., 2019a; Oliveira et al., 2019). Nevertheless, given that humans are exposed to a mixture of PAHs, the HBM of other OHPAHs along with 1OHPYR would provide a better overall assessment of PAH exposure (Dobraca et al., 2018; Fan et al., 2012). In fact, urinary metabolites of naphthalene, fluorene, phenanthrene, chrysene, and benzo(a)pyrene are also commonly used as biomarkers of exposure to PAHs in many HBM studies (CDC, 2019; Health Canada, 2017; Hoseini et al., 2018; Murawski et al., 2020; Urbancova et al., 2020). In Spain, the BIOAMBIENT.ES project was the first national-level HBM survey to show urinary levels of OHPAHs in a representative sample of Spanish adults in 2009 (Bartolomé et al., 2015). But in the case of children, only one HBM study on PAHs has been conducted so far in Spain (Freire et al., 2009), and thus there is a lack of updated data on their exposure to PAHs.

The main objective of our study was to evaluate the exposure of children living in the Valencian Region (Spain) to PAHs by measuring 11 urinary OHPAHs levels in their urine samples. We also investigated the influence of dietary habits and socio-demographic characteristics on PAH exposure of children. Likewise, hazard quotients and hazard indexes were estimated to assess the risk associated to PAH exposure.

## 2. Materials and methods

### 2.1. Study design

The present research is part of the BIOVAL study, a cross-sectional HBM program carried out by the Public Health Directorate of the Regional Government of València (Spain) in 2016, whose objective was to assess the risk and the exposure to PAHs and other food pollutants for children between 5 and 12 years of age living in the Valencian Region. The complete study design was previously described by Pérez et al., (2017). In short, parents of children who studied in Valencian primary schools were asked to participate in the BIOVAL study. The participants had to meet the following inclusion criteria:

(i) Children aged between 5 and 12 years old.
(ii) No metabolic disease or urinary problems.
(iii) Residence in the Valencian Region: Castelló de la Plana, València or Alacant provinces.

In total, 666 children were randomly recruited from 25 primary schools located in 16 municipalities of the entire Valencian territory. However, only 566 children provided a sufficient volume of urine or had a creatinine concentration within the range 30–300 $mg{\cdot}dl^{-1}$ (Barr et al., 2005) to carry out the analysis of the 11 PAH metabolites. The representativeness of the sample was guaranteed because: i) as recommended by the International Federation of Clinical Chemist (IFCC), more than 120 randomly-selected children were included in the BIOVAL study (Pérez et al., 2017); ii) the percentage of participants recruited by province was in accordance with the proportionality of the geographical distribution of this population (Pérez et al., 2017) (Fig. 1); ii) 50% of the children aged between 6 and 8 years old, and 50% between 9 and 11 years old; and, iii) sex equality was achieved: 50% of children were males and 50% were females.

### 2.2. Sample and data collection

Prior to the sample collection, an informed consent form was signed by the parents who showed interest in their children taking part in the study. The parents certified in this document that they were accurately and adequately informed about the BIOVAL study and voluntarily consented to their children's participation in it.

Each participant was provided with a kit containing the urine collection instructions, disposable gloves and a sterile 100-ml polypropylene bottle. They collected their first-morning void urine samples at home, which were then kept at 4 °C in a portable refrigerator, before being transported to the Biobank for Biomedical Research and Public Health of the Valencian Region (IBSP-CV Biobank, Spain; PT13/0010/

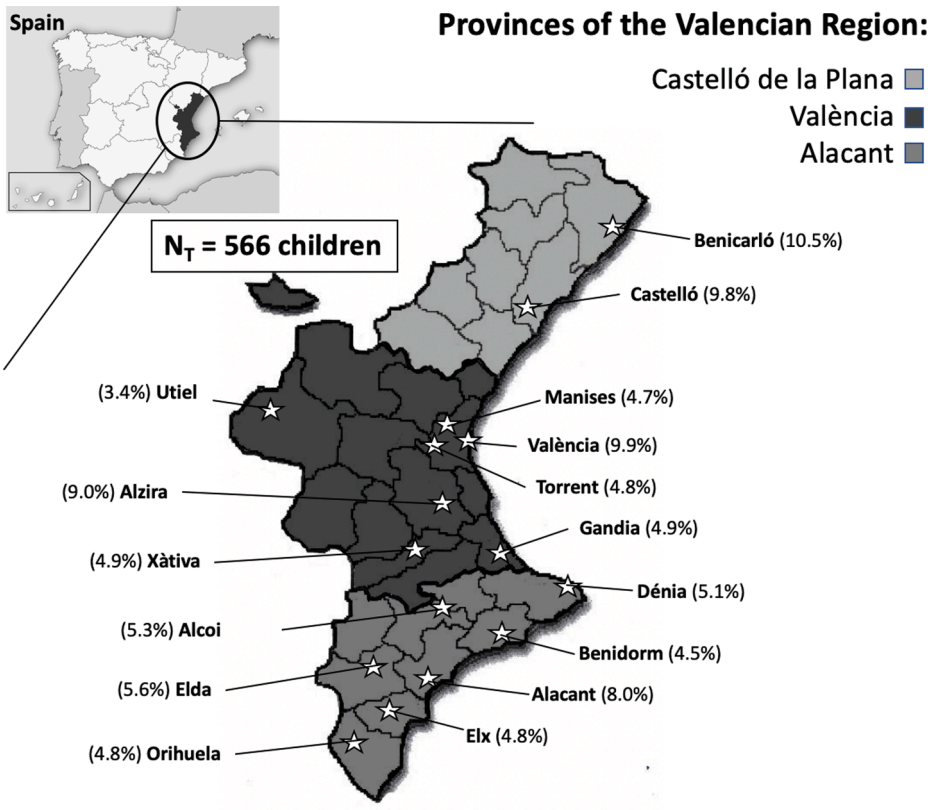


**Fig. 1.** Geographical distribution of the children participating in the BIOVAL program along the three provinces of the Valencian Region.

0064). The samples were integrated into the Spanish National Biobank Network and the Valencian Biobanking Network, where they were stored in 10-ml aliquots at −80 °C until analysis.

The parents of the donors were asked to complete a questionnaire on dietary data, lifestyle, and general socio-demographic characteristics of their children. The purpose of this questionnaire was to gather information on the participants in order to statistically relate it to the measurements conducted on their urine samples. Table 1 summarizes the socio-demographic characteristics and eating habits that were extracted from the participants' questionnaires for the PAH study.

### 2.3. Ethical approval xxx

The study received ethical clearance from the Ethics Committee for Clinical Research of the Directorate General of Public Health and Centre for Advanced Research in Public Health of the Valencian Government. After informed consent was obtained from each participant, all the samples were collected and then stored in the IBSP-CV BioBank. All personal data were dealt with (collected, handled, transferred, and analyzed) in a secure setting and were not kept for a period longer than necessary for the information to be processed. The actual processing of the data was compliant with all ethics and legal considerations at national and EU level, in particular the General Data Protection Regulation.

### 2.4. Chemical analysis

We analyzed the urine samples from the BIOVAL program in the Public Health Laboratory of València (Spain) using the method, reagents and the standard solutions fully described in Fernández et al., (2021). Table S1 lists the 11 OHPAHs determined in the present study, together with their corresponding internal standards (IS).

In brief, 2.5 ml of urine was spiked with 10 μL of β-glucuronidase/arylsulfatase, 1 ml of acetate buffer (pH = 5) and the IS working solution (150–500 ng·ml$^{-1}$). The mixture was incubated 15 h at 37 °C. After the enzymatic hydrolysis, the mixture was subjected to a liquid–liquid extraction with ethyl acetate (2 × 4 ml). Then, the total organic layer was transferred to a Supel™ QuE Z-Sep tube and shaken for 1 min. After centrifugation, the purified extract was evaporated under a $N_2$ stream until dryness. Finally, the dry residue was dissolved in 100 μL of water: methanol (40:60) containing 0.1% of acetic acid. The final extract was 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 mm × 2.1 mm, 2.6 μm), and coupled to a Finnigan TSQ Quantum Ultra 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. (2021).

We measured urinary creatinine using the Jaffé's reaction at the University Hospital Doctor Peset in València (Spain) in order to correct differences in the OHPAH levels due to urine dilution. In accordance with international guidelines, samples with urinary concentrations of creatinine out of the range 30–300 mg·dl$^{-1}$ were excluded from the study (Barr et al., 2005).

### 2.5. Statistical analysis

To perform the statistical analysis, we used IBM SPSS® Statistics (version 26.0) and R software (version 3.5.2). First, the unquantified OHPAH concentrations (<LoQ) were estimated by applying the maximum likelihood estimation method, recommended by the European Food Safety Authority (EFSA, 2010). The obtained OHPAH levels in ng·ml$^{-1}$ and μg·g creatinine$^{-1}$ were summarized by the following descriptors: detection frequency (DF), arithmetic mean (AM), geometric mean (GM), 25th, 50th, 75th, and 95th percentiles (P25, P50, P75, and

**Table 1**
Lifestyle, sociodemographic characteristics and food consumption habits of the children participating in the BIOVAL programme ($n_T = 566$).

| Variable | n (%) | Mean (minimum–maximum) |
|---|---|---|
| ***Children's characteristics*** | | |
| Body Mass Index (BMI, kg·m$^{-2}$) | | 18 (12–45) |
| *Missing data* | 16 (3%) | |
| Age (years) | | 8 (5–12) |
| Province | | |
| Alacant | 205 (36%) | |
| Castelló de la Plana | 126 (22%) | |
| València | 235 (42%) | |
| Home location | | |
| Urban | 441 (78%) | |
| Rural | 22 (4%) | |
| Other | 102 (18%) | |
| *Missing data* | 1 (0.2%) | |
| Sex | | |
| Male | 285 (50%) | |
| Female | 281 (50%) | |
| Country of birth | | |
| Spain | 555 (98%) | |
| Foreign | 8 (1%) | |
| *Missing data* | 3 (1%) | |
| Physical activity | | |
| 3 or more days per week | 299 (53%) | |
| 1 or 2 days per week | 207 (36.5%) | |
| Occasionally or never | 60 (10.5%) | |
| ***Parents' characteristics*** | | |
| Country of birth | | |
| Both parents were Spanish | 490 (87%) | |
| At least one of the parents was foreign | 58 (10%) | |
| *Missing data* | 18 (3%) | |
| Maximum level of education | | |
| Without studies or primary studies | 58 (10%) | |
| Secondary studies | 167 (30%) | |
| University studies | 341 (60%) | |
| Employment situation | | |
| Both unemployed | 38 (7%) | |
| Only one parent employed | 164 (29%) | |
| Both employed | 364 (64%) | |
| ***Food consumption in the last 72 h (grams)*** | | **Mean (minimum–maximum)** |
| Eggs | | 65 (0–360) |
| Meat products | | 484 (0–1545) |
| Fishery products | | 262 (0–1300) |
| Dairy products | | 996 (0–2820) |
| Vegetables | | 399 (0–1798) |
| Chips | | 26 (0–210) |
| Fruits and nuts | | 840 (0–2835) |
| Legumes | | 101 (0–1000) |
| Cereals | | 498 (0–1655) |
| Bakery products | | 75 (0–350) |
| Oils and fats | | 17 (0–120) |
| ***Frequency of consumption of packaged food (number of products·month$^{-1}$)*** | | **Mean (minimum–maximum)** |
| Canned food | | 0.4 (0–4) |
| Plastic-packed food | | 6 (0–21) |
| Carton-packed food | | 0.9 (0–4) |
| Food in glass containers | | 0.2 (0–6) |

P95), and maximum and standard deviation (SD). In addition, OHPAHs with the same PAH parent were converted to nmol·l$^{-1}$ and added together as follows: sum of hydroxynaphthalenes (∑OHNAPs) = 1OHNAP + 2OHNAP; sum of hydroxyfluorenes (∑OHFLUs) = 2OHFLU + 3OHFLU; and sum of hydroxyphenanthrenes (∑OHPHENs) = 1OHPHEN + 2OHPHEN + 3OHPHEN + 4OHPHEN + 9OHPHEN. To be able to study correlations among the PAH metabolites, we used Spearman's test. While qualitative variables were described with absolute frequencies and percentages, quantitative variables were summarized by their median, minimum and maximum values (Table 1).

As the data was not normally distributed, we applied the logarithm (log) of each OHPAH chemical family to build an exposure prediction model including urinary creatinine levels and variables from Table 1 as independent variables, which were treated as continuous if they were quantitative parameters (age, BMI and food consumption habits), or as dichotomous (sex of the children) or polychotomous variables (province, home location, country of birth, physical activity, and parents' characteristics), depending on their number of categories. For this purpose, we performed linear regression models for log-transformed ∑OHNAPs, ∑OHFLUs, ∑OHPHENs, and 1OHPYR concentrations, using a forward stepwise selection procedure based on the corrected Akaike information criterion (AICc) (Konishi and Kitagawa, 2008). Additionally, we divided the numerical explanatory variables by two times their SD to obtain directly-comparable regression coefficient magnitudes (Gelman et al., 2008). A p-value less than or equal to 0.05 was considered as statistically significant. The coefficients estimated for each variable included in the final model reflected the change in the log of PAH metabolite levels in the urine of children.

Finally, the following assumptions were verified: normality of the residuals with the Shapiro-Wilk test, non-autocorrelation using the Durbin-Watson statistic, homoscedasticity by scatter plots, and non-multicollinearity of explanatory variables with tolerance and condition indexes.

### 2.6. Risk assessment

Due to the fact that the main source of PAH exposure in non-smokers is diet (EFSA, 2008), we assessed the risk of exposure to PAHs in the target children by considering only the dietary ingestion route. To this end, we calculated the estimated daily intake (EDI) of naphthalene, fluorene, phenanthrene, and pyrene by combining urinary levels of their metabolites (∑OHNAPs, ∑OHFLUs, ∑OHPHENs and 1OHPYR, respectively) with their toxicokinetic properties in the human body. GM and P95 distribution levels were used to calculate EDIs, which were stratified according to the age of the participants (5–8 years old, and 9–12 years old), using equation (1) below (Peng et al., 2020) and the parameter values presented in Table S2.

$$EDI = \frac{C_U \times V_{24h} \times MW}{F_{UE} \times BW} \quad (1)$$

EDI = estimated daily intake of the PAH parent compound (ng·kg-bw$^{-1}$·day$^{-1}$)
$C_U$ = molar concentration of the sum of OHPAHs with the same PAH parent in urine (GM or P95 in nmol·l$^{-1}$)
$V_{24h}$ = total urinary volume excreted within 24 h for children (0.66 l·day$^{-1}$) (Aylward et al., 2011)
MW = molecular weight of the parent PAH compound (g·mol$^{-1}$)
$F_{UE}$ = fraction of the oral intake dose of PAH excreted as urinary OHPAH (ranging from 0.08 to 1) (Li et al., 2012; Motorykin et al., 2015)
BW = mean body weight of the target population (27 kg for 5–8 years old children, and 39 kg for 9–12 years old children)

The non-cancer risk from a single exposure was assessed by means of a hazard quotient (HQ), calculated as the ratio of EDI for each PAH to its reference dose for oral exposure (RfD) (Table S2), as follows (Miri et al., 2018; Peng et al., 2020):

$$HQ_i = \frac{EDI_i}{RfD_i} \quad (2)$$

Additionally, we evaluated the cumulative risk of exposure to PAH mixtures by adding up individual HQs of PAHs according to equation (3) (Peng et al., 2020). This parameter is known as the hazard index (HI):

$$HI_{PAHs} = \sum HQ_i \quad (3)$$

A HQ < 1 is interpreted as an acceptable risk from an exposure to a

**Table 2**
Descriptive analysis of the OHPAHs levels (ng·ml$^{-1}$) in urine from Valencian children (n = 566).[a]

| Parent compound | Metabolites | LoQ | DF (%) | P25 | P50 | AM | GM | P75 | P95 | Maximum | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 1OHNAP | 0.01 | 87 | 0.2 (0.3) | 0.6 (0.6) | 1.0 (1.0) | 0.3 (0.4) | 1.2 (1.1) | 3.1 (3.2) | 17.2 (12.4) | 1.4 (1.4) |
| | 2OHNAP | 0.01 | 100 | 5 (6) | 11 (11) | 18 (18) | 10 (11) | 20 (20) | 53 (52) | 382 (265) | 28 (24) |
| | [c,d]∑OHNAPs | – | – | 42 (48) | 81 (80) | 132 (129) | 79 (82) | 151 (150) | 389 (368) | 2647 (1836) | 196 (165) |
| Fluorene | 2OHFLU | 0.01 | 99 | 0.11 (0.12) | 0.17 (0.17) | 0.25 (0.25) | 0.17 (0.18) | 0.27 (0.25) | 0.55 (0.55) | 16.99 (17.61) | 0.73 (0.75) |
| | 3OHFLU | 0.05 | 37 | -[b] | -[b] | 0.10 (0.10) | -[b] | 0.07 (0.07) | 0.29 (0.30) | 2.42 (2.55) | 0.26 (0.29) |
| | [c,e]∑OHFLUs | – | – | 0.8 (0.8) | 1.2 (1.2) | 1.9 (1.9) | 1.2 (1.3) | 1.9 (1.7) | 5.2 (5.0) | 106.5 (110.4) | 4.7 (4.9) |
| Phenanthrene | 1OHPHEN | 0.01 | 97 | 0.06 (0.07) | 0.11 (0.11) | 0.16 (0.16) | 0.10 (0.10) | 0.18 (0.17) | 0.39 (0.41) | 4.35 (4.93) | 0.26 (0.28) |
| | 2OHPHEN | 0.01 | 96 | 0.02 (0.03) | 0.04 (0.04) | 0.06 (0.06) | 0.04 (0.04) | 0.07 (0.07) | 0.15 (0.14) | 1.26 (1.31) | 0.08 (0.08) |
| | 3OHPHEN | 0.01 | 99 | 0.05 (0.06) | 0.08 (0.09) | 0.12 (0.11) | 0.08 (0.09) | 0.14 (0.13) | 0.30 (0.28) | 2.47 (2.56) | 0.15 (0.15) |
| | 4OHPHEN | 0.01 | 78 | 0.01 (0.01) | 0.02 (0.02) | 0.04 (0.04) | 0.02 (0.02) | 0.04 (0.04) | 0.11 (0.10) | 0.60 (0.62) | 0.05 (0.05) |
| | 9OHPHEN | 0.01 | 86 | 0.02 (0.02) | 0.04 (0.04) | 0.06 (0.06) | 0.03 (0.03) | 0.07 (0.07) | 0.18 (0.19) | 1.65 (1.87) | 0.10 (0.10) |
| | [c,f]∑OHPHENs | – | – | 1.1 (1.2) | 1.6 (1.6) | 2.2 (2.2) | 1.7 (1.7) | 2.6 (2.4) | 5.0 (4.9) | 39.0 (40.4) | 2.7 (2.8) |
| Pyrene | 1OHPYR | 0.01 | 98 | 0.07 (0.07) | 0.11 (0.10) | 0.13 (0.13) | 0.10 (0.10) | 0.16 (0.15) | 0.29 (0.29) | 0.84 (1.20) | 0.10 (0.10) |
| Benzo(a)pyrene | 3OHBAP | 0.05 | 5 | -[b] | -[b] | -[b] | -[b] | -[b] | -[b] | -[b] | -[b] |
| [c,g]∑OHPAHs | | – | – | 46 (51) | 85 (84) | 137 (133) | 85 (88) | 157 (158) | 398 (374) | 2653 (1840) | 198 (166) |

LoQ = Quantification Limit; DF = Detection Frequency (% samples > LoQ); P = percentile; AM = Arithmetic mean; GM = Geometric mean; SD = Standard deviation.
[a] Values within parenthesis are the urinary levels adjusted by creatinine (μg·g Cre$^{-1}$).
[b] This parameter was not calculated due to the low DF of this metabolite.
[c] Units: nmol·l$^{-1}$ (nmol·g Cre$^{-1}$).
[d] ∑OHNAPs = 1OHNAP + 2OHNAP.
[e] ∑OHFLUs = 2OHFLU + 3 OHFLU.
[f] ∑OHPHENs = 1OHPHEN + 2OHPHEN + 3OHPHEN + 4OHPHEN + 9OHPHEN.
[g] ∑OHPAHs = ∑OHNAPs + ∑OHFLUs + ∑OHPHENs + 1OHPYR + 3OHBAP.

specific PAH and a HI < 1 means that the cumulative exposure to a certain PAH mixture is little concern to public health.

According to the Panel on Contaminants in the Food Chain (CONTAM Panel), only PAHs for which oral carcinogenicity data are available can help to assess the carcinogenic potency of a PAH mixture (EFSA, 2008). For this reason, the only possible indicators of cancer risk are the following eight PAHs (PAH8): benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, chrysene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene (EFSA, 2008). And therefore, since naphthalene, fluorene, phenanthrene, and pyrene are not included in this group, the risk of cancer from exposure to PAHs could not be assessed in the present study.

## 3. Results

In the end, a total of 566 urine samples with a creatinine concentration ranging from 33 to 266 mg·dl$^{-1}$ (mean = 104 mg creatinine ·dl$^{-1}$ urine) were considered in the descriptive and statistical analysis. Table 1 summarizes the parameters obtained from the questionnaires regarding the socio-demographic characteristics, lifestyle habits, and the dietary patterns of the donors used in the research. Out of the 566 children, 50% were boys and 50% were girls; 51% were aged between 5 and 8 years old and 49% between 9 and 12 years old. Most of the children (93%) had at least one parent employed, and 78% of them lived in urban areas. Anomalous BMI were found in 16 children (3%), who had a BMI greater than 25 kg·m$^{-2}$, four of which presented a BMI greater than 30 kg·m$^{-2}$. In contrast, 62% of the children had a BMI lower than 18 kg·m$^{-2}$.

### 3.1. Urinary levels of OHPAHs

Table 2 shows the descriptive statistics of the urinary concentrations of OHPAH (in ng·ml$^{-1}$ and μg·g creatinine$^{-1}$) in the target population. As can be observed, all PAH metabolites were detected in more than 78% of the urine samples, with the exception of 3OHBAP and 3OHFLU, with DFs of 5% and 37%, respectively, since these two metabolites had the highest LoQ (0.05 ng·ml$^{-1}$). It is worth noting that 2OHNAP was found in 100% of the analyzed samples.

Regarding OHPAH urinary concentrations, the highest levels were found for the metabolites of naphthalene, 1OHNAP and 2OHNAP, with GMs of 0.3 and 10 ng·ml$^{-1}$, respectively. By contrast, the phenanthrene metabolites 4OHPHEN, 9OHPHEN, and 2OHPHEN had the lowest GMs of all the metabolites: 0.02, 0.03, and 0.04 ng·ml$^{-1}$, respectively. The urinary concentrations of the remaining OHPAHs ranged from 0.08 (3OHPHEN) to 0.17 ng·ml$^{-1}$ (2OHFLU), while reference values ($RV_{95}$) were in the range of 0.11 (4-hydroxyphenanthrene) to 53 ng·ml$^{-1}$ (2-hydroxynaphthalene). Overall, more than seven metabolites of PAHs were detected in approximately 90% of the children.

### 3.2. Correlations and predictors of exposure to PAHs

Table S3 includes the Spearman's correlation matrix calculated for the urinary levels of the OHPAHs. According to this matrix, all PAH metabolites were highly and directly related to each other, indicating, in all probability, similar sources.

Fig. 2 represents the results of the multiple regression model obtained for each PAH family along with its standardized coefficients (β) and their corresponding 95% confidence intervals (CIs). The final regression coefficients ($R^2$) obtained were between 0.160 and 0.227. The obtained regression formulas for predicting OHPAHs concentrations in urine and more detailed information on these prediction models are shown in Table S4.

As shown by Fig. 2, the age of the children was negatively related to all OHPAHs. In contrast, a positive correlation was found with urinary creatinine. In the case of OHNAPs, children who had foreign parents tended to have lower urinary concentrations of these metabolites than those with Spanish parents, but given that only 10% of the participants

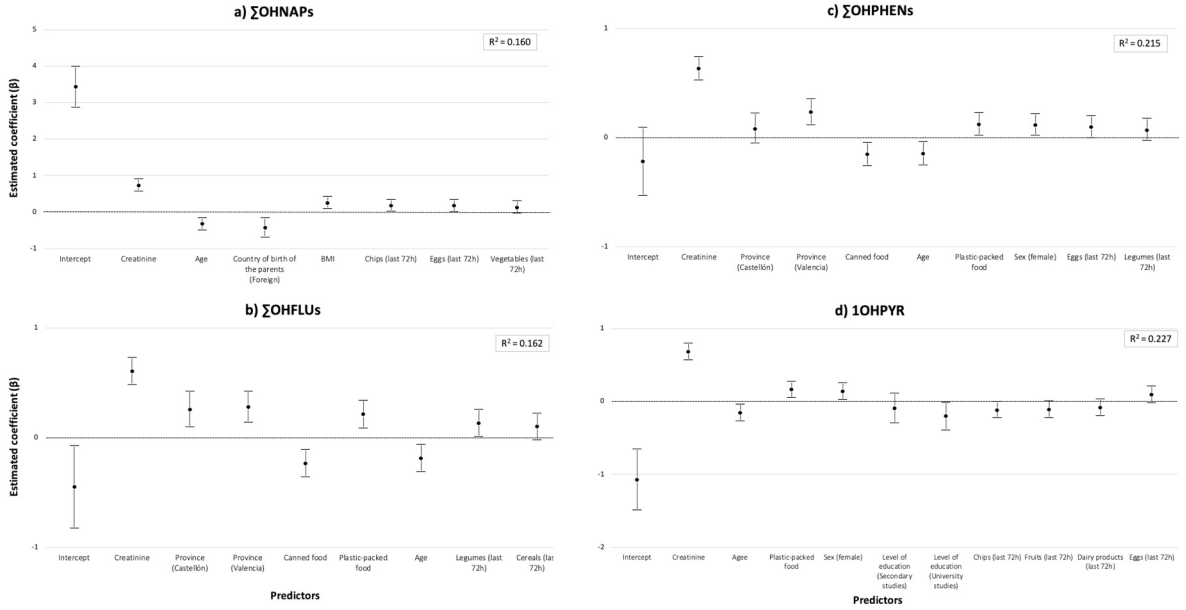


**Fig. 2.** Standardized coefficients of the linear regression models fitted for the log-transformed urinary concentrations of a) ΣOHNAPs, b) ΣOHFLUs, c) ΣOHPHENs, and d) 1OHPYR, with their corresponding 95% confidence intervals and coefficients of determination ($R^2$).

were foreign, this finding was not considered highly relevant. Moreover, higher OHNAPs levels were found in children with higher BMIs. In addition, urinary levels of ∑OHFLUs and ∑OHPHENs were significantly different depending on the province where the children lived. In general, participants living in the València province, and to a lesser extent in Castelló de la Plana, had higher levels of these metabolites in their urine samples. If sex differences are taken into account, it can be observed that girls presented higher ∑OHPHENs and 1OHPYR concentrations than boys. Furthermore, the higher the parents' education level was, the lower the 1OHPYR urinary levels were.

Regarding dietary habits of the participants, children who consumed a higher number of chips and legumes in the last 72 h presented higher ∑OHNAPs and ∑OHFLUs concentrations in their urine, respectively. By contrast, a negative association was found between the intake of chips and urinary levels of 1OHPYR. Moreover, the consumption frequency of food packaged in plastic was highly and positively related with all OHPAH concentrations, except for ∑OHNAPs.

Another relevant finding with a clear tendency to significance (p-values close to 0.05) were that eggs consumption appeared to be related to higher urinary levels of ∑OHNAPs, ∑OHPHENs and 1OHPYR.

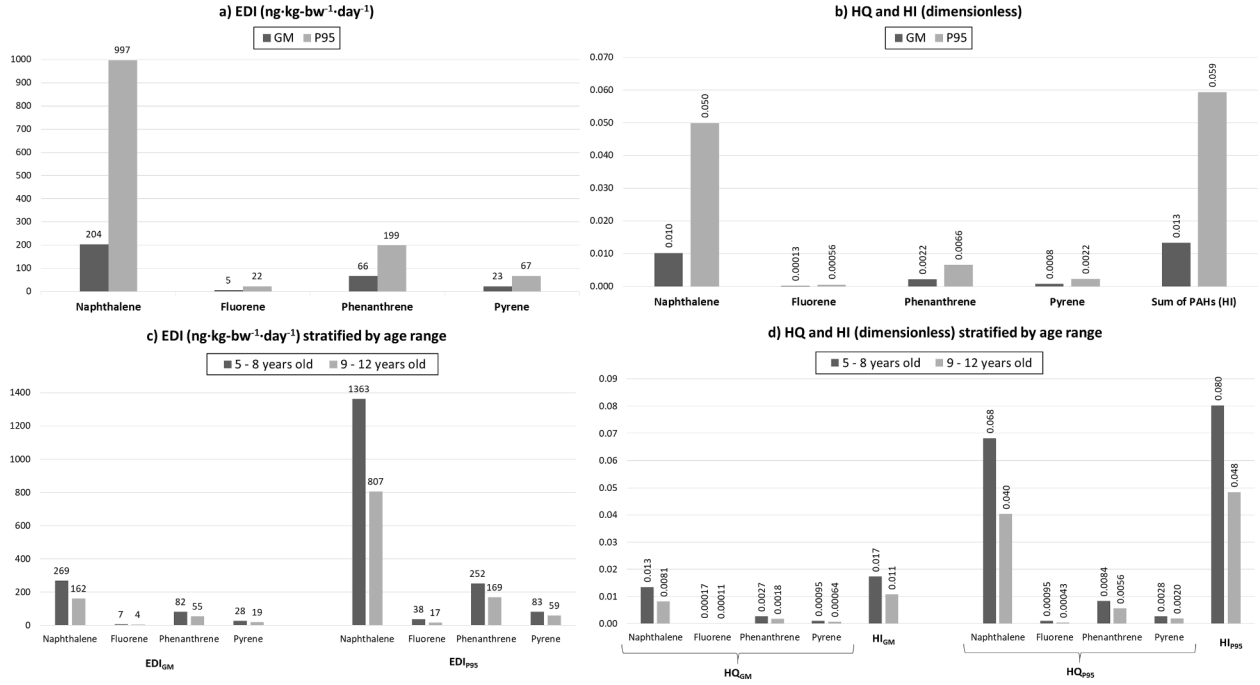


**Fig. 3.** a) Estimated daily intakes (EDI, in ng·kg-bw$^{-1}$·day$^{-1}$) at GM and P95 for naphthalene, fluorene, phenanthrene and pyrene; and, b) Hazard quotients and hazard indexes at GM and P95 for PAHs, c) Estimated daily intakes (EDI, in ng·kg-bw$^{-1}$·day$^{-1}$) stratified by age range; and, d) Hazard quotients and hazard indexes stratified by age range.

### 3.3. Risk assessment

EDIs, HQs, and HIs calculated for naphthalene, fluorene, phenanthrene, and pyrene are represented in Fig. 3. We did not assess exposure to benzo(a)pyrene due to the low DF (5%) of its urinary metabolite (3OHBAP) in the analyzed samples.

As illustrated by Fig. 3, naphthalene was the PAH with the highest EDI (204 and 997 ng·kg-bw$^{-1}$·day$^{-1}$ for GM and P95, respectively), followed by phenanthrene (66 and 199 ng·kg-bw$^{-1}$·day$^{-1}$), pyrene (23 and 67 ng·kg-bw$^{-1}$·day$^{-1}$), and finally, fluorene (5 and 22 ng·kg-bw$^{-1}$·day$^{-1}$). Broadly speaking, EDIs of children aged 5–8 years old were approximately twice that of children aged 9–12 years old.

Concerning the risk estimation represented in Fig. 3, HQs were in the range of 0.00013 (fluorene) and 0.010 (naphthalene) at GM level, and of 0.00056 (fluorene) and 0.050 (naphthalene) at P95 level. Similarly, the cumulative risk to PAHs resulted in HIs far below 1, with naphthalene being the major contributor to the risk of exposure to the assessed PAH mixture at both distribution levels. As expected, children from 5 to 8 years old obtained higher values of HQ and HI than children aged 9–12. Fortunately, given that all HQs and HIs were lower than 1, no apparent non-cancer health risk associated with the exposure to PAHs in the target children could be determined.

## 4. Discussion

In the present study, 2OHNAP was the predominant metabolite in the PAHs' total internal dose, accounting for 87% of the ∑OHPAHs, followed by 1OHNAP (7%), ∑OHPHENs (3%), ∑OHFLUs (2%), and 1OHPYR (1%). This composition profile is very similar to what was obtained in children in Portugal (Oliveira et al., 2017), Poland (Sochacka-Tatara et al., 2018), Germany (Murawski et al., 2020), China (Yu et al., 2021), Canada (Health Canada, 2017; Ratelle et al., 2020), USA (CDC, 2019), and Australia (Thai et al., 2020). In the case of 3OHBAP, the metabolite of the only known carcinogenic PAH (benzo(a)pyrene), we observed very low detection rates (5%), which is in line with other HBM studies in the Czech Republic (Urbancova et al., 2020), Spain (Fernández et al., 2021), Portugal (Oliveira et al., 2017), Canada (Health Canada, 2017; Ratelle et al., 2020), and China (Fan et al., 2012), despite having different LoQs. The main reason is that high-molecular weight PAHs, those with more than four fused rings, are largely eliminated through feces (Li et al., 2012; Likhachev et al., 1992).

If we consider the obtained concentrations in a global context, we can find several similarities and differences among children around the world. Table 3 shows the mean OHPAH concentrations observed in children at school age in countries around the world.

In addition, Fig. 4 represents the $RV_{95}$ used to compare the exposure of children from the BIOVAL study with the following population-representative surveys: the German Environmental Survey, 2014–2017 (GerES V) (Murawski et al., 2020); the National Health and Nutrition Examination Survey (NHANES) (CDC, 2019); and the Canadian Health Measures Survey (CHMS) (Health Canada, 2017). It should be taken into account that these studies are not methodologically homogeneous, in that they include different types of urine samples, age ranges of children, and sampling years, as well as the fact that they employ other analytical methods and/or report different LoQs.

Firstly, our study reports mean levels of 2OHNAP between 2 and 25 times higher than levels observed in children from other countries of America, Europe, Africa, and Asia, these levels only being lower than those of children living in polluted areas located in China (Fan et al., 2012; Lin et al., 2020) and Korea (Yoon et al., 2012). In fact, the $RV_{95}$ derived for 2OHNAP in the present study (53 ng·ml$^{-1}$) is approximately twice the $RV_{95}$ observed for children in Germany (24 ng·ml$^{-1}$), USA (19 ng·ml$^{-1}$), and Canada (19 ng·ml$^{-1}$), showing that exposure of Spanish children to naphthalene is apparently high and should be reduced (Schulz et al., 2011), given that naphthalene is considered by the International Agency for Research on Cancer as a possible carcinogenic substance (IARC, 2021). This fact could be attributed to a higher exposure to PAH sources in Spanish children compared to other countries, such as environmental tobacco smoke (ETS), food and cooking methods, or indoor/outdoor air quality (Hoseini et al., 2018; Huang et al., 2019b; Morgan et al., 2015; Murawski et al., 2020; Oliveira et al., 2019). However, further analyses are required to draw firm conclusions.

By contrast, the opposite trend was found to be the case for 1OHNAP, with children from the BIOVAL study having the lowest levels compared to the rest of the world, except for newborns living in the Czech Republic (Urbancova et al., 2020). In addition, the lowest $RV_{95}$ for 2OHNAP was observed for children living in Spain.

Secondly, North-American (CDC, 2019; Health Canada, 2017) and Australian children (Thai et al., 2020) showed similar $RV_{95}$ and mean levels of 2OHFLU and 3OHFLU, respectively, to those obtained in the present research. On the other hand, there were some Asian (Fan et al., 2012; Hoseini et al., 2018; Lin et al., 2020; Yu et al., 2021) and European countries (Murawski et al., 2020; Sochacka-Tatara et al., 2018) which reported higher mean concentrations of these fluorene metabolites. Furthermore, the GerES V survey reported the highest $RV_{95}$ for fluorene metabolites in children of a similar age (Fig. 4), probably because it is referred to the sum of 2OHFLU and 9-hydroxyfluorene (9OHFLU) (Murawski et al., 2020).

In general, lower levels of OHPHENs have been observed in children under study, except from those living in the USA (CDC, 2019) and Australia (Thai et al., 2020), in which lower levels of 1OHPHEN, 3OHPHEN, and 2OHPHEN were reported, respectively. Likewise, $RV_{95}$ derived for OHPHENs in Spanish children appeared to be lower than or in the same range as those reported for German, American, and Canadian children.

Finally, the children in our study obtained mean levels and $RV_{95}$ of 1OHPYR that were generally lower than or comparable to other nationalities. The highest mean concentrations of 1OHPYR were found in Mexico (Martínez-salinas et al., 2012; Sánchez-Guerra et al., 2012), and some countries belonging to Asia (Hemat et al., 2012; Naksen et al., 2017; Shahsavani et al., 2017; Wang et al., 2017; Yoon et al., 2012) and Africa (Tuakuila et al., 2015), probably because people from less developed countries are more exposed to PAH pollution due to deficient air pollution control in these areas and the use of biomass fuels in households (Adeniji et al., 2018; EUROSTAT, 2020; Hemat et al., 2012).

When comparing our findings to another study carried out on Spanish children, urinary concentrations of 1OHPYR were slightly higher in the Valencian children of the present study (2016) than in children living in Granada (2005–2006) (Freire et al., 2009).

The statistical analysis showed that consumption of chips, legumes, and pre-packaged food, as well as several socio-demographic characteristics, such as sex, BMI, age, educational level, and home location, were the most relevant factors affecting OHPAH urinary levels in children. Possible reasons for these findings are discussed in the following paragraphs.

Concerning food consumption habits, a positive association was observed between ∑OHNAPs and the intake of potato chips. This is in accordance with Purcaro et al., 2006, who affirmed that fried food was an important source of exposure to low-molecular PAHs. This association was also observed in an HBM project conducted on Spanish pregnant women (Llop et al., 2008). Surprisingly, the opposite trend was found for 1OHPYR. This fact may be explained by the high variability of PAH concentrations produced after the frying process, which can be very different depending on the cooking conditions (Purcaro et al., 2006).

Legumes were a food group contributing to ∑OHFLUs levels in the present study, since the more intake of legumes, the higher the urinary levels of fluorene metabolites in children. In fact, Bartolomé et al., (2015) found associations between OHPAHs and legumes similar to those of the present study. However, as reported by several Spanish total diet studies, legumes are minor contributors of PAHs (Domingo and Nadal, 2015; Ibáñez et al., 2005; Martí-Cid et al., 2008).

Another important finding was that plastic-wrapped food was

**Table 3**
OHPAHs concentrations (geometric mean) reported in children at school age and other population groups in countries around the world.*

| Continent | Reference | Country, City/ Region | Target population | Sampling year | N | Units | 1OHNAP | 2OHNAP | 2OHFLU | 3OHFLU | 1OHPHEN | 2OHPHEN | 3OHPHEN | 4OHPHEN | 9OHPHEN | 1OHPYR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Europe** | **This study** | Spain, Valencian Region | Children (5–11 years old) | 2016 | 566 | ng/ml (μg/g Cre) | 0.3 (0.4) | 10 (11) | 0.17 (0.18) | <0.05 | 0.10 (0.10) | 0.04 (0.04) | 0.08 (0.09) | 0.02 (0.02) | 0.03 (0.03) | 0.10 (0.10) |
| | Freire et al., (2009) | Spain, Granada | Children (4 years old) | 2005–2006 | 174 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.07 |
| | Sochacka-Tatara et al., (2018) | Poland, Krakow | Children (3 years old) | 2004–2007 | 202/218 | ng/ml | 1.92 | 4.22 | 0.47 | 0.18 | 0.32 | 0.08 | 0.20 | 0.05 | NA | 0.18 |
| | Murawski et al., (2020) | Germany | Children (3–17 years old) | 2015–2017 | 516 | ng/ml | 0.79 | 4.23 | 0.39 | NA | 0.14 | 0.09 | 0.13 | 0.05 | 0.06 | 0.10 |
| | Urbancova et al., (2020)[c] | Czech Republic | Newborns | 2016–2017 | 330 | ng/ml | 0.12 | 2.28 | 0.07 | NA | NA | NA | NA | NA | NA | 0.05 |
| | Cirillo et al. (2006)[a] | Italy, Campania | Children (7–9 years old) | 2004 | 30 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.17 |
| | Hansen et al., (2006)[b] | Denmark | Children (3–13 years old) | 1994–1995 | 204 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.23 |
| | De Craemer et al., (2016)[c] | Belgium | Adolescents (14–16 years old) | 2013 | 20 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.18[j] |
| | Mucha et al., (2006) | Ukraine, Kiev (city) | Children (3 years old) | 1998 | 42 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.30 |
| | Mucha et al., (2006) | Ukraine, Mariupol (steel mill and coking facility) | Children (3 years old) | 1998 | 48 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.52 |
| **America** | CDC (2019) | USA | Children (6–11 years old) | 2013–2014 | 399 | ng/ml | 0.80 | 3.03 | 0.12 | 0.06 | 0.07 | 0.04[d] | 0.05[d] | 0.02[d] | NA | 0.13 |
| | Health Canada (2017) | Canada | Children (6–11 years old) | 2014–2015 | 510 | ng/ml | 0.62 | 3.80 | 0.21 | 0.08 | 0.13 | 0.04 | 0.08 | 0.02 | 0.03 | 0.10 |
| | Martínez-salinas et al. (2012)[a,e] | Mexico (rural areas exposed to biomass combustion) | Children (6–12 years old) | 2009 | 90 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1.54 |
| | Martínez-salinas et al. (2012)[a],f | Mexico (urban areas exposed to traffic and brick furnace emissions) | Children (6–12 years old) | 2009 | 67 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.34 |
| | Sánchez-Guerra et al., (2012)[a] | Mexico (petrochemical areas) | Children (6–10 years old) | 2008 | 82 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.65 |
| **Oceania** | Thai et al., (2020) | Australia, South East Queensland region | Children (5–14 years old) | 2017 | 100 | ng/ml | 1.70 | 4.50 | 0.12 | 0.04 | 0.36[k] | 0.01 | 0.36[k] | 0.08 | NA | 0.16 |
| **Asia** | | | | 2015 | 165 | | 0.86 | 1.95 | 0.58 | NA | 0.29 | 0.25 | NA | 0.10 | 0.10 | 0.44 |

(*continued on next page*)

**Table 3** (*continued*)

| Continent | Reference | Country, City/ Region | Target population | Sampling year | N | Units | 1OHNAP | 2OHNAP | 2OHFLU | 3OHFLU | 1OHPHEN | 2OHPHEN | 3OHPHEN | 4OHPHEN | 9OHPHEN | 1OHPYR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yu et al., (2021) | China, Shenzhen, Guangdong Province | Children (8–11 years old) | | | ng/ml | | | | | | | | | | |
| | Lin et al., (2020) | South China (e-waste recicling area) | Children (3–14 years old) | 2015–2016 | 19 | μg/g Cre | 9.55 | 10.60 | 0.90 | 0.05 | 0.13[l] | 0.13 | 0.23 | 0.05 | 0.13[l] | 0.16 |
| | Liu et al., (2017) | China, Chongqing (urban area) | Children (7–13 years old) | 2015 | 1207 | ng/ml | NA | 2.57 | 0.84 | NA | NA | NA | NA | NA | 2.55 | 0.18 |
| | Fan et al., (2012)[a] | China (residential area) | Children (4–6 years old) | 2008 | 35 | μg/g Cre | NA | 5.91 | 3.58 | NA | NA | 0.53 | 0.15 | 0.29 | 6.78 | 0.27 |
| | Fan et al., (2012)[a] | China (polluted area) | Children (4–6 years old) | 2008 | 39 | μg/g Cre | NA | 11.60 | 6.00 | NA | NA | 0.55 | 0.64 | 0.40 | 4.13 | 1.23 |
| | Hoseini et al., (2018) | Iran, Tehran | Children (<12 years old) | 2013–2014 | 14 | ng/ml | 0.60 | 1.71 | 0.27 | NA | NA | NA | NA | NA | 0.06 | 0.15 |
| | Shahsavani et al., (2017)[g] | Iran, Shiraz | Children (9–12 years old) | 2015 | 115 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 2.28 |
| | Miri et al., (2018) | Iran | Children (5–7 years old) | 2017 | 200 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.26 |
| | Poursafa et al. (2018)[e] | Iran, Isfahan | Children (6–18 years old) | 2014–2016 | 186 | ng/ml | 0.81 | 1.05 | NA | NA | NA | NA | NA | NA | 0.08 | 0.06 |
| | Kelishadi et al., (2018) | Iran, Isfahan | Children (6–18 years old) | 2015–2016 | 150 | ng/ml | 0.36 | 0.42 | NA | NA | NA | NA | NA | NA | 0.11 | 0.10 |
| | Naksen et al., (2017)[h] | Thailand (biomass burning-exposed area) | Children (9–12 years old) | 2015 | 202 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.82 |
| | Ruchirawat et al., (2007)[a] | Thailand, Chonburri (rural area) | Male children (9–13 years old) | NR | 61 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.19 |
| | Ruchirawat et al., (2007)[a] | Thailand, Bangkok (urban area) | Male children (9–13 years old) | NR | 105 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.35 |
| | Hemat et al., (2012)[c] | Afghanistan | Children (2–9 years old) | 2007 | 13 | ng/ml | NA | NA | NA | NA | 1.34 | 1.12[m] | 1.24 | 0.13 | 1.12[m] | 1.56 |
| | Lee et al., (2007) | Korea (areas near a steel mill) | Children (7–15 years old) | 2004 | 1012 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.43 |
| | Yoon et al., (2012)[f] | Korea, Seoul/ Incheon (metropolitan area) | Children (10–12 years old) | 2002–2003 | 197 | ng/ml | NA | 18.55 | NA | NA | NA | NA | NA | NA | NA | 0.80[n] |
| | Yoon et al., (2012)[f] | | | 2002–2003 | 125 | ng/ml | NA | 27.8 | NA | NA | NA | NA | NA | NA | NA | 1.14[n] |

(*continued on next page*)

**Table 3** (*continued*)

| Continent | Reference | Country, City/ Region | Target population | Sampling year | N | Units | 1OHNAP | 2OHNAP | 2OHFLU | 3OHFLU | 1OHPHEN | 2OHPHEN | 3OHPHEN | 4OHPHEN | 9OHPHEN | 1OHPYR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Korea, Pohang (industrial area) | Children (10–12 years old) | | | | | | | | | | | | | |
| | Puttaswamy et al., (2020) | India (rural areas with solid biomass fuel cooking) | Children (<1 years old) | NR | 39 | ng/ml | NA | 2.15 | NA | NA | NA | NA | NA | NA | NA | 0.45 |
| | Wang et al., (2017) | Taiwan | Children (3 years old) | 2010 | 453 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1.94 |
| | Alghamdi et al., (2015)[i] | Saudi Arabia (area near to an oil refinery) | Male children (10–12 years old) | 2013 | 167 | ng/ml | NA | NA | NA | NA | 0.19 | 0.08[m] | 0.18 | 0.04 | 0.08[m] | 0.30 |
| | Alghamdi et al., (2015)[i] | Saudi Arabia (area near to a highway) | Male children (10–12 years old) | 2013 | 187 | ng/ml | NA | NA | NA | NA | 0.13 | 0.07[m] | 0.15 | 0.03 | 0.07[m] | 0.19 |
| | Alghamdi et al., (2015)[i] | Saudi Arabia (area near to the Red Sea) | Male children (10–12 years old) | 2013 | 132 | ng/ml | NA | NA | NA | NA | 0.15 | 0.08[m] | 0.14 | 0.03 | 0.08[m] | 0.17 |
| | Mori et al., (2011) | Japan, Tokio (suburban area) | Children (3–6 years old) | 2007 | 134 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.13 |
| | Chen et al., (2015)[a] | Mongolia, Ulaanbaatar (warm/ moderate season) | Children (11–15 years old) | 2011–2012 | 320 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.27 |
| | Chen et al., (2015)[a] | Mongolia, Ulaanbaatar (cold season) | Children (11–15 years old) | 2011–2012 | 320 | μg/g Cre | NA | NA | NA | NA | NA | NA | NA | NA | NA | 0.58 |
| **Africa** | Bortey-Sam et al., (2017)[j] | Ghana, Kumasi | Young people (3–20 years old) | 2015 | 36 | ng/ml | NA | 4.99 | NA | NA | 0.13[l] | 0.15 | NA | 0.11 | 0.13[l] | 0.14 |
| | Tuakuila et al., (2015) | Congo, Kinshasa | Children (6–14 years old) | 2011 | 60 | ng/ml | NA | NA | NA | NA | NA | NA | NA | NA | NA | 6.10 |

NA: not analyzed.
NR: not reported.
[*] 3OHBAP was not included in this table because we did not have statistically comparable data to compare our results with other studies. In addition, very few articles reported levels for this metabolite.
[a] Value converted from μmol/mol creatinine to μg/g Creatinine using the molecular weight of creatinine and OHPAHs.
[b] Value converted from μmol/mol creatinine to ng/ml using the mean of creatinine levels (0.3 mmol/l) and molecular weight of 1OHPYR.
[c] Median values.
[d] These values correspond to a survey conducted between 2011 and 2012.
[e] Calculated mean of 4 different groups.
[f] Calculated mean of 2 different groups.
[g] Value converted from ng/g creatinine to ng/ml using the mean of creatinine levels (156 mg/dl).
[h] Arithmetic mean.
[i] Calculated mean of 3 different groups.
[j] Adjusted by specific gravity.
[k] Sum of 1OHPHEN and 3OHPHEN.
[l] Sum of 1OHPHEN and 9OHPHEN.
[m] Sum of 2OHPHEN and 9OHPHEN.
[n] Conjugated 1OHPYR.

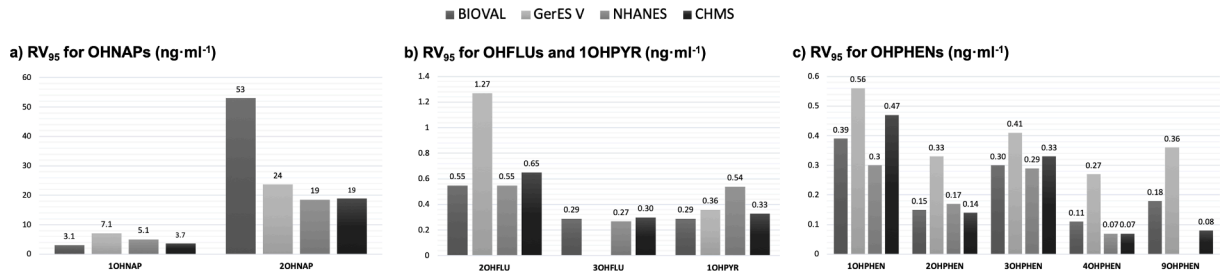


**Fig. 4.** Comparison among reference values ($RV_{95}$) in children reported by the following HBM surveys: the GerES V study (Germany, 2015–2017)* (Murawski et al., 2020); the NHANES (USA, 2013–2014) (CDC, 2019); the CHMS (Canada, 2014–2015) (Health Canada, 2017); and, the BIOVAL study (Spain, 2016) (Present study), for urinary OHPAHs: a) OHNAPs, b) OHFLUs and 1OHPYR, and c) OHPHENs.

identified as a possible source of exposure to PAHs in the children under study. Similarly, children participating in the GerES V showed higher urinary OHPAH levels if they tended to chew plastic objects (Murawski et al., 2020). This is in line with recent studies by Li et al. (2017) and Schweighuber et al. (2019), who studied the presence of PAHs in polystyrene-based products in contact with foodstuffs, concluding that low-molecular weight PAHs can be generated in the production and processing of these materials, as well as being present in the dye used for their coloring. In addition, they also reported that PAH migration from these plastic materials into foods is possible, which requires further attention (Li et al., 2017; Schweighuber et al., 2019).

With regard to inhalation of polluted indoor and outdoor air, several studies support that it may play an important role in the internal exposure to PAHs (De Craemer et al., 2016; Freire et al., 2009; Oliveira et al., 2017; Yu et al., 2021). However, although many authors observed higher OHPAHs concentrations in children living in urban areas (high pollution) compared to those living in rural areas (low pollution) (Cirillo et al., 2006; Hansen et al., 2006; Oliveira et al., 2019; Ruchirawat et al., 2007; Yu et al., 2021), our results showed no differences between children living in these locations. By contrast, it was observed that the province where the children lived had a significant effect on their OHPAH concentrations, as reported in other previous studies (Dobraca et al., 2018; Lee et al., 2009), with participants living in València appearing to be more exposed to PAHs than children living in Castelló de la Plana or Alacant. Therefore, the association of OHPAH levels with location of residence may suggest that airborne exposure is an important contributor, but no conclusions could be drawn with this information alone.

In recent studies, low household income has been associated with higher levels of 1OHPYR and other OHPAHs in urine (Jain, 2020; Keir et al., 2020; Liu et al., 2017), but this characteristic was not considered in the present research. Nevertheless, lower concentrations of 1OHPYR were found in children whose parents had a high level of education, which is also related to the socio-economic status of the family (De Craemer et al., 2016; Dobraca et al., 2018; Lampert et al., 2018). Chuang et al. (1999) suggested that the increased PAH exposure of low-income people is likely to be related to poor air quality, which may be considerably influenced by ETS and household location (proximity to industrial or dense traffic areas).

Another relevant finding was that the urinary levels of PAH metabolites were lower in older children. This has been also observed in other HBM studies on children from other countries (Chen et al., 2015; Jain, 2020; Lee et al., 2007; Mori et al., 2011; Oliveira et al., 2017; Palazzi et al., 2019; Thai et al., 2016; Yu et al., 2016). The reason for this could be that small children have not fully developed metabolic and physiologic functions, leading to a reduced capability to degrade PAHs and more susceptibility to these substances (Hoseini et al., 2018; Huang et al., 2019a; Oliveira et al., 2017; Palazzi et al., 2019; Yu et al., 2016). Also, very young children frequently play on the floor, increasing the inhalation or dermal absorption of PAHs from carpets and soil (Hoseini et al., 2018; Huang et al., 2019a; Oliveira et al., 2019; Poursafa et al., 2017). However, other authors report no associations between urinary OHPAHs and age (Bartolomé et al., 2015; Bortey-Sam et al., 2017; Chen et al., 2015; Llop et al., 2008), while one study finds that this association is positive (Liu et al., 2017).

Regarding sex, females presented higher ∑OHPHENs and 1OHPYR levels in their urines. Despite several authors not reporting any relationship with this aspect (Bartolomé et al., 2015; Chen et al., 2015; Cirillo et al., 2006; Huang et al., 2006; Mori et al., 2011; Mucha et al., 2006; Thai et al., 2016), differences between the sexes have been supported by many recent studies (Bortey-Sam et al., 2017; Hoseini et al., 2018; Huang et al., 2019a; Keir et al., 2020; Liu et al., 2017; Yu et al., 2016). This finding is possibly explained by sex-related differences in absorption and metabolism of PAHs (Keir et al., 2020; Oliveira et al., 2017), as well as by other behaviors (e.g. participation in different indoor and outdoor activities) (Yu et al., 2016).

In addition, BMI tended to increase ∑OHNAPs concentrations in the children under study. This correlation between some OHPAHs and BMI has been previously described by other authors (Alghamdi et al., 2015; Bartolomé et al., 2015; Jain, 2020; Murawski et al., 2020; Poursafa et al., 2017), revealing the fat-soluble properties of PAHs (Liu et al., 2017).

In a risk assessment context, lower EDIs were found in the present study when comparing the median daily intake of pyrene of most European countries (0.05 μg/kg-day) (HBM4EU, 2019) and several Asian countries (Guo et al., 2013; Yu et al., 2021). In addition, the children aged 5–8 years old in our research presented higher EDIs and HQs for naphthalene, and higher HIs at both distribution levels (GM and P95) than the children aged 9–12 years old and female non-smokers living in València (2015) (Fernández et al., 2021), evidencing that younger children might be at greater risk of exposure to PAHs.

However, the cumulative risk of exposure to PAHs expressed by HI values was far below 1 in all cases, suggesting that this exposure is unlikely to have a significant impact on children's health. In any case, given the high urinary levels of 2OHNAP obtained in the present research, further studies are recommended to monitor PAH internal exposure over time, especially in younger children, as well as to identify other significant factors contributing to PAH exposure in children living in Spain.

## 5. Conclusions

In the present research, nine urinary biomarkers of exposure to PAHs were detected in more than 78% of children living in the Valencian Region (Spain). $RV_{95}$ for urinary metabolites of naphthalene, fluorene, phenanthrene and pyrene of Spanish children aged 5–12 years were derived from the present study. According to the urinary composition profiles, the highest levels were found for metabolites of naphthalene, which were responsible for 94% of the total internal exposure to PAHs. Overall, the obtained OHPAH concentrations were lower or in the same range as children of other nationalities. Nonetheless, 2OHNAP levels are much higher than the average levels described worldwide, showing that more attention should be paid to Spanish children's exposure. Age, BMI,

dietary habits, province of residence, and plastic-wrapped food intake were identified as relevant predictors of exposure to PAHs. In terms of exposure, no potential health risk due to PAH exposure was found for Spanish children.

## 6. Strengths and limitations

The questionnaire used for the data collection was a general questionnaire designed for the assessment of exposure to several food and environmental contaminants. Therefore, some specific questions related to PAHs exposure, such as ETS, were not collected and consequently, they were not included in the statistical analysis.

On the other hand, a major strength of the present study was the collection of urine samples from a large children population with wide geographical distribution, being a representative sample of the children living in this region. Thus, the sample size of the study together with the high detection frequencies observed for the majority of OHPAHs led to a high statistical power of the study results. Finally, this is the first study to report urinary levels of 11 OHPAHs in Spanish children, highly contributing to the knowledge of PAH exposure in this country.

## CRediT authorship contribution statement

**Sandra F. Fernández:** Methodology, Validation, Investigation, Writing - original draft, Visualization, Software, Formal analysis, Data curation. **Olga Pardo:** Writing - review & editing, Supervision, Funding acquisition. **Cristina S. Hernández:** Writing - original draft. **Borja Garlito:** Writing - review & editing. **Vicent Yusà:** Conceptualization, Writing - review & editing, Supervision, Project, administration. **:** .

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This study was supported by the GV/2019/137 and ACIF/2019/083 projects, funded by the Generalitat Valenciana. The authors would like to thank Dr. Joan Quiles for his contribution to designing the food survey, as well as to all the participating children and their parents for their enthusiasm and commitment. Likewise, we would like to thank the headteachers of the public schools for their support and cooperation. In particular, we would like to acknowledge the effort of all members of the BIOVAL task force (BIOVAL team of the Public Health Centres) and the IBSPCV BioBank (PT13/0010/0064) which are part of the Spanish National Biobank Network and also the Valencian Biobanking Network for their collaboration.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106535.

## References

Adeniji, A.O., Okoh, A.I., Okoh, O.O., 2018. Analytical methods for polycyclic aromatic hydrocarbons and their global trend of distribution in water and sediment: a review. In: Recent Insights in Petroleum Science and Engineering. INTECH. DOI: 10.5772/intechopen.71163.

Alghamdi, M.A., Alam, M.S., Stark, C., Mohammed, N., Harrison, R.M., Shamy, M., Khoder, M.I., Shabbaj, I.I., Göen, T., 2015. Urinary metabolites of polycyclic aromatic hydrocarbons in Saudi Arabian schoolchildren in relation to sources of exposure. Environ. Res. 140, 495–501. https://doi.org/10.1016/j.envres.2015.04.023.

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., 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., Román, J., 2015. Urinary polycyclic aromatic hydrocarbon metabolites 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.

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Asante, K.A., Baidoo, E., Obirikorang, C., Saengtienchai, A., Isoda, N., Nimako, C., Mizukawa, H., Ishizuka, M., 2017. Oxidative stress and respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (PAHs) in Kumasi, Ghana. Environ. Pollut. 228, 311–320. https://doi.org/10.1016/j.envpol.2017.05.036.

CDC, 2019. Fourth National Report on Human Exposure to Environmental Chemicals. Fourth Natl. Rep. Hum. Expo. to Environ. Chem. 1–529.

Chen, Y.T., Huang, Y.K., Luvsan, M.E., Gombojav, E., Ochir, C., Bulgan, J., Chan, C.C., 2015. The influence of season and living environment on children's urinary 1-hydroxypyrene levels in Ulaanbaatar, Mongolia. Environ. Res. 137, 170–175. https://doi.org/10.1016/j.envres.2014.11.022.

Chuang, J.C., Callahan, P.J., Lyu, C.W., Wilson, N.K., 1999. Polycyclic aromatic hydrocarbon exposures of children in low-income families. J. Expo. Anal. Environ. Epidemiol. 9, 85–98. https://doi.org/10.1038/sj.jea.7500003.

Cirillo, T., Montuori, P., Mainardi, P., Russo, I., Triassi, M., Amodio-Cocchieri, R., 2006. Multipathway Polycyclic aromatic hydrocarbon and Pyrene exposure among children living in Campania (Italy). J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst Environ. Eng. 41, 2089–2107. https://doi.org/10.1080/10934520600867854.

De Craemer, S., Croes, K., van Larebeke, N., Sioen, I., Schoeters, G., Loots, I., Nawrot, T., Nelen, V., Campo, L., Fustinoni, S., Baeyens, W., 2016. Investigating unmetabolized polycyclic aromatic hydrocarbons in adolescents' urine as biomarkers of environmental exposure. Chemosphere 155, 48–56. https://doi.org/10.1016/j.chemosphere.2016.04.017.

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. Toxicol. 86, 144–153. https://doi.org/10.1016/j.fct.2015.10.002.

EFSA, 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA J. 2010; 8(3)1557 8, 1–96. DOI: 10.2903/j.efsa.2010.1557.

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.

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, Authorisation and Restriction of Chemicals (REACH) as regards pol. Off. J. Eur. Union. DOI: 10.36950/benius.2013.12.

European Commission, 2006. COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union.

EUROSTAT, 2020. Quality of life indicators - natural and living environment [WWW Document]. URL https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Quality_of_life_indicators_-_natural_and_living_environment#Exposure_to_pollution.2C_grime_and_other_environmental_problems (accessed 11.1.20).

Fan, R., Wang, D., Mao, C., Ou, S., Lian, Z., Huang, S., Lin, Q., Ding, R., She, J., 2012. Preliminary study of children's exposure to PAHs and its association with 8-hydroxy-2'-deoxyguanosine in Guangzhou, China. Environ. Int. 42, 53–58. https://doi.org/10.1016/j.envint.2011.03.021.

Fernández, S.F., Pardo, O., Pastor, A., Yusà, V., 2021. Biomonitoring of polycyclic aromatic hydrocarbons in the urine of lactating mothers: Urinary levels, association with lifestyle factors, and risk assessment 268. DOI: 10.1016/j.envpol.2020.115646.

Freire, C., Abril, A., Fernández, M.F., Ramos, R., Estarlich, M., Manrique, A., Aguirre, A., Ibarluzea, J., Olea, N., 2009. Urinary 1-hydroxypyrene and PAH exposure in 4-year-old Spanish children. Sci. Total Environ. 407, 1562–1569. https://doi.org/10.1016/j.scitotenv.2008.10.068.

Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg. Environ. Health 220, 94–97. https://doi.org/10.1016/j.ijheh.2017.01.007.

Gelman, A., 2008. Scaling regression inputs by dividing by two standard deviations. Stat. Med. 27, 2865–2873. https://doi.org/10.1002/sim.3107.

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

Hansen, Å.M., Raaschou-Nielsen, O., Knudsen, L.E., 2006. Urinary 1-hydroxypyrene in children living in city and rural residences in Denmark. Sci. Total Environ. 363, 70–77. https://doi.org/10.1016/j.scitotenv.2005.06.017.
HBM4EU, 2019. Reporting for first set of substances Additional Deliverable Report.
HBM4EU, 2018. SCOPING DOCUMENT. PAHs and air pollutants.
Health Canada, 2017. Fourth Report on Human Biomonitoring of Environmental Chemicals in Canada. Results of the Canadian Health Measures Survey Cycle 4 (2014–2015).
Hemat, H., Wittsiepe, J., Wilhelm, M., Müller, J., Göen, T., 2012. High levels of 1-hydroxypyrene and hydroxyphenanthrenes in urine of children and adults from Afghanistan. J. Expo. Sci. Environ. Epidemiol. 22, 46–51. https://doi.org/10.1038/jes.2011.33.
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.
Huang, W., Caudill, S.P., Grainger, J., Needham, L.L., Patterson, D.G., 2006. Levels of 1-hydroxypyrene and other monohydroxy polycyclic aromatic hydrocarbons in children: a study based on U.S. reference range values. Toxicol. Lett. 163, 10–19. https://doi.org/10.1016/j.toxlet.2005.08.003.
Huang, X., Deng, X., Li, W., Liu, S., Chen, Y., Yang, B., Liu, Q., 2019a. Internal exposure levels of polycyclic aromatic hydrocarbons in children and adolescents: a systematic review and meta-analysis. Environ. Health Prev. Med. 24 https://doi.org/10.1186/s12199-019-0805-9.
Huang, X., Deng, X., Li, W., Liu, S., Chen, Y., Yang, B., Liu, Q., 2019b. Internal exposure levels of polycyclic aromatic hydrocarbons in children and adolescents: a systematic review and meta-analysis. Environ. Health Prev. Med. 24, 1–15. https://doi.org/10.1186/s12199-019-0805-9.
IARC, 2021. List of Classifications – IARC Monographs on the Identification of Carcinogenic Hazards to Humans [WWW Document]. URL https://monographs.iarc.fr/list-of-classifications (accessed 1.11.20).
Ibáñez, R., Agudo, A., Berenguer, A., Jakszyn, P., Tormo, M.J., Sanchéz, M.J., Quirós, J. R., Pera, G., Navarro, C., Martinez, C., Larrañaga, N., Dorronsoro, M., Chirlaque, M. D., Barricarte, A., Ardanaz, E., Amiano, P., González, C.A., 2005. Dietary intake of polycyclic aromatic hydrocarbons in a Spanish population. J. Food Prot. 68, 2190–2195. https://doi.org/10.4315/0362-028X-68.10.2190.
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.
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 metabolites—empirical analyses of Cycle 2 (2009–2011) CHMS data. J. Expo. Sci. Environ. Epidemiol. 2 https://doi.org/10.1038/s41370-020-0208-4.
Kelishadi, R., Sobhani, P., Poursafa, P., Amin, M.M., Ebrahimpour, K., Hovsepian, S., Mansourian, M., Najafi, R., Hashemipour, M., 2018. Is there any association between urinary metabolites of polycyclic aromatic hydrocarbons and thyroid hormone levels in children and adolescents? Environ. Sci. Pollut. Res. 25, 1962–1968. https://doi.org/10.1007/s11356-017-0577-y.
Konishi, S., Kitagawa, G., 2008. Information Criteria and Statistical Modeling. Springer.
Lampert, T., Hoebel, J., Kuntz, B., Kroll, L.E., 2018. Socioeconomic status and subjective social status measurement in KiGGS Wave 2 3, 12–14. DOI: 10.17886/RKI-GBE-2018-033.
Lee, K.H., Vermeulen, R., Lenters, V., Cho, S.H., Kang, D., Strickland, P.T., 2009. Determinants of urinary 1-hydroxypyrene glucuronide in South Korean children. Int. Arch. Occup. Environ. Health 82, 961–968. https://doi.org/10.1007/s00420-008-0385-2.
Lee, M.S., Eum, K. Do, Zoh, K.D., Kim, T.S., Pak, Y.S., Paek, D., 2007. 1-Hydroxypyrene as a biomarker of PAH exposure among subjects living in two separate regions from a steel mill. Int. Arch. Occup. Environ. Health 80, 671–678. https://doi.org/10.1007/s00420-007-0178-z.
Li, S.Q., Ni, H.G., Zeng, H., 2017. PAHs in polystyrene food contact materials: an unintended consequence. Sci. Total Environ. 609, 1126–1131. https://doi.org/10.1016/j.scitotenv.2017.07.262.
Li, Z., Romanoff, L., Bartell, S., Pittman, E.N., Trinidad, D.A., McClean, M., Webster, T.F., Sjödin, A., 2012. Excretion Profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. Chem. Res. Toxicol. 25, 1452–1461. https://doi.org/10.1021/tx300108e.
Likhachev, A.J., Beniashvili Sh., D., Bykov, V.J., Dikun, P.P., Tyndyk, M.L., Savochkina, I. V., Yermilov, V.B., Zabezhinski, M.A., 1992. Biomarkers for individual susceptibility to carcinogenic agents: Excretion and carcinogenic risk of benzo[a] pyrene metabolites. Environ. Health Perspect. 98, 211–214. DOI: 10.1289/ehp.9298211.
Lin, M., Tang, J., Ma, S., Yu, Y., Li, G., Fan, R., Mai, B., An, T., 2020. Insights into biomonitoring of human exposure to polycyclic aromatic hydrocarbons with hair analysis: a case study in e-waste recycling area. Environ. Int. 136, 105432 https://doi.org/10.1016/j.envint.2019.105432.
Liu, H., Xu, C., Jiang, Z.Y., Gu, A., 2016. Association of polycyclic aromatic hydrocarbons and asthma among children 6–19 years: NHANES 2001–2008 and NHANES 2011–2012. Respir. Med. 110, 20–27. https://doi.org/10.1016/j.rmed.2015.11.003.
Liu, S., Liu, Q., Ostbye, T., Story, M., Deng, X., Chen, Y., Li, W., Wang, H., Qiu, J., Zhang, J., 2017. Levels and risk factors for urinary metabolites of polycyclic aromatic hydrocarbons in children living in Chongqing, China. Sci. Total Environ. 598, 553–561. https://doi.org/10.1016/j.scitotenv.2017.04.103.
Llop, S., Ballester, F., Estarlich, M., Ibarluzea, J., Manrique, A., Rebagliato, M., Esplugues, A., Iñiguez, C., 2008. Urinary 1-hydroxypyrene, air pollution exposure and associated life style factors in pregnant women. Sci. Total Environ. 407, 97–104. https://doi.org/10.1016/j.scitotenv.2008.07.070.
Luzardo, O.P., Rodríguez-Hernández, Á., Quesada-Tacoronte, Y., Ruiz-Suárez, N., Almeida-González, M., Henríquez-Hernández, L.A., Zumbado, M., Boada, L.D., 2013. Influence of the method of production of eggs on the daily intake of polycyclic aromatic hydrocarbons and organochlorine contaminants: an independent study in the Canary Islands (Spain). Food Chem. Toxicol. 60, 455–462. https://doi.org/10.1016/j.fct.2013.08.003.
Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2008. Evolution of the dietary exposure to polycyclic aromatic hydrocarbons in Catalonia, Spain. Food Chem. Toxicol. 46, 3163–3171. https://doi.org/10.1016/j.fct.2008.07.002.
Martínez-Salinas, R.I., Elena Leal, M., Batres-Esquivel, L.E., Domínguez-Cortinas, G., Calderón, J., Díaz-Barriga, F., Pérez-Maldonado, I.N., 2010. Exposure of children to polycyclic aromatic hydrocarbons in Mexico: assessment of multiple sources. Int. Arch. Occup. Environ. Health 83, 617–623. https://doi.org/10.1007/s00420-009-0482-x.
Martínez-salinas, R.I., Pérez-Maldonado, I.N., Batres-Esquivel, L.E., Flores-Ramírez, R., Díaz-Barriga, F., 2012. Assessment of DDT, DDE, and 1-hydroxypyrene levels in blood and urine samples in children from Chiapas Mexico. Environ. Sci. Pollut. Res. 19, 2658–2666. https://doi.org/10.1007/s11356-012-0758-7.
Miri, M., Alahabadi, A., Ehrampoush, M.H., Ghaffari, H.R., Sakhvidi, M.J.Z., Eskandari, M., Rad, A., Lotfi, M.H., Sheikhha, M.H., 2018. Environmental determinants of polycyclic aromatic hydrocarbons exposure at home, at kindergartens and during a commute. Environ. Int. 118, 266–273. https://doi.org/10.1016/j.envint.2018.06.006.
Morgan, M.K., Jones, P.A., Sobus, J.R., Chuang, J.C., Wilson, N.K., 2015. Using urinary biomarkers to evaluate polycyclic aromatic hydrocarbon exposure in 126 preschool children in Ohio. Int. J. Environ. Health Res. 25, 628–639. https://doi.org/10.1080/09603123.2014.1003039.
Mori, T., Yoshinaga, J., Suzuki, K., Mizoi, M., Adachi, S. ichi, Tao, H., Nakazato, T., Li, Y. S., Kawai, K., Kasai, H., 2011. Exposure to polycyclic aromatic hydrocarbons, arsenic and environmental tobacco smoke, nutrient intake, and oxidative stress in Japanese preschool children. Sci. Total Environ. 409, 2881–2887. DOI: 10.1016/j.scitotenv.2011.04.028.
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.
Mucha, A.P., Hryhorczuk, D., Serdyuk, A., Nakonechny, J., Zvinchuk, A., Erdal, S., Caudill, M., Scheff, P., Lukyanova, E., Shrkiryak-Nyzhnyk, Z., Chislovska, N., 2006. Urinary 1-hydroxypyrene as a biomarker of PAH exposure in 3-year-old Ukrainian children. Environ. Health Perspect. 114, 603–609. https://doi.org/10.1289/ehp.7898.
Murawski, A., Roth, A., Schwedler, G., Schmied-Tobies, M.I.H., Rucic, E., Pluym, N., Scherer, M., Scherer, G., Conrad, A., Kolossa-Gehring, M., 2020. Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Int. J. Hyg. Environ. Health 226, 113491. https://doi.org/10.1016/j.ijheh.2020.113491.
Naksen, W., Kawichai, S., Srinual, N., Salrasee, W., Prapamontol, T., 2017. First evidence of high urinary 1-hydroxypyrene level among rural school children during smoke haze episode in Chiang Mai Province, Thailand. Atmos. Pollut. Res. 8, 418–427. https://doi.org/10.1016/j.apr.2016.11.002.
Oliveira, M., Slezakova, K., Delerue-Matos, C., do Carmo Pereira, M., Morais, S., 2017. Assessment of exposure to polycyclic aromatic hydrocarbons in preschool children: Levels and impact of preschool indoor air on excretion of main urinary monohydroxyl metabolites. J. Hazard. Mater. 322, 357–369. DOI: 10.1016/j.jhazmat.2016.10.004.
Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2019. Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: A review on indoor and outdoor exposure levels, major sources and health impacts. Environ. Int. 124, 180–204. https://doi.org/10.1016/j.envint.2018.12.052.
Palazzi, P., Hardy, E.M., Appenzeller, B.M.R., 2019. Biomonitoring of children exposure to urban pollution and environmental tobacco smoke with hair analysis – A pilot study on children living in Paris and Yeu Island. France. Sci. Total Environ. 665, 864–872. https://doi.org/10.1016/j.scitotenv.2019.02.177.
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.
Pérez, R., Domenech, E., Coscollà, C., Yusà, V., 2017. Human Biomonitoring of food contaminants in Spanish children: design, sampling and lessons learned. Int. J. Hyg. Environ. Health 220, 1242–1251. https://doi.org/10.1016/j.ijheh.2017.07.006.
Poursafa, P., Amin, M.M., Hajizadeh, Y., Mansourian, M., Pourzamani, H., Ebrahim, K., Sadeghian, B., Kelishadi, R., 2017. Association of atmospheric concentrations of polycyclic aromatic hydrocarbons with their urinary metabolites in children and adolescents. Environ. Sci. Pollut. Res. 24, 17136–17144. https://doi.org/10.1007/s11356-017-9315-8.
Poursafa, P., Dadvand, P., Amin, M.M., Hajizadeh, Y., Ebrahimpour, K., Mansourian, M., Pourzamani, H., Sunyer, J., Kelishadi, R., 2018. Association of polycyclic aromatic hydrocarbons with cardiometabolic risk factors and obesity in children. Environ. Int. 118, 203–210. https://doi.org/10.1016/j.envint.2018.05.048.

Purcaro, G., Navas, J.A., Guardiola, F., Conte, L.S., Moret, S., 2006. Polycyclic aromatic hydrocarbons in frying oils and snacks. J. Food Prot. 69, 199–204. https://doi.org/10.4315/0362-028X-69.1.199.

Puttaswamy, N., Saidam, S., Rajendran, G., Arumugam, K., Gupton, S., Williams, E.W., Johnson, C.L., Panuwet, P., Rajkumar, S., Clark, M.L., Peel, J.L., Checkley, W., Clasen, T., Balakrishnan, K., Barr, D.B., 2020. Cross-validation of biomonitoring methods for polycyclic aromatic hydrocarbon metabolites in human urine: Results from the formative phase of the Household Air Pollution Intervention Network (HAPIN) trial in India. J. Chromatogr. B Anal. Technol. Biomed Life Sci. 1154, 122284 https://doi.org/10.1016/j.jchromb.2020.122284.

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.

Ruchirawat, M., Settachan, D., Navasumrit, P., Tuntawiroon, J., Autrup, H., 2007. Assessment of potential cancer risk in children exposed to urban air pollution in Bangkok, Thailand. Toxicol. Lett. 168, 200–209. https://doi.org/10.1016/j.toxlet.2006.09.013.

Sánchez-Guerra, M., Pelallo-Martínez, N., Díaz-Barriga, F., Rothenberg, S.J., Hernández-Cadena, L., Faugeron, S., Oropeza-Hernández, L.F., Guaderrama-Díaz, M., Quintanilla-Vega, B., 2012. Environmental polycyclic aromatic hydrocarbon (PAH) exposure and DNA damage in Mexican children. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 742, 66–71. https://doi.org/10.1016/j.mrgentox.2011.12.006.

Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int. J. Hyg. Environ. Health 215, 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007.

Schweighuber, A., Himmelsbach, M., Buchberger, W., Klampfl, C.W., 2019. Analysis of polycyclic aromatic hydrocarbons migrating from polystyrene/divinylbenzene-based food contact materials. Monatshefte fur Chemie 150, 901–906. https://doi.org/10.1007/s00706-019-2377-1.

Shahsavani, S., Dehghani, M., Hoseini, M., Fararouei, M., 2017. Biological monitoring of urinary 1-hydroxypyrene by PAHs exposure among primary school students in Shiraz, Iran. Int. Arch. Occup. Environ. Health 90, 179–187. https://doi.org/10.1007/s00420-016-1184-9.

Sochacka-Tatara, E., Majewska, R., Perera, F.P., Camann, D., Spengler, J., Wheelock, K., Sowa, A., Jacek, R., Mróz, E., Pac, A., 2018. Urinary polycyclic aromatic hydrocarbon metabolites among 3-year-old children from Krakow, Poland. Environ. Res. 164, 212–220. https://doi.org/10.1016/j.envres.2018.02.032.

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 metabolites 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.

Thai, P.K., Heffernan, A.L., Toms, L.M.L., Li, Z., Calafat, A.M., Hobson, P., Broomhall, S., Mueller, J.F., 2016. Monitoring exposure to polycyclic aromatic hydrocarbons in an Australian population using pooled urine samples. Environ. Int. 88, 30–35. https://doi.org/10.1016/j.envint.2015.11.019.

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.

The European Parliament and the Council of the European Union, 2000. DIRECTIVE 2000/60/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.

Tuakuila, J., Kabamba, M., Mata, H., Mbuyi, F., 2015. Tentative reference values for environmental pollutants in blood or urine from the children of Kinshasa. Chemosphere 139, 326–333. https://doi.org/10.1016/j.chemosphere.2015.06.039.

Urbancova, K., Dvorakova, D., Gramblicka, T., Sram, R.J., Hajslova, J., Pulkrabova, J., 2020. Comparison of polycyclic aromatic hydrocarbon metabolite concentrations in urine of mothers and their newborns. Sci. Total Environ. 723, 138116 https://doi.org/10.1016/j.scitotenv.2020.138116.

US EPA, 2014. Priority Pollutant List.

Wang, I.J., Karmaus, W.J.J., Yang, C.C., 2017. Polycyclic aromatic hydrocarbons exposure, oxidative stress, and asthma in children. Int. Arch. Occup. Environ. Health 90, 297–303. https://doi.org/10.1007/s00420-017-1198-y.

Yoon, H.S., Lee, K.M., Lee, K.H., Kim, S., Choi, K., Kang, D., 2012. Polycyclic aromatic hydrocarbon (1-OHPG and 2-naphthol) and oxidative stress (malondialdehyde) biomarkers in urine among Korean adults and children. Int. J. Hyg. Environ. Health 215, 458–464. https://doi.org/10.1016/j.ijheh.2012.02.007.

Yu, Y., Peng, M., Liu, Y., Ma, J., Wang, N., Ma, S., Feng, N., Lu, S., 2021. Co-exposure to polycyclic aromatic hydrocarbons and phthalates and their associations with oxidative stress damage in school children from South China. J. Hazard. Mater. 401, 123390 https://doi.org/10.1016/j.jhazmat.2020.123390.

Yu, Y., Wang, Q., Li, L., Liu, Z., Sun, P., Zhang, Y., Lin, H., Xiang, M., Li, H., Lin, B., 2016. Particle-associated ambient benzo[a]pyrene and levels of urinary 1-hydroxypyrene in a non-occupationally exposed population of adults and children in Lanzhou, China. Bull. Environ. Contam. Toxicol. 96, 303–308. https://doi.org/10.1007/s00128-016-1741-7.