Oral bioavailability reveals an overestimation of the toxicity of polycyclic aromatic hydrocarbons in atmospheric particulate matter

Joel Sánchez-Piñero1 · Paula Gómez-Meijide1 · Estefanía Concha-Graña1 · Jorge Moreda-Piñeiro1 · Soledad Muniategui-Lorenzo1 · Purificación López-Mahía1

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Abstract
Polycyclic aromatic hydrocarbons (PAHs) in atmospheric particulate matter have adverse effects on human health, yet total PAH concentrations should overestimate the toxicity compared to the bioavailable amount of PAHs. To explore this hypothesis, we measured PAHs oral bioavailability in vitro in particulate matter with aerodynamic diameter lower than 10 µm (PM10) using a test that mimics the human digestive system. This assay combines the use of simulated gastrointestinal fluids and a dialysis membrane to simulate intestinal absorption. Results show that oral PAH bioavailability was below 5%, with fluorene, anthracene, acenaphthene and phenanthrene as the most bioavailable PAHs. Data suggest no carcinogenic risk of oral bioavailable PM10-bound PAHs following a health risk assessment via inhalation-ingestion by using benzo(a)pyrene-equivalent carcinogenic concentration and hazard indexes. To our best knowledge, this is the first research study of in vitro oral bioavailability estimation of PM10-associated PAHs.

Keywords Oral bioavailability · Polycyclic aromatic hydrocarbons · Particulate matter · Physiologically based extraction · Health risk assessment

Introduction
Inhalation of atmospheric particulate matter represents a significant exposure pathway for humans. Several studies have associated particulate matter exposure with an increase in morbidity and mortality due to its negative outcomes on human health, being particulate matter with aerodynamic diameter ≤ 10 µm (PM10) and ≤ 2.5 µm (PM2.5) the most studied particulate matter fractions due to their potential to penetrate and deposit in different regions of respiratory system after inhalation (Burnett et al. 2018). Also, particle-bound pollutants are thought to have a key role in adverse health effects of particulate matter, which can be get into the body by means of inhalation (Galvão et al. 2018). Among PM-bound pollutants, polycyclic aromatic hydrocarbons (PAHs) are a well-known group of compounds widely studied because of their high ubiquity in environmental samples and their adverse effects on human health (Abdel-Shafy and Mansour 2016). PAHs occurrence in particulate matter fractions has been attributed mainly as a result of organic materials combustion (Mesquita et al. 2014). Nevertheless, considering total particle-bound pollutant concentrations provides an overestimation of their toxicity and a non-realistic evaluation of the exposition and health risk assessment. In order to improve risk assessment, it is also important to consider how pollutants are assimilated by exposed people. In recent years, scientific community has been focused on the pollutant fraction that can be dissolved in biological fluids (i.e. bioaccessible fraction) and the fraction that diffuse across biological membranes to reach systemic circulation once dissolved (i.e. bioavailable fraction), to address a more realistic evaluation. For this purpose, different methodologies have been described in the literature to perform bioaccessibility/bioavailability estimation, distinguishing between in vivo methods and in vitro methods (using synthetic body fluids and physiological human conditions) to assess the maximum concentration of pollutant that can be dissolved.
(bioaccessible fraction), in combination with absorption sinks, semi-permeable dialysis membranes or cultured cells models to assess fraction of pollutant bioavailable (i.e. enters the blood stream) (Miller et al. 1981; Ruby et al. 1999; Kastury et al. 2017). In vivo methods may be unethical, expensive and impractical for large scale testing (Nemmar et al., 2013). On contrary, in vitro assays offer an attractive alternative to in vivo assays, due to the simplicity, rapidity, easy-control, low cost, high precision and reproducibility (Kastury et al. 2017). However, there is standardization shortage, which results in important differences in body fluid compositions and physiological human extraction conditions.

After air inhalation during breathing, particles come into the body and are deposited in different regions of respiratory depending on the particle properties (mainly the size) (Kastury et al. 2017). Concerning the deposition, particles with aerodynamic diameter < 2.5 µm are more likely to reach the alveolar region of lungs (where pollutants might be dissolved into lung fluids and absorbed to blood (inhalation bioavailability), while particles with aerodynamic diameter in the range of 2.5–10 µm are deposited in tracheobronchial region, which could be subjected to mucociliary clearance and expelled by coughing/sneezing or ingested. Those particles conducted to the gastrointestinal tract could be an important absorption pathway of particle-bound pollutants (oral bioaccessibility/bioavailability). Several oral in vitro bioaccessibility approaches have been applied mainly for metal(loid)s in particulate matter (Falta et al. 2008; Mukhtar and Limbeck 2011; Uzu et al. 2011; Hu et al. 2012; Puls et al. 2012; Sysalová et al. 2014; Mohr et al. 2017; Kastury et al. 2018; Nie et al. 2018; Gao et al. 2018) and indoor/outdoor dust (Turner and Simmonds 2006; Turner and Ip 2007; Turner and Hefzi 2010; Turner 2011; Boisa et al. 2014; Bradham et al. 2014; Huang et al. 2014a, b; Patinha et al. 2015; Goix et al. 2016; Padoan et al. 2017) samples. However, in vitro oral bioaccessibility studies of organic pollutants in particulate matter were not found in the literature, while there were some studies applied to indoor/outdoor dust and soils for organic compounds, i.e. PAHs (Tang et al. 2006; Kang et al. 2011; Collins et al. 2013; Li et al. 2015; Kademoglou et al. 2018a), organophosphate flame retardants (Quintana et al. 2017), semi-volatile organic compounds (Raffy et al. 2018), polychlorinated biphenyls (Wang et al. 2013) and polybrominated diphenyl ethers (Yu et al. 2011; Kademoglou et al. 2018b; Gao et al. 2019b). The stringent analytical requirements (target pollutants enrichment, due to the low levels; and pollutant isolation from the synthetic fluids matrix) for organic pollutant quantification in bioaccessible/bioavailable fractions might explain the scarce information. Regarding in vitro oral bioavailable studies, in vitro physiologically based extraction test methodology developed by Miller et al. (Miller et al. 1981) was extensively used because of showing well correlations for metals with in vivo studies. According to the literature, some researches have based on this methodology (with some modifications) to assess metal(loid)s oral bioavailability in foods (Wolfge et al. 2002; Haro-Vicente et al. 2006; Moreda-Piñeiro et al. 2013, 2015a), while it was recently applied to PM₁₀ samples (Moreda–Piñeiro et al. 2019).

Thus, the main objective of this research is the novel application and validation of an in vitro physiologically based extraction test, based on the use of a dialysis membrane to simulate the intestinal absorption (Miller et al. 1981; Moreda–Piñeiro et al. 2019), to assess the oral bioavailability of PAHs in PM₁₀. Furthermore, the exposure and health risk assessment based on total and oral bioavailable contents of PAHs obtained were estimated and discussed.

**Experimental**

**PM₁₀ sample collection**

An automatic high-volume sampler DIGITEL DHA-80 (Hegnau, Switzerland) was used to samples collection at an urban site of A Coruña city (northwest of Spain). Description of the sampling site was previously reported (Moreda-Piñeiro et al. 2015b). Atmospheric particulate matter was collected on Ahlstrom Munksjö MK360 (Falun, Sweden) quartz fibre filters, 150 mm of diameter, at 30 m³ h⁻¹ for 24 h, 00:00 to 23:59 (Coordinated Universal Time), from 1st January to 31st December 2015 according to the European Norm 12,341 (EN 12,341:2015) (UNE 2015). Due to the low PAHs levels expected in oral bioavailable fractions and limited PM₁₀ filter amounts, monthly pooled samples (total filter area of 8.5 cm²) were made by combining one portion (0.28 cm²) of each daily PM₁₀ sample collected during each month. Monthly PM₁₀ mass ranged from 0.69 mg (September) to 1.0 mg (March).

**In vitro oral bioavailability procedure**

In vitro oral PAHs bioavailable fractions were obtained following the methodology as described in the Supplementary Material.

**Total and oral bioavailable PAHs extraction and quantification procedures**

Procedures for PAHs extraction and quantification in bioavailable fractions and in PM₁₀ samples are summarized in Supplementary Material; details for validation procedures (Tables S1-3) are also shown in this section.
Human health risk assessment of target PM$_{10}$-associated PAHs via inhalation-ingestion was performed basing on United States Environmental Protection Agency’s (USEPA) Inhalation Dosimetry Methodology (as described in Supplementary Material) considering three scenarios (residents in the studied area (scenario I); residents working outside the studied area (scenario II); and workers not living in the area (scenario III), Table S4) (USEPA 2009, 2014; Hernández-Pellón et al. 2018).

Carcinogenicity of PM$_{10}$-associated PAHs relatively to benzo(a)pyrene-equivalent toxic concentration was calculated as described in Supplementary Material (USEPA 1993).

Results and discussion

PM10 concentration levels and total PAH content

The daily PM$_{10}$ mass concentrations ranged between 3 and 57 µg m$^{-3}$. There is only two samples that exceed the daily limit value of 50 µg m$^{-3}$ set by the European Commission, which cannot exceed more than 35 times per year (Directive 2008/50/EC) (EU 2008). Despite this, the annual mean PM$_{10}$ concentration corresponds to 20 µg m$^{-3}$, which is below the annual mean value of 40 µg m$^{-3}$ set in the European Directive. The monthly average PM$_{10}$ mass concentrations ranged between 17 µg m$^{-3}$ (September) and 26 µg m$^{-3}$ (March).

Monthly average PAHs concentrations obtained are shown in Figure S1, while detailed information is given in supporting material (Table S5). The contribution of each PAH to the Σ16PAHs (Figure S1) was dominated by benzo(e)pyrene (19–27%), chrysene (13–19%), benzo(b)fluoranthene + benzo(j)fluoranthene (8–20%) and indene(1,2,3-cd)pyrene (9–15%), while low contribution was achieved for the carcinogenic PAHs (benzo(b)fluoranthene + benzo(j)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene and indene(1,2,3-cd)pyrene). As shown in the graph, fluoranthene, benzo(a)pyrene, benzo(e)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene and indene(1,2,3-cd)pyrene were found lower than limits of quantification. Furthermore, oral bioavailable carcinogenic PAHs plus non-carcinogenic PAHs concentrations represented 17–35% and 64–82% of the total oral bioavailable PAHs content, respectively. Results of monthly average PAH oral bioavailable concentrations as well as total carcinogenic PAHs and total non-carcinogenic PAHs are given in Table S6, while oral monthly average PAH bioavailability ratios (B$_{av}$ expressed as percentage), calculated using the following formula, are shown in Fig. 1:

$$B_{av}(\%) = \frac{[PAH]_{oral bio available fraction}}{[PAH]_{total content}} \times 100$$  \hspace{1cm} (1)

where [PAH]$_{oral bio available fraction}$ is the monthly average PAH concentration obtained after in vitro oral bioavailability (pg m$^{-3}$) and [PAH]$_{total content}$ is the total monthly average PAH concentration (pg m$^{-3}$).

Oral PAHs bioavailability percentages obtained for each PAH were below 5% (Fig. 1). As shown in the graph, fluoranthene offered the highest monthly average oral bioavailability ratio ± standard deviation (2.8 ± 2.1%), followed by anthracene (1.4 ± 1.1%), acenaphthene (1.2 ± 1.7%) and phenanthrene (0.71 ± 0.27%). Regarding fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(e)pyrene, benzo(b)fluoranthene + benzo(j)fluoranthene, benzo(k)anthracene (< 0.21–3.4) > chrysene (0.25–2.6) > pyrene (< 0.05–2.9) > fluorene (< 0.02–1.3) > acenaphthene (< 0.10–0.97) > naphthalene (< 0.11–0.95) > benzo(a)anthracene (< 0.02–0.14). Oral bioavailable concentrations of anthracene, benzo(b)fluoranthene + benzo(j)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene and indene(1,2,3-cd)pyrene were found lower than limits of quantification. Furthermore, oral bioavailable carcinogenic PAHs plus non-carcinogenic PAHs concentrations represented 17–35% and 64–82% of the total oral bioavailable PAHs content, respectively. Results of monthly average PAH oral bioavailable concentrations as well as total carcinogenic PAHs and total non-carcinogenic PAHs are given in Table S6, while oral monthly average PAH bioavailability ratios (B$_{av}$ expressed as percentage), calculated using the following formula, are shown in Fig. 1:

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where [PAH]$_{oral bio available fraction}$ is the monthly average PAH concentration obtained after in vitro oral bioavailability (pg m$^{-3}$) and [PAH]$_{total content}$ is the total monthly average PAH concentration (pg m$^{-3}$).
fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, bioavailability percentages obtained were below 0.5%. Naphthalene dialyzability percentages were not assessed because naphthalene concentrations in most of the dialysate fractions extracts were lower than limit of quantification. The low percentages obtained show that a low amount of PM10-bound inhaled PAHs could be available in the blood through intestinal absorption.

Although 4–6 ring-condensed PAHs (benzo(e)pyrene, chrysene, indene(1,2,3-cd)pyrene and benzo(b)fluoranthene + benzo(j)fluoranthene, BbF + BjF; benzo(k)fluoranthene, BkF; benzo(a)pyrene, BaP; dibenz(a,h)anthracene, DBahA; benzo(g,h,i)perylene, BghiP; and indeno(1,2,3-cd)pyrene, IP) were the most predominant in monthly PM10 samples (considering total concentrations), the highest bioavailability ratios were obtained for 3 ring-condensed PAHs (fluorene, anthracene, acenaphthene and phenanthrene). This trend might be attributed to an increasing hydrophobicity (as increasing the condensed ring number) that obstructs PAHs mobilization from PM10 to biological simulated fluids (aqueous based), as published for other simulated fluids (Li et al. 2019; Sánchez-Piñero et al. 2021). Furthermore, the different chemical composition (different particulate matter sources) of PM10 samples could explain the high standard deviation of monthly average oral bioavailability ratios.

There are no oral PM10-bounded PAHs bioavailability data in the literature. Therefore, oral bioavailability ratios obtained in this study will be compared with oral bioaccessibility ratios found in the literature. Although there are different bioaccessibility protocols reported in the literature to assess oral bioaccessible fractions of PAHs in soil (Tang et al. 2006; Collins et al. 2013; Li et al. 2015; Gao et al. 2019a) and indoor dust (Kang et al. 2011) samples, oral bioavailable ratios shown in Fig. 1 are lower than those previously published for bioaccessibility ratios. Oral PAHs bioaccessibility ratios in air conditioning filter dust, ranging from 2 to 17% (Kang et al. 2011), and soil, ranging from 3.9 to 54.9% (Tang et al. 2006), were assessed using a physiologically based extraction test. Within this context, the lower values obtained in this study might be attributed to the inclusion of a dialysis membrane filled with aqueous buffer solution, which hinders the capture of PM10-released PAHs (Fig. 1). Furthermore, several in vitro bioaccessibility approaches for

![Figure 1](image-url)
organic compounds involved the use of sorption sinks (i.e. silicone sheet \( \text{Gao et al. 2019b} \), Tenax TA® absorption sink \( \text{Li et al. 2015; Kademoglou et al. 2018b} \) and activated carbon sink \( \text{Collins et al. 2013} \)) to capture pollutants as they are released from matrix. The addition of a silicone sheet as sorption skin was observed to increase PAH bioaccessibility ratios up 44–67% \( \text{Gao et al. 2019b} \). In addition, activated carbon and Tenax TA® were used as sorption skins, increasing bioaccessibility ratios up to 16–31% in contaminated soils \( \text{Li et al. 2015} \) and up to 15–75% in spiked soil samples \( \text{Collins et al. 2013} \). According to the results obtained in the present study, the values are even much lower than those reported when including a sorption sink. Nevertheless, the inclusion of a sorption sink could create a sorption gradient higher than reality that might lead to overestimation.

### Human health risk assessment of PM10-bound PAHs

Human health risk assessment was performed based on the inhalation dosimetry methodology for different scenarios and benzo(a)pyrene-equivalent carcinogenic concentrations approach, both models proposed by USEPA.

| PAHs | Scenario I Adults Total | Oral bioavailable | Scenario I Children Total | Oral bioavailable | Scenario II Adults Total | Oral bioavailable | Scenario II Children Total | Oral bioavailable | Scenario III Total | Oral bioavailable |
|------|-------------------------|-------------------|---------------------------|-------------------|--------------------------|-------------------|---------------------------|-------------------|---------------------|-------------------|
| Naph | 81.3                    | 0.91              | 24.4                      | 0.27              | 40.7                     | 0.46              | 24.2                      | 0.27              |
| Ace  | 109                     | 0.93              | 32.6                      | 0.28              | 54.4                     | 0.46              | 32.4                      | 0.28              |
| Fl   | 46.6                    | 2.1               | 14.0                      | 0.62              | 23.3                     | 1.0               | 13.9                      | 0.62              |
| Phe  | 332                     | 3.3               | 99.7                      | 0.97              | 166                      | 1.6               | 98.9                      | 0.97              |
| Ant  | 98.0                    | 0.25              | 29.4                      | 0.075             | 49.0                     | 0.12              | 29.2                      | 0.074             |
| Ft   | 1710                    | 3.4               | 513                       | 1.0               | 855                      | 1.7               | 509                       | 1.0               |
| Pyr  | 1200                    | 2.8               | 359                       | 0.83              | 598                      | 1.4               | 356                       | 0.83              |
| BaA  | 201                     | 0.075             | 60.2                      | 0.023             | 100                      | 0.038             | 59.7                      | 0.022             |
| Chry | 586                     | 0.70              | 176                       | 0.21              | 293                      | 0.35              | 174                       | 0.21              |
| BeP  | 2790                    | 1.2               | 836                       | 0.36              | 1390                     | 0.60              | 829                       | 0.36              |
| BbF + BjF | 604                     | 0.021             | 181                       | 0.0062            | 302                      | 0.010             | 180                       | 0.0061            |
| BkF  | 171                     | 0.039             | 51.3                      | 0.012             | 85.5                     | 0.020             | 50.9                      | 0.012             |
| BaP  | 264                     | 0.021             | 79.3                      | 0.0062            | 132                      | 0.010             | 78.6                      | 0.0061            |
| DahA | 112                     | 0.0021            | 33.5                      | 0.0006            | 55.9                     | 0.0010            | 33.3                      | 0.0006            |
| BghiP| 1130                    | 0.0096            | 340                       | 0.0027            | 566                      | 0.0048            | 337                       | 0.0029            |
| IP   | 390                     | 0.0014            | 117                       | 0.0004            | 195                      | 0.0007            | 116                       | 0.0004            |

Total and oral bioavailable PAH concentrations in monthly pooled PM\(_{10}\) samples were used for health risk assessment. Limit of quantification/2 criterion was considered for concentration values below limits of quantification. Exposure concentrations, carcinogenic risk and carcinogenic hazard index were evaluated for PM\(_{10}\)-associated PAHs in the area studied. Maximum values of exposure concentrations estimated for each scenario are shown in Table 1, while carcinogenic risk and carcinogenic hazard index maximum values are shown in Table 2. Regarding exposure concentrations values, benzo(e)pyrene and fluoranthene are the most exposed PAHs for all scenarios considering total and oral bioavailable PAH concentrations, respectively. The highest exposure concentrations values were obtained for the most conservative scenario (scenario I for adults), with values of 2790 (total PAH concentrations) and 3.4 (oral bioavailable PAH concentrations) pg m\(^{-3}\) for benzo(e)pyrene and fluoranthene, respectively. Furthermore, estimated carcinogenic risks do not exceed the acceptable threshold lifetime cancer risk 1.0 × 10\(^{-6}\) set by USEPA, being benzo(a)pyrene the PAH that showed the highest values for all scenarios considering both total and oral bioavailable PAH concentrations. Maximum carcinogenic hazard index values estimated.
Table 2 Maximum carcinogenic risk for polycyclic aromatic hydrocarbons, (PAH) and carcinogenic hazard index (HIc) values estimated for each scenario, considering total and oral bioavailable concentrations. Benzo(a)anthracene, BaA; chrysene, Chry; benzo(b)fluoranthene + benzo(j)fluoranthene, BbF + BjF; benzo(k)fluoranthene, BkF; benzo(a)pyrene, BaP; dibenz(a,h)anthracene, DBahA; and indeno(1,2,3-cd)pyrene, IP

| PAHs          | Scenario I       | Scenario II      | Scenario III     |
|---------------|------------------|------------------|------------------|
|               | Adults Total     | Oral bioavailable | Total Oral bioavailable | Total Oral bioavailable |
| BaA            | $1.2 \times 10^{-9}$ | $4.5 \times 10^{-12}$ | $3.6 \times 10^{-9}$ | $1.4 \times 10^{-12}$ |
| Chrysene       | $3.5 \times 10^{-10}$ | $4.2 \times 10^{-13}$ | $1.1 \times 10^{-10}$ | $1.3 \times 10^{-13}$ |
| BbF + BjF      | $6.6 \times 10^{-8}$ | $2.3 \times 10^{-12}$ | $2.0 \times 10^{-8}$ | $6.8 \times 10^{-13}$ |
| BkF            | $1.0 \times 10^{-9}$ | $2.4 \times 10^{-13}$ | $3.1 \times 10^{-10}$ | $7.1 \times 10^{-14}$ |
| BaP            | $1.6 \times 10^{-7}$ | $1.2 \times 10^{-11}$ | $4.8 \times 10^{-8}$ | $3.7 \times 10^{-12}$ |
| DahA           | $6.7 \times 10^{-8}$ | $1.2 \times 10^{-12}$ | $2.0 \times 10^{-8}$ | $3.7 \times 10^{-13}$ |
| IP             | $2.3 \times 10^{-8}$ | $8.2 \times 10^{-14}$ | $7.0 \times 10^{-9}$ | $2.5 \times 10^{-14}$ |
| HIc            | $3.3 \times 10^{-7}$ | $2.1 \times 10^{-11}$ | $9.9 \times 10^{-8}$ | $6.3 \times 10^{-12}$ |

for each scenario do not exceed the cumulative cancer risk set in $1.0 \times 10^{-4}$, showing values of $3.3 \times 10^{-7}$ (total PAH concentrations) and $2.1 \times 10^{-11}$ (oral bioavailable PAH concentrations) for scenario I (adults). Then, no carcinogenic risk was found for PM$_{10}$-associated PAHs in the studied area.

Health risk assessment based on benzo(a)pyrene-equivalent carcinogenic concentrations was also performed. Benzo(a)pyrene-equivalent values obtained varied from 2100 to 5610 pg m$^{-3}$ and 0.17 to 1.4 pg m$^{-3}$ considering total and oral bioavailable PAH concentrations, respectively. Benzo(a)pyrene-equivalent concentrations exceeded the guideline value of 1.0 ng m$^{-3}$ for benzo(a)pyrene (Directive 2004/107/EC) (EU 2004) when total PAH concentrations were used. However, no exceedances of benzo(a)pyrene limit value were observed when oral bioavailable PAH concentrations were used. In addition, lifetime cancer risk values estimated considering total and oral bioavailable PAH concentrations are shown in Fig. 2a–b, respectively. Attending to the graphs, all monthly average PM$_{10}$ samples exceeded the acceptable carcinogenic risk limit of $1.0 \times 10^{-6}$ for individual carcinogens when total PAH concentrations are considered (Fig. 2a). Nevertheless, none of them exceeded the cumulative cancer risk ($1.0 \times 10^{-4}$) for multiple carcinogens (USEPA 2001; Davie-Martin et al. 2017). Furthermore, lifetime risk calculated basing on oral bioavailable concentrations were much lower than the acceptable carcinogenic risk limit of $1.0 \times 10^{-6}$ (Fig. 2b). Additionally, benzo(a)pyrene-equivalent concentrations approach offered slightly higher values than carcinogenic hazard index as a result of considering a high number of PAHs in risk calculation.

Estimated life cancer risk values assessed using total PAH concentrations are higher (by a factor of $10^{4}$) than values achieved using oral bioavailable PAH concentrations. This great difference could be resulted in an overestimation of cancer risks when considering total concentrations instead of using bioaccessible/bioavailable concentrations. Within this context, many authors have already reported the necessity to consider bioaccessibilities and bioavailabilities on exposure and health risk models, providing a more realistic assessment (Huang et al. 2014b, 2018; Kastury et al. 2018; Raffy et al. 2018; Gao et al. 2018; Moreda-Piñeiro et al. 2019). In addition, it is important to point out that our health risk assessment could be overestimated even using oral bioavailable concentrations because of not considering other parameters such as PM$_{10}$ deposition in different lung regions and clearance rates.

Conclusion

The present study describes a novel in vitro methodology for oral bioavailable PM$_{10}$-associated PAHs estimation that encompasses a first in vitro physiologically based extraction test using gastrointestinal fluids in combination with a dialysis membrane to simulate intestinal absorption, which would provide a better understanding of how substances can interact with organisms. Also, procedure for the analysis of PAHs in oral PM$_{10}$ bioavailable fractions was successfully validated, offering a simple, sensitive and accurate methodology to bioavailable PAHs quantification.

Applicability of the proposed method has been demonstrated by the analysis of a set of PM$_{10}$ samples. Oral bioavailable ratios of investigated PAHs were found to be below 5%, showing that a low amount of PM$_{10}$-bound PAHs could be available through intestinal absorption after inhalation. Among them, fluorene was found to be the most orally bioavailable, followed by anthracene, acenaphthene and phenanthrene (averaged bioavailable ratios of 2.8%, 1.4%, 1.2% and
0.71%, respectively), while carcinogenic PAHs (benzo(b)fluoranthene + benzo(j)fluoranthene, chrysene, indene(1,2,3-cd)pyrene, benzo(a)pyrene, benzo(k)fluoranthene, benzo(a)anthracene and dibenz(a,h)anthracene) offered the lowest oral bioavailabilities (bioavailable ratios below 0.5%). Moreover, bioavailability percentages were observed to decrease when the number of condensed PAHs rings increases, which might be attributed to the increase in PAHs hydrophobicity as reported for other simulated biological fluids.

Furthermore, carcinogenic hazard index values and lifetime cancer risks based on benzo(a)pyrene-equivalent toxic concentrations by using oral bioavailable concentrations did not exceed cumulative cancer risk (1.0 × 10^{-6}) set by USEPA, suggesting no carcinogenic risk was found to oral bioavailable PAHs in PM_{10} bound samples studied. However, a brief comparison among carcinogenic hazard index values using both total and bioavailable concentrations was performed, which resulted in an overestimation (by an average factor of almost 10^{5}) of PM_{10}-associated risks PAHs. Attending to this, inclusion of bioavailabilities in human health risk assessment models would be a step towards a more realistic assessment, as well as the development of pollutants’ bioavailability-based regulations that achieve the combination of pollutants limit levels safe for humans and ecosystems together with a realistic management of resources.

Finally, future efforts should be focused on the standardization of in vitro bioavailability methods so as to obtain more comparable data, as well as the suitability of including bioavailabilities and other biologically relevance parameters (such as deposition in lung regions and clearance rates) in current risk assessment models and toxicology studies.

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