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RESEARCH ARTICLE

Urinary levels of monohydroxylated polycyclic aromatic hydrocarbons in Brazilian children and health risk assessment: a human biomonitoring-based study

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Abstract

Monitoring human exposure to polycyclic aromatic hydrocarbons (PAHs) is a public health concern. Children are a vulnerable subgroup of the population with limited human biomonitoring data worldwide. Thus, this study aimed to measure the levels of seven PAH metabolites in urine from Brazilian children and provide risk assessment values for this exposure. Our data show naphthalene was the major contributor to children's exposure to PAHs, with a 100% detection rate. Children in urban regions presented higher exposure to PAHs, with higher concentrations of 2-naphthol in the southeast (1.09 ng/mL, $p < 0.05$). Furthermore, the highest concentration of 2-naphthol was found in older children ($p = 0.02$), suggesting a possible difference in dietary habits. Exposure to the carbaryl insecticide is suggested based on the high concentrations of 1-naphthol (1.29 ng/mL) and considering the ratio 1-naphthol/2-naphthol (1.78). Moreover, the positive correlation between the metabolites of fluorine and pyrene also suggests exposure to PAHs by petrol combustion. The risk assessment of the PAH exposure was evaluated using the estimated daily intake (EDI) for two naphthalene metabolites in the study with a 100% detection rate. The EDI was 14.47 ng/kg BW/day. The risk assessment to the PAH exposure revealed a non-carcinogenic risk profile, with a hazard quotient of 0.71. To the best of our knowledge, this study is the first to provide levels of PAHs in Brazilian children.

Keywords Polycyclic aromatic hydrocarbons · OH-PAHs · Naphthalene, Brazilian children, Human biomonitoring · Children exposure

Introduction

Polycyclic aromatic hydrocarbons (PAHs) present a basic structure consisting of at least two fused aromatic rings and are formed from the pyrolysis or incomplete combustion of organic matter through human activity, including coal combustion, biomass, wood-burning, vehicle exhaust, tobacco smoke, and industrial emissions or from natural processes. These pollutants are used commercially as intermediates in plasticizer, pigment, dye, and pesticide production. PAHs are ubiquitous organic compounds that contribute to global pollution in urban areas (Andersson and Achten 2015; Oliveira et al. 2017, 2019; Palazzi et al. 2019; Santos et al. 2019). Therefore, they can be found in all environmental compartments and biological samples, accumulating in adipose tissue, liver, kidneys, brain, and mammary glands after chronic exposure (Oliveira et al. 2017; Dobraca et al. 2018; Gachanja and Maritim 2018; Santos et al. 2019). Thus, human exposure to PAHs is of great concern. The exposure occurs primarily through ingestion, inhalation, and dermal contact. Contaminated water and food are the principal sources of PAH ingestion for non-occupationally exposed individuals (Oliveira et al. 2017, 2019; Palazzi et al. 2019).

Several human studies have suggested a relationship between PAH exposure and the occurrence of adverse effects on human reproductive, endocrine, central nervous systems,

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cardiovascular systems, respiratory problems, and skin allergies (Agency for Toxic Substances And Disease Registry (ATSDR) 2009; Kim et al. 2013). The United States Environmental Protection Agency (U.S. EPA) has listed sixteen PAHs as priority pollutants, and the International Agency for Research on Cancer (IARC) has classified some of them as carcinogens or possible carcinogens to humans (IARC, reference). For example, benzo[a]pyrene is considered a carcinogen, belonging to group 1A in the IARC classification, and naphthalene is classified as a possible carcinogen for humans (group 2B, IARC) (International Agency for Research on Cancer (IARC) 1983; Andersson and Achten 2015; Agency for Toxic Substances 7Disease Registry (ATSDR) 2017; Oliveira et al. 2017).

PAHs are metabolized into different hydroxylated derivatives, called mono-hydroxylated polycyclic aromatic hydrocarbon metabolites (OH-PAHs). Initially, PAHs are oxidated by hepatic cytochrome P450 monooxygenases, and reactive epoxide intermediates are formed. After that, in the second metabolism step, occurs reduction or hydrolysis of the epoxides to OH-PAH, followed by the conjugation of OH-PAHs with glucuronic acid. Then, the metabolites are excreted in the urine. The principal metabolites formed and detected in urine samples include 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, and 1-hydroxypyrene. On account of the rapid metabolism, the PAHs do not accumulate in the human body. The urinary levels of their metabolites are considered biomarkers of internal dose that can reflect the short-term exposure to these chemicals (Ribeiro 2001; Elovaara et al. 2003; Li et al. 2006; Srogi 2007; Xia et al. 2009; Ma and Harrad 2015; Moustafa et al. 2015; Centers for Disease Control and Prevention (CDC) 2018; Santos et al. 2019; Yu et al. 2021). Moreover, the 1-hydroxynaphthalene (1-naphthol) and 2-hydroxynaphthalene (2-naphthol) are biomarkers to evaluate human exposure to naphthalene. Unlike naphthalene, the carbaryl, a broad-spectrum carbamate insecticide, is only metabolized as 1-naphthol. Thus, by calculating the ratio between the metabolites, it is possible to suggest human exposure to both chemicals (Meeker et al. 2007; Sun et al. 2008; Thai et al. 2020; Torres-Moreno et al. 2022).

Children are a vulnerable population subgroup to develop adverse effects by exposure to PAHs and other chemicals. Their physiological systems are not fully developed, especially the central nervous system—the induction of genetic damage early in life and the greater vulnerability of children exposed to environmental genotoxicants. Given the smaller body weight and higher calorie intake per kilogram, children can be exposed to higher levels of OH-PAHs than adults. Then, children are becoming a group of growing interest, as in research on age-dependent reactions to environmental exposures; and between regulatory agencies to ensure minimal health risk (Pedersen et al. 2007; Oliveira et al. 2017; Dobraca et al. 2018; Sinha and Banda 2018).

Human biomonitoring (HB) programs have been implemented in various countries by public health agencies to assess human exposure to environmental pollutants. On the other hand, in Brazil, few HB studies are describing the prevalence of emerging pollutants in children (Rocha et al. 2017, 2018). The evaluation of the prevalence of PAHs and the assessment of the levels in Brazilians may provide support in a subsequent determination of reference values and the association with adverse health effects in exposed groups (Oliveira et al. 2017; Sinha and Banda 2018). Considering the scarcity of data in Brazil, the present study aimed to establish total urinary concentrations of polycyclic aromatic hydrocarbon metabolites in children from all geographic regions to assess exposure levels. Furthermore, the risk assessment of this exposure was also estimated.

Material and methods

Chemicals/reagents and solutions

Seven monohydroxylated polycyclic aromatic hydrocarbon metabolites selected in this study included the 1-hydroxy-naphthalene (1OH-NAP), 2-hydroxy-naphthalene (2OH-NAP), 9-hydroxy-fluorene (9OH-FLU), 2-hydroxy-fluorene (2OH-FLU), 9-hydroxy-phenanthrene (9OH-PHE), 1-hydroxy-pyrene (1OH-PYR), and 3-hydroxy-benzo[a] pyrene (3OH-B[a]P). The 1-hydroxy-naphthalene-*d7* (1OH-NAP-*d7*) was used as an internal standard. All native analytical standards and the labeled internal standard were purchased from Toronto Research Chemicals® (North York, Ontario, Canada), Accustandard® (New Haven, CT, USA), and Sigma-Aldrich® (St Louis, MO, USA). The individual stock solutions of all compounds were prepared in HPLC grade methanol (Sigma-Aldrich®, St Louis, MO, USA) and stored at – 20 °C in an amber glass until analysis. The working solutions and serial dilutions relative to the points on the calibration curve were prepared in ethyl acetate (Sigma-Aldrich®, St Louis, MO, USA) and stored at 4 °C. The derivatizing reagent used was N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), obtained from Sigma-Aldrich® (St Louis, MO, USA). Methanol and ethyl acetate were HPLC grade and obtained from J. T. Baker (Phillipsburg, NJ, USA). The high-purity water (resistivity 18.2 MΩ cm) was obtained from a Milli-Q water purification system (Millipore RiOs-DITM, Bedford, MA, USA). Synthetic urine was employed in the calibrations curves and to evaluate the method performance. The reagents (analytical grade from Sigma-Aldrich®) present in the urine synthetic included potassium chloride, sodium

chloride, urea, citric acid, ascorbic acid, potassium phosphate, creatinine, sodium hydroxide, sodium bicarbonate, and sulfuric acid. The enzyme β-glucuronidase *Helix pomatia* (100,000 units/mL from Sigma-Aldrich®) was used to enzymatic hydrolyze.

Study population and collection of urine samples

Two hundred urine samples were randomly selected from urban resident school-aged children of both sexes (6–14 years old). Participants are from 21 states of all Brazilian regions (Southeast, South, Central-west, Northeast, and North), as described in Table S1. The characteristics of the studied population, including distributions by gender and age, are given in Table S2. The selected number of samples was based on the population of each region. Based on that, the number of samples was proportional to the population of each region. We also take into account gender and age. Children with pathologies in acute or chronic conditions, such as kidney problems, and alterations in parameters that assess kidney and liver functions, which could limit the volunteer's ability to participate in the study, were excluded from the study.

Children's parents or their legal guardians were informed about the study, guaranteeing them the right to participate or not. After the legal guardian agreed to participate, they signed written informed consent. Urine samples were then collected at the school in each study region. For this, children were instructed to deposit the urine in a universal collection bottle (previously provided). After collection, a volume of 10 mL of each urine was packed, identified, and stored frozen at a local partner laboratory at − 20 °C before sending to Ribeirão Preto, State of São Paulo (University of São Paulo), for analysis. All samples were transported to Ribeirão Preto frozen in dry ice. At Ribeirão Preto, the samples were stored at − 80 °C to preserve their stability for several years, as previously reported in the literature (Liao et al. 2012; Zhang et al. 2013). These samples are part of a biobank created at the University of São Paulo in Ribeirão Preto.

The Institutional Ethical Review Board of the School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil, approved this study (CAAE 29,299,720.7.0000.5403). Ethical approval is according to the Declaration of Helsinki.

Instrumental analysis and sample preparation

The instrumental analysis of OH-PAHs was performed employing gas chromatography coupled to mass spectrometry–ISQ single quadrupole (Thermo Fisher Scientific®, Waltham, MA, USA), from previously reported studies by Jiang et al. (2019) and Li et al. (2015). The chromatographic separation was carried out on column FS-CAP SLB-5MS from Sigma-Aldrich® (30 m × 0.25 mm × 0.25 µm; 5% diphenyl polysiloxanes, 95% dimethylpolysiloxane), and helium was used as carrier gas at a flow of 1 mL/min. The initial temperature of the chromatographic column oven was 100 °C, maintained for 2 min, followed by a heating ramp at 15 °C/min to 210 °C, with a subsequent increase of 20 °C/min until 280 °C, holding for 4 min, finally an increase of 10 °C/min until 300 °C, maintained for 6.17 min. The total run time was 26 min. The injector temperature was maintained at 250 °C. A volume of 2 µL was injected into the GC–MS system, performed in splitless mode with valve opening after 1 min. The detection system used was the mass spectrometric operated in electron impact ionization mode (EI). The temperatures of the ionization source and the transfer line to the mass spectrometer were maintained at 300 °C and 290 °C, respectively. The data were acquired using *Full Scan* mode and the selective ion monitoring (SIM) of each native standard and labeled internal standard (ISTD). The mass spectrum (m/z) of SIM of all OH-PAHs and ISDT are described in Table S3 (Supplementary Information). Data acquisition and quantification were performed using the Thermo Xcalibur™ version 2.2 (Thermo Fisher Scientific®) program.

The sample preparation method used was based on a previously reported study by Onyemauwa et al. (2009), employing solid-phase extraction (SPE). Before the sample was treated to the determination of the total concentration of OH-PAHs in the urines from Brazilian children, hydrolysis with an *β*-glucuronidase enzyme solution was performed (Rocha et al. 2016, 2017). First, an amount of 1.5 mL of the homogenized urine was transferred to a conical-bottom polypropylene tube, and 100 µL of enzyme solution and 25 µL of the internal standard solution at a final concentration of 20.0 ng/mL were added to the sample. The enzyme solution containing 1.0 mol/L ammonium acetate solution containing 2000 units/mL of β-glucuronidase was prepared daily. After that, the samples were incubated at 37 °C overnight. Following this step, the samples were subjected to extraction by SPE. The SPE procedure was carried out employing a cartridge Oasis HLB sorbent (Oasis HLB 3 cc Vac Cartridge, 60 mg Sorbent per Cartridge, 30 µm from Waters®, Milford, MA, USA) for each 1.5 mL of the hydrolyzed urine sample. The samples were the previous dilution with 500 µL of phosphate buffer pH 7.0 before the SPE procedure. Methanol and phosphate butter pH 7.0 were used to condition the cartridge. After that, the samples were carried out and then eluted with ethyl acetate. The residues were dried in a speed dry vacuum concentrator (model Christ RCV 2–25 CO Plus and Christ CT 04–50 SR—Martin Christ®, Germany). Subsequently, the dry residue was reconstituted in 100 µL of the mixture of ethyl acetate: BFSTA with 1% TMCS. The derivation step

was performed at 60 °C for 60 min. Then, 2 µL was injected into the GC–MS system.

Quality control and method performance

PAHs are ubiquitous organic compounds and can be found in all environmental compartments and biological samples. So, there is no human urine exempt from these chemicals. Therefore, the evaluation of analytical parameters of the method performance was realized employing synthetic urine as the matrix in the sample preparation method. The analytical parameters were evaluated according to the Food and Drug Administration bioanalytical method recommendations, including linearity, the lower limit of detection (LLOD), the lower limit of quantification (LLOQ) precision, accuracy, and matrix effect (Food and Drug Administration (FDA) 2018). During the analysis, procedural blanks, matrix spike samples, and quality control were included in the instrumental analysis together with the samples. To increase the analysis reproducibility used an isotopically labeled internal standard. The internal standard level (1OH-NAP-*d*7) was fixed at 20 ng/mL in all the studies. The calibration curves were performed by least-squares linear regression analysis of weighted analysis of the ratio between peak areas of the analytes and the internal standard with seven different concentrations and realized in triplicate. The linearity showed levels in the range of 0.1 to 50 ng/mL for all OH-PAHs, except 3OH-B[a]P that was 0.5 to 50 ng/mL.

Risk assessment of the children exposure to PAHs

The majority of children's exposure to PAHs is through dietary ingestion since they are non-occupationally exposed population and non-smokers. Considering that the children are a vulnerable population subgroup to develop adverse effects by exposure to chemicals, the risk assessment of this exposure is essential. In this research, the exposure to PAHs was evaluated by determining the urinary levels, an internal dose biomarker (European Food Safety Authority (EFSA) 2008). After that, the risk assessment was carried out by the estimated daily intake (EDI) calculation of the parent PAH compound according to previously reported studies by Yu et al. (2021) and Fernández et al. (2021), as follows:

$$EDI = \frac{C \times V \times \mathrm{MW1}}{f \times \mathrm{BW} \times \mathrm{MW2}}$$

where C represents the geometric mean of the urinary concentration of the OH-PAHs (ng/mL), V is the total volume of the urine excreted in 24 h (12 mL/kg body weight/day), MW_1 corresponds to the molecular weight of the parent PAH compound, MW_2 is the molecular weight of the PAH metabolite, BW is the mean bodyweight of the children according to age, and finally, f-value corresponding to the ratio of OH-PAH excreted in the urine.

Data analysis

The statistical analysis of this study was realized employing the RStudio® software version 4.0.3. All statistical analyses were realized in only concentrations higher than the LLOD value. Geometric mean, median, percentiles, and minimum–maximum values of OH-PAHs urinary levels were calculated, and the results reported herein were for volume-based concentrations (ng/mL). Detected concentrations below the LLOD value were substituted with LLOD divided by the square root of 2 (United States Environmental Protection Agency (USEPA) 1994). Spearman's correlation evaluated the relationship between polycyclic hydrocarbons metabolites. Multiple regression models (regression analysis) were performed, followed by an adequate post-test to obtain the statistical difference between the concentrations found in the urine samples from children of different Brazilian regions, age groups, and gender. Values of p less than 0.05 ($p < 0.05$) were accepted as a level of significance.

Results and discussion

Quality control and method performance

The linearity of the method was performed by constructing three analytical calibration curves using fortified synthetic urine with OH-PAH concentrations of 0.1–0.5–1.0–2.0–10–20–50 ng/mL and internal standard at 20 ng/mL. Linearity was established by linear regression using the least-squares method and evaluated by relating the ratio of the analyte areas and the internal standard versus the nominal concentrations of the analytes. All correlation coefficients (r) obtained were higher than 0.98, indicating good linearity. The LLODs and LLOQs have been calculated as 3 and 10 times the standard deviation of ten replicate analyses of the lowest calibration standard divided by the value of the regression slope. The LLOD of the majority OH-PAHs in urine was 0.03 ng/mL and 0.15 ng/mL to 3-OHB[a]P. The LLOQ values were used as the first point of the calibration curves, corresponding to 0.1 ng/mL and 0.5 ng/mL (3-OHB[a]P). All coefficients of variation obtained in the precision evaluation and the percentage of recovery (%) in the accuracy studies were lower than 15%. Then, all results obtained are shown in Table S4 (Supplementary Information). Synthetic urine samples did not show endogenous interference in the analysis of OH-PAHs by GC–MS. The CVs of the normalized matrix factors (FMNs) for all analyzed samples were less than 15% (Table S4), indicating the absence of matrix effect in the proposed method.

Occurrence of hydroxylated polycyclic aromatic hydrocarbons in Brazilian children

This study is the first to assess Brazilian children to PAH exposure to the best of our knowledge. The detection rate (%), geometric mean, median, percentiles, and range (minimum–maximum) of OH-PAH urinary concentrations (ng/mL) measured in 200 urine samples of Brazilian children are summarized in Table 1. The concentrations of OH-PAHs according to every geographic region are described in Table S5 (Supplementary Information). The composition of OH-PAHs measured in urine samples from all Brazilian geographic regions is shown in Fig. 1. The PAH metabolites in the study were quantified in all samples. Besides, a detection rate of 100% for the naphthalene metabolites was obtained (1OH-NAP and 2OH-NAP). Other PAH metabolites presented the detection ratio of 61% for 2OH-FLU and 9OH-PHE, 45% for 9OH-FLU, 43% for 3OH-B[a]P, and 21% for 1OH-PYR. Moreover, the 1OH-NAP showed the highest urinary concentration, corresponding to 1.29 (0.05–38.52) ng/mL, followed by the 2OH-NAP, with a value of 0.73 (0.05–9.84) ng/mL. The sum of all analyzed PAH metabolites (∑OH-PAHs) showed a concentration of 6.85 (0.63–62.39) ng/mL. A possible reason for the lower concentrations found for the high-molecular-weight PAHs in urine is due to these compounds being primarily eliminated through feces, as the metabolites of pyrene (0.53 ng/mL) and benzo[a]pirene (0.21 ng/mL) (Fernández et al. 2021; Torres-Moreno et al. 2022).

PAHs can be sourced from natural, domestic, mobile, industrial, and agricultural sources. Burning fossil fuels is considered one of the primary sources of exposure to PAHs. Thus, PAH concentrations increase significantly in urban environments and are mainly influenced by vehicular emissions. Individual variability concerning exposure to environmental pollutants is directly influenced by temporal and spatial differences, and by the heterogeneity of individuals according to lifestyle habits (Oliveira et al. 2017; Dobraca et al. 2018; Gachanja and Maritim 2018; Santos et al. 2019). Human exposure to phenanthrene and fluorene is most abundant through domestic sources and vehicle emissions (Ratelle et al. 2020). The geometric mean of 9OH-PHE was 0.26 (0.03–56.17) ng/mL, with the highest value to the southeast (1.09 ng/mL). According to data from statistical correlation analysis, it is possible to assess co-exposure between PAH classes. The evaluation of the relationship between the concentrations of a PAH metabolite pair has been used to identify the potential PAH sources

Table 1 Urinary levels of polycyclic aromatic hydrocarbon metabolites (ng/mL) in Brazilian children ($n = 200$)

Urinary levels	1OH-NAP	2OH-NAP	9OH-FLU	2OH-FLU	9OH-PHE	1OH-PYR	3OH-B[a]P	∑OH-PAHs
DR (%)	100	100	45	61	61	21	43	-
GM	1.29	0.73	0.13	0.27	0.26	0.53	0.21	6.90
25th	0,62	0,31	0,02	0,02	0,02	0,27	0,11	3,81
50th (median)	1.33	0.71	0.02	0.43	0.39	0.48	0.11	6.78
75th	3.12	1.58	1.39	1.62	1.33	0.77	0.54	11.68
Minimum	0.05	0.05	0.08	0.104	0.03	0.13	0.11	0.63
Maximium	3.52	9.84	13.38	29.55	56.17	8.28	1.33	62.39

Abbreviations: *DR%*, detection rate %; *GM*, geometric mean; *∑OH-PAHs*, the sum of all polycyclic aromatic hydrocarbon metabolites

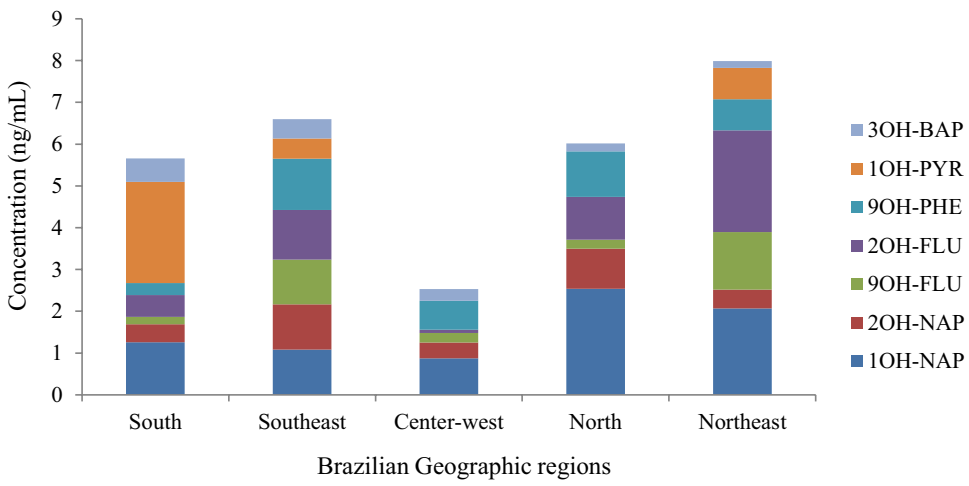

Fig. 1 Composition of polycyclic aromatic hydrocarbon metabolites measured in children's urine from all Brazilian geographic regions

(Azevedo et al. 2013). The statistic data of correlation analysis is described in Fig. 2. Ratelle et al. (2020) showed a statistically significant association between phenanthrene and fluorene concentrations and the daily consumption of meat and smoked. In this study, both metabolites showed similar detection rates and concentrations. Besides, 2OH-FLU and 9OH-PHE showed a positive correlation, with a significant value of 0.285. Fluorene and pyrene are related to the combustion of oil and the burning of gasoline. Our data showed a positive and statistically significant correlation between both fluorine and pyrene metabolites. The correlation between 9OH-FLU and 1OH-PYR was 0.581, and between 2OH-FLU and 1OH-PYR was 0.495. A potential source of human exposure to benzopyrene is cigarette smoke and occupational exposure in the steel industry. In this study, benzopyrene metabolite (3OH-B[a]P) presented a low concentration in the samples, with a geometric mean of 0.21 ng/mL and a 43% detection rate. Furthermore, 3OH-B[a]P showed a negative correlation with 2OH-NAP (−0.320) and 9OH-FLU (−0.374). Considering that children are a

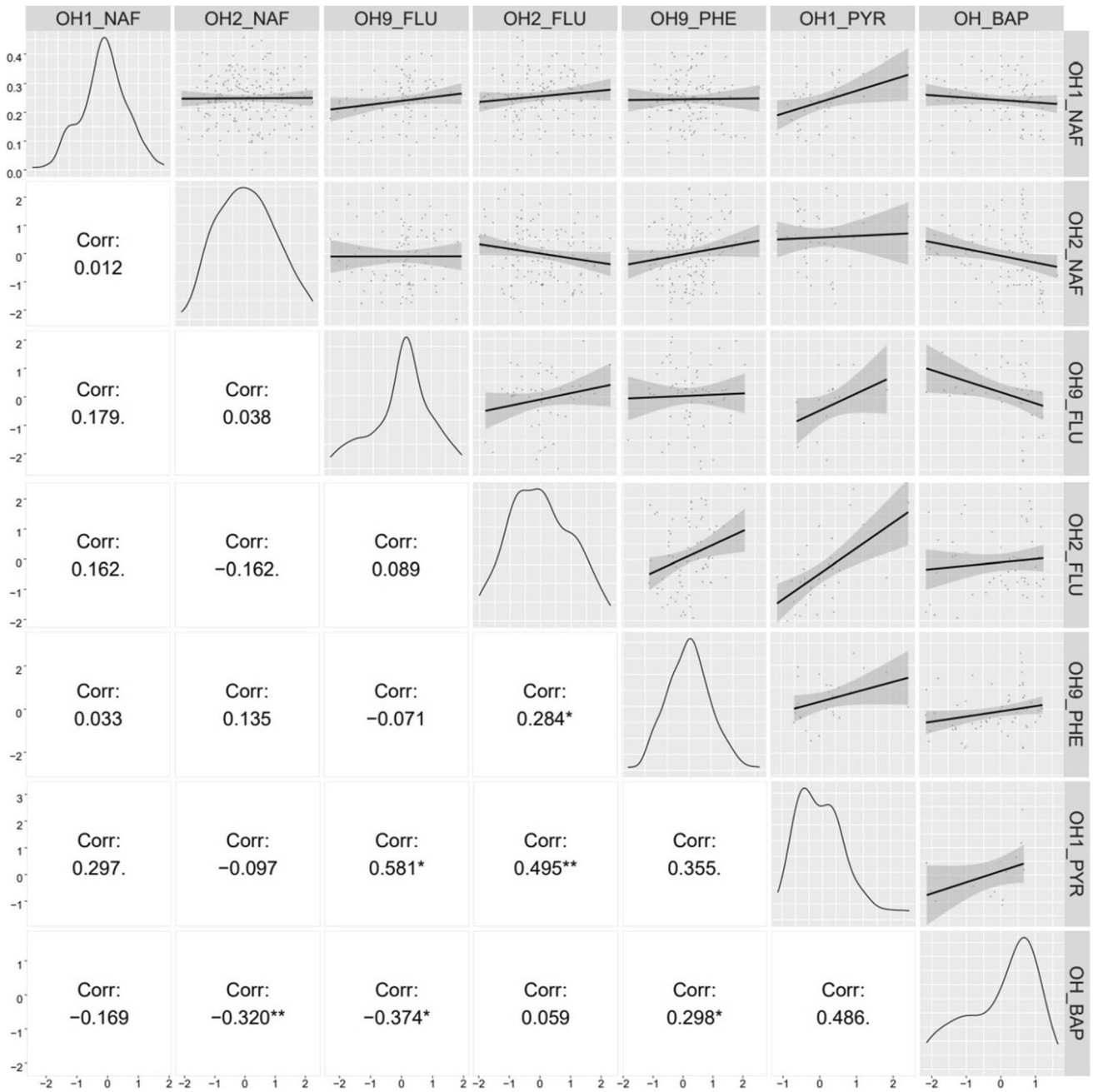

Fig. 2 Correlation between polycyclic aromatic hydrocarbon metabolites of this study

non-occupationally exposed population and non-smokers, these data might suggest a low exposure of the Brazilian children to benzopyrene. 1-naphthol is a metabolite formed from naphthalene and insecticide carbaryl. On the other hand, naphthalene is metabolized in both metabolites (1-naphthol and 2-naphthol). 1-naphthol was found in high concentrations in all samples, with a geometric mean of 1.29 (0.05–3.52) ng/mL. Since 2-naphthol has a similar structure to 1-NAP, it is utilized to assess the environmental exposure to carbaryl and naphthalene in epidemiology research. In this study, the ratio of 1-naphthol and 2-naphthol (1 N/2 N) was calculated to investigate the possible sources of exposure for Brazilian children. The ratio 1 N/2 N was 1.78 (0.55–6.29). This high value suggests a potential exposure to the insecticide carbaryl. Therefore, our findings provided a short understanding of the Brazilian population's exposure to PAHs and potentially carbaryl insecticide.. However, continuous biomonitoring is required to assess whether the high level of OH-PAHs is constant over time.

The samples of this study were collected in all Brazilian geographic regions (south, southeast, center-west, north, and northeast). The larger ∑OH-PAH concentration was determined in the northeast region with a value of 8.53 ng/mL, followed by north (7.71 ng/mL), central-west (6.89 ng/mL), southeast (6.56 ng/mL), and then south (6.45 ng/mL). Concerning the concentration difference found between each region of the country, the data analysis showed results with statistically significant ($p < 0.05$) only to 2OH-NAP, with the highest value in the southeast region (1.09 ng/mL). The statistical difference was found between southeast and central-west ($p = 0.0003$), southeast and northeast ($p = 0.0002$), and southeast and south ($p = 0.0000008$). These statistical analyses are described in Table 2. Brazil presents a natural and cultural diversity, several social inequalities, and contrasts. That way, these results suggest a potential variation in children's PAH exposures across the country. Besides, in Brazil, 84.72% of the population lives in urban areas. Some literature studies have reported the most prevalence of PAHs in urban and industrial areas. Besides, vehicle exhaust and petrochemical emissions might be important exposure sources of PAHs for children. The southeast Brazilian region is the most populated and industrialized. There is a large concentration of automotive and petrochemical industries and an extensive flow of vehicles in urban areas, contributing significantly to the emission of PAHs and other pollutants. The consumption of food prepared at high temperatures, like grilling and smoking meat, has been reported to increase PAH exposure and is considered the main dietary source of PAHs (Llorca et al. 2017; Duque et al. 2019; Bonilla-Bedoya et al. 2020; Ratelle et al. 2020; Souza et al. 2020; Yu et al. 2021).

Table 2 Urinary concentration of 2-hydroxylated-naphthalene in Brazilian children ($n = 200$) separated according to geographic region. The values are expressed as geometric mean (ng/mL)

Variable	Contrast	GM (ng/mL)	*p*-value*
2OH-NAP	Southeast and Center-west	1.09 and 0.38	0.0003
2OH-NAP	Southeast and Northeast	1.09 and 0.45	0.0002
2OH-NAP	Southeast and South	1.09 and 0.43	0.0000008
2OH-NAP	Southeast and North	1.09 and 0.96	$p > 0.05$

*Significance level is $p < 0.05$

Some of the literature data have shown the presence of PAHs in different environmental samples in Brazil, corroborating with the human exposure data shown in this study. The physical–chemical properties of the PAHs determine their destination in the environment. Therefore, the highest PAH concentrations are commonly found in soil, sediment, atmospheric suspended particulates, and animal origin food. Oliveira et al. (2020) determined the concentration of eighteen PAHs in fish samples, and the found values of the sum of PAHs were between 1.32 and 18 ng/g weight wet. The atmospheric concentrations of PAHs determined in samples from Brazil were 0.70 to 90 ng/m^3 in passive air (Meire et al. 2019) and 0.106 to 8.57 µg/g in street dust (Franco et al. 2017). Maciel et al. (2015) determined the concentration of sixteen PAHs in the urbanized tropical estuary, and the maximum value found was 497.6 µg/g. In the reported study by Resende et al. (2018), the PAH concentration in soil was between 15.8 and 39.4 ng/g. Thus, these data might suggest a considerable PAH exposure by ingestion of dust in urban areas. In addition, the soil and sediment may be considered a primary source of PAH contamination for food. Lopes et al. (2017) determined PAH metabolites in Brazilian maté drinkers and suggested that this habit might increase the urinary concentrations of some PAH metabolites and smoke cigarettes. Several studies describe potential exposure sources to PAHs. Since all populations are exposed to PAHs, biomonitoring studies should be routine, mainly of sensitive groups like children and pregnant women. Furthermore, to better assess the difference in exposure between Brazilian geographic regions, further studies are needed to determine PAHs in environmental samples and air particles to verify the actual and potential exposure sources. Some factors may influence the urinary levels of OH-PAHs, such as age groups and genders. Therefore, the statistical analysis was also performed to evaluate the differences between measured concentrations of the 1OH-NAP, 2OH-NAP, and ∑OH-PAH, according to gender and age group (6–14). These statistical analyses are described in Table 3. Gender did not show a significant difference in urinary concentrations of PAH metabolites, and a similar result was also reported by Yu et al. (2021). In this study, the data suggest that the children of both genders might have similar behavior habits, and their exposure doses of PAHs are similar. This research showed

Table 3 Statistical description of naphthalene metabolites and the sum of all OH-PAHs analyzed in young and old, male and female Brazilian children ($n=200$). The values are expressed as geometric mean (ng/mL)

Variable	Group 1	Group 2	p-value**
1OH-NAP	Female	Male	0.852
2OH-NAP	Female	Male	0.625
∑OH-PAHs	Female	Male	0.983
1OH-NAP	High (11–14*)	Low (6–10*)	0.279
2OH-NAP	High	Low	**0.02**
∑OH-PAHs	High	Low	0.851

*Years old; **bold: significance level is $p<0.05$

a statistically significant difference between the 2OH-NAP concentrations and the studied age groups. For the statistical analysis, the children were separated into two groups, younger and older. The highest concentration of 2OH-NAP was found in older children ($p=0.02$), suggesting a possible difference in dietary habits of the children group, as related by Fernández et al. (2021).

Our results were compared with previously published studies in other countries. The comparison is described in Table 4. In all mentioned studies, the children's exposure was evaluated from the perspective of the internal dose determination of the PAH metabolites, and the most abundant PAH metabolites quantified in children were of the naphthalene, mainly 2OH-NAP, corroborating the findings of this research (Urbancova et al. 2017; Murawski et al. 2020; Ratelle et al. 2020; Urbancova et al. 2020; Fernández et al. 2021; Hudson-Hanley et al. 2021; Yu et al. 2021). Naphthalene is ubiquitous and human exposure is high worldwide since it is one of the constituent chemicals of urban air pollution. Naphthalene is used to produce moth repellant, the carbamate insecticide, surface-active agents, and resins. Human exposure may also occur by burning fossil fuels and organic material, vehicle exhaust emissions, and cigarette smoke. In the reported study by Urbancova et al. (2017), the concentrations of ΣOH-PAHs were approximately 3 times higher in newborns' urine from an industrialized location in the Czech Republic than in a no polluted locality. Considering the mentioned ∑OH-PAH concentrations in a global context, it is possible to find similarities among exposure children in different countries, like the similar values between our results (6.85 ng/mL) and those reported by Murawski et al. (2020) in Germany (6.02 ng/mL). Similar values may suggest that total PAH deposition fluxes are similar in various urban areas (Azevedo et al. 2013). A considerable difference can also be observed between the values obtained in the reported study by Fernández et al. (2021) in Spain (10.84 ng/mL), and by Yu et al. (2021) in China (3.86 ng/mL). The differences between the concentrations found for OH-PAHs can be explained by the diversity in food consumption and behavior habits among the populations worldwide.

Risk assessment of the Brazilian children exposure to PAHs

The risk assessment of the PAH exposure was performed by estimated daily intake (EDI) calculation for the two naphthalene metabolites since it showed a 100% detection rate. The EDI was calculated separately for each sample. The geometric mean concentration used in the formula was the sum of two naphthalene metabolites (1OH-NAP and 2OH-NAP). The total volume of urine excreted for children was 12 mL/kg BW/day, and for each sample, this value was multiplied by the mean of BW of the child

Table 4 Comparison of the sum OH-PAH concentration determined in Brazilian children samples and the positive detection rate of the naphthalene metabolites with other studies reported in other countries. The values are expressed in ng/mL

∑OH-PAH	Detection rate (%) of naphthalene metabolites	Population age	Country	Reference
6.85 (0.63–62.39)	100% for both	6–14	Brazil	This study
3.86 (0.36–36.5)	99.4% 2OH-NAP 67.5% 1OH-NAP	8–11	China	Yu et al. (2021)
10.84	100% 2OH-NAP 87% 1OH-NAP	5–12	Spain	Fernández et al. (2021)
6.02	100% 2OH-NAP 96% 1OH-NAP	3–17	Germany	Murawski et al. (2020)
7.08	Naphthalene was the most abundant PAHs	NHANES 2013–2014 (6–17)	USA	Hudson-Hanley et al. (2021)
2.90	100% 2OH-NAP 75% 1OH-NAP	Newborn	Czech Republic	Urbancova et al. (2017)
5.15 (μg/g creatinine)	100% 2OH-NAP 67% 1OH-NAP	Newborn	Czech Republic	Urbancova et al. (2020)

relative to age and gender. According to a survey over families' budget, these BW data are carried out by the Brazilian Institute of Geography and Statistics (IBGE –Brazilian Institute of Geography and Statistics, Brazil 2010; IBGE –Brazilian Institute of Geography and Statistics, Brazil 2011). All these data are described in Table S6 (Supplementary Information). The f-value used was 1 since the hydroxylated naphthalene is 100% excreted in the urine. Each sample was its respective value and then the geometric mean of all samples was calculated. The EDI obtained in this study was 14.47 (1.02–208.88) ng/kg BW/day.

The hazard quotient (HQ) also was determined aim assess non-carcinogenic risk to naphthalene. To calculate the HQ used the ratio of EDI determined and the reference dose (RfD), which was stipulated as 20 for naphthalene, according to the United States Environmental Protection Agency (Yu et al. 2021). The HQ of this study was 0.72, and values below 1 (risk level) meant that the chemical shows a potential non-carcinogenic risk. This result is according to literature data about naphthalene toxicity (group 2B, IARC). The low molecular weight PAHs, like naphthalene and fluorene, are associated with acute toxic effects.

In comparison, high molecular weight molecules, like pyrene and benzopyrene, are carcinogenic to humans (IARC, 1983; Andersson and Achten 2015; Oliveira et al. 2017; Ratelle et al. 2020). The extensive exposure to naphthalene makes PAHs a priority to human biomonitoring. However, our results of HQ suggest that this exposure might not pose a health risk. PAHs are considered the most common organic contaminants in the environment. Due to high urinary levels of OH-PAHs determined in this research, more studies need to monitor PAH internal dose over time. Furthermore, the additional determination of biomarkers of effect is necessary to identify potential health implications.

This study provides for the first data on exposure to PAHs in Brazilian children and the risk assessment values for this exposure. On the other hand, we also acknowledge limitations in the present study. For example, the questionnaire did not consider the family environment, socio-economic status, detailed diet, and others. Brazil is a vast country with natural and cultural diversity and social inequalities and contrasts. Then, it is difficult to rule out confounding factors, mainly considering a random collection of the urine samples, as is the case of the present study.

Conclusion

To the best of our knowledge, this is the first study to assess the exposure of Brazilian children to polycyclic aromatic hydrocarbons. Overall, our findings suggest that the risks from PAHs could be neglected since Brazil does not have any current legislation associated with the presence of PAHs in environmental, food, and air particle samples. Besides, in all analyzed urine samples, PAH metabolites were quantified and the naphthalene metabolites showed a 100% detection rate. The 1OH-NAP is considered a metabolite of both naphthalene and carbaryl insecticide, while 2OH-NAP is exclusively naphthalene's metabolite. Thus, the high concentrations of 1-naphthol and the ratio 1 N/2 N suggest potential exposure to the carbaryl insecticide in the Brazilian population. The positive correlation between the metabolites of fluorine and pyrene suggests exposure to PAHs through the oil combustions and burning of gasoline. The low concentrations found for the benzopyrene's metabolite may be consistent with the low exposure to benzopyrene since this PAH is usually found in cigarette smoke and occupational exposure in the steel industry. Furthermore, the results of this research may infer that children in urban regions presented higher exposure to PAHs, with higher concentrations of 2OH-NAP determined in the southeast ($p < 0.05$). The highest concentration of 2OH-NAP was found in older children ($p = 0.02$), suggesting a possible difference in dietary habits of the children group. The risk assessment showed a non-carcinogenic risk profile to naphthalene exposure (HQ < 1). However, more studies are required to monitor PAH internal dose in vulnerable populations over time.

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CRediT authorship contribution Marilia Cristina Oliveira Souza: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft.

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Anthony Santana: writing—review and editing.

Andres Dobal Campiglia: funding acquisition, writing—review and editing.

Fernando Barbosa: conceptualization, funding acquisition, project administration, supervision, writing—review and editing.

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Data availability All data generated or analyzed during this study are with the corresponding author, and, if necessary, she is available for taking any question about the datasets and these can be requested by reasonable request.

Declarations

Ethics approval and consent to participate This study was approved by the Institutional Ethical Review Board of the School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil (CAAE 29299720.7.0000.5403).

Consent for publication The informed consent was obtained from the legal guardian of the children.

Conflict of interest The authors declare no competing interests.

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