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Inhalation bioaccessibility of PAHs in $PM_{2.5}$: Implications for risk assessment and toxicity prediction

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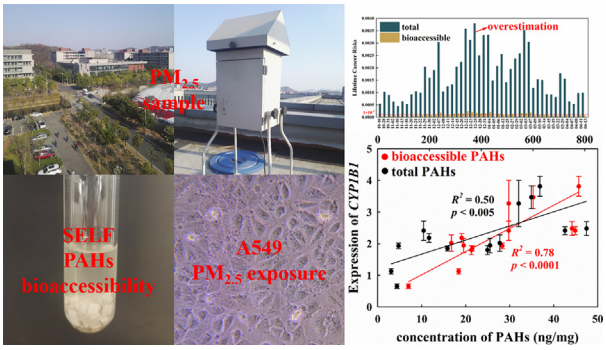
HIGHLIGHTS

- Inhalation bioaccessibility of $PM_{2.5}$-associated PAHs was measured by simulated epithelial lung fluid.
- Life time cancer risk posed by $PM_{2.5}$-associated PAHs was calculated with PAH inhalation bioaccessibility.
- Bioaccessible concentration of PAHs can better predict toxicity posed by $PM_{2.5}$-associated PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, 46 $PM_{2.5}$ samples collected from Nanjing, China were analyzed for total PAH concentration, with 14 samples assessed for PAH inhalation bioaccessibility and dioxin toxicity. The concentration of 19 PAH compounds in $PM_{2.5}$ ranged from 4.03 to 102 ng m^{-3}. When PAH inhalation bioaccessibility was assessed using simulated epithelial lung fluid, mean bioaccessibility values ranged from 3.21% (Benzo(c)fluorene) to 44.2% (Acenaphthylene). Benzo(a)pyrene concentration in 50% of the $PM_{2.5}$ samples exceeded the Chinese air quality standard of 2.5 ng m^{-3}, however, when bioaccessibility was considered, all samples were below the criterion. Similarly, the cancer risk probability for all $PM_{2.5}$ samples was $>10^{-4}$ incidences on the basis of total PAH concentration, while only 37% of samples posed a risk $>10^{-4}$ after incorporation of bioaccessibility. Dioxin toxicity of $PM_{2.5}$-bound PAHs was also investigated by characterizing mRNA expression of cytochrome P450 superfamily members in human lung cells (A549 cell). Compared to total PAH concentration, the use of bioaccessible concentration was better at predicting dioxin toxicity of $PM_{2.5}$-associated PAHs (correlation coefficient $R^2 = 0.40$–0.83 with $p < 0.05$). This study indicates that PAH inhalation bioaccessibility is an important consideration when assessing and predicting the risk posed by $PM_{2.5}$ particles, which is particularly important for countries with deteriorating air quality.

1. Introduction

Air particles with a diameter <2.5 μm ($PM_{2.5}$) have become a major research issue worldwide because of their potential impact on human

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health (Cifuentes et al., 2000). The adverse health effects of $PM_{2.5}$ are thought to largely depend on particle-associated contaminants, such as heavy metals, polychlorinated dibenzo-p-dioxins, and polycyclic aromatic hydrocarbons (PAHs) (Liacos et al., 2012). It has been demonstrated that approximately 1.6% of lung cancer occurrence in China may be attributable to the inhalation of PAHs from polluted air (Bandowe et al., 2014).

When assessing exposure associated with inhalation of air particles, an unknown is quantifying the dose that is absorbed into the systemic circulation (i.e. the bioavailable fraction). The default assumption presumes that all contaminants in inhaled $PM_{2.5}$ particles are solubilized in lung fluid and absorbed. However, contaminant bioavailability may be significantly reduced due to the binding of contaminants to air particles and their limited release under lung fluid conditions (Boisa et al., 2014; Li et al., 2016a). As a result, quantifying contaminant bioavailability has the potential to impact exposure assessment and risk calculations which, in turn, may influence management requirements to reduce human exposure to air particles. Although in vivo assays using animal models are an accurate method to measure contaminant inhalation bioavailability, these assays are complicated, laborious and expensive. Consequently, in vitro assays using simulated lung fluid (e.g., simulated epithelial lung fluid, SELF) have been developed as they are simple and practical to use (Boisa et al., 2014; Wiseman and Zereini, 2014; Zereini et al., 2012). Simulated lung fluids have been used to measure metal bioaccessibility in $PM_{2.5}$. For example, Boisa et al. (2014) used SELF to measure Pb inhalation bioaccessibility in PM_{10} from soil and mining waste. However, limited studies have applied SELF in vitro assays to organic contaminants (such as PAHs) in $PM_{2.5}$.

Toxicity induced by PAHs in $PM_{2.5}$ has been the focus of numerous studies and regulatory guidelines. PAHs may be metabolized to active intermediates by the cytochrome P450 1 (CYP1) superfamily members before eliciting adverse health effects (Billet et al., 2008). *AhR* and *ARNT* genes play a vital role in the regulation of CYP enzymes and the mediation of PAH-induced gene expression. *AhRR* inhibits *AhR* signaling through competition with *AhR* by binding with *ARNT* (Haarmann-Stemmann and Abel, 2006). $PM_{2.5}$-associated PAHs may induce the expression of *AhR*/*ARNT*-regulated genes such as *AhRR*, *CYP1A1* and *CYP1B1,* and the expression is related to the formation of PAH-active metabolites which are responsible for dioxin toxicity (Borgie et al., 2015). When reviewing the literature in this area, PAH toxicity was largely dependent on the bioaccessibility across different soil or sediment samples (Hawthorne et al., 2006). However, the relationship between PAH bioaccessibility in $PM_{2.5}$ and adverse effects through exposure to $PM_{2.5}$-associated PAHs has yet to be investigated.

In this study, both winter and spring $PM_{2.5}$ samples were collected from Nanjing, China with PAH concentration, inhalation bioaccessibility, and dioxin toxicity measured. This information was utilized to determine how inhalation bioaccessibility considerations may be used to improve the assessment of risk posed by $PM_{2.5}$-associated PAHs. It was hypothesized that the concentration of bioaccessible PAHs would be a better predictor of dioxin toxicity compared to total $PM_{2.5}$-associated PAH concentration.

2. Materials and methods

2.1. Chemicals and reagents

The 16 USEPA priority PAHs, including naphthalene (Nap), acenaphthylene (Acl), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (FA), pyrene (Pyr), chrysene (Chr), benzo(a)anthracene (BaA), benzo(k)fluoranthene (BkF), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBahA), indeno(1,2,3-cd)pyrene (Ind) and benzo(g,h,i)perylene (BghiP), were investigated in this study. In addition, three more PAHs, benzo(c)fluorene (BcF), dibenzo(a,e)fluoranthene (DBaeF), and dibenzo(a,i)pyrene (DBaiP), were also included, which have been reported to be more toxic (USEPA (U.S. Environmental Protection Agency), 2010) or have higher relative mutagenic potency (Machala et al., 2001) compared to Benzo(a)pyrene. The mixed standard of 16 EPA priority PAHs was purchased from J&K Scientific (Shanghai, China) with purity >98%. Standards of the other three PAHs were purchased from Accu Standard (CT, USA) and Cambridge Isotope Laboratories (MA, USA) with purity >99%. Chrysene-d_{12}, which was used as an internal standard, was purchased from Cambridge Isotope Laboratories (MA, USA). Other chemicals used in this study were high-performance liquid chromatography (HPLC) or analytical grade. Cell culture medium and relevant reagents, including Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) and antibiotic-antimycotic (AA), were purchased from Thermo Fisher Scientific Inc. (GIBCO, USA). Cell culture plates and dishes were obtained from Corning Inc. (NY, USA).

2.2. $PM_{2.5}$ sampling and analysis

The sampling site was located on the roof of QianPansheng Building (about 25 m above ground) of Nanjing University on Xianlin campus. $PM_{2.5}$ was collected for 16 h per day using a high volume air sampler (KC1000, Laoshan Electronic Instrument Company, Qingdao, China) at a flow rate of 1 m^3 min^{-1} with quartz microfiber filters (Whatman, 203 mm × 254 mm). A total of 46 $PM_{2.5}$ samples were collected from October 16, 2015 to April 13, 2016 (November 2, 2015 to January 12, 2016 for Winter, and February 25, 2016 to April 13, 2016 for Spring). Samples collected from October 16, 2015 to October 31, 2015 were not taken into consideration in season comparison, but were discussed in bioaccessibility and toxicity study. $PM_{2.5}$ samples were collected once every 3 or 4 days except on rainy days. In addition, if Air Quality Index (AQI) was above 150 or under 50, an additional sample was collected. The temperature of sampling day was 0–16 °C in winter and 4–22 °C in spring. Wind velocity was 3–14 km/h in winter and 5–14 km/h in spring, and relative humidity was 60–95% in winter and 46–84% in spring. Before sampling, filters were wrapped in aluminum foil and baked at 500 °C for 4 h. Filters were then equilibrated for 48 h in a desiccator. After sampling, filters were wrapped in aluminum foil and stored at −20 °C until analysis, organic carbon (OC) and elemental carbon (EC) in $PM_{2.5}$ were determined from a 0.81 cm^2 punch taken from each quartz microfiber filter with a semi-continuous carbon analyzer (Model-4, Sunset Lab, USA). OC and EC in $PM_{2.5}$ on filters were analyzed following a modified protocol of the National Institute of Occupational Safety and Healthy (NIOSH 5040) (Chen et al., 2017). Briefly, total carbon, including OC and EC, was converted to CO_2 and measured with a non-dispersive infrared absorption CO_2 sensor. Total carbon was separated into OC and EC by dividing their peak area by the internal calibration peak made by methane gas (5% CH_4 in He).

2.3. Total and bioaccessible concentrations of PAHs in $PM_{2.5}$

One-eighth of the filer was cut into pieces with a ceramic knife and extracted three times with 10 mL dichloromethane (DCM) and acetone solution (1:1, v/v) for 30 min in an ultrasonic bath (SCOENTZ, SB-800 DTD, China). The combined extracts were filtered through anhydrous sodium sulfate and concentrated by rotary evaporation (IKA®RV10, Germany). The residue was then dissolved in 2 mL n-hexane, filtered through 0.45 μm PTFE filters (ANPEL, China) and stored in a freezer at −20 °C until analysis.

Simulated epithelial lung fluid (SELF, pH = 7.4) was used to measure inhalation bioaccessibility of PAHs in $PM_{2.5}$ (Boisa et al., 2014). The detailed compositions of SELF can be found in the supporting information as Table S1. Briefly, 1/8 of the filter was cut into pieces (about 0.25 cm^2 for each piece) and added to 20 mL SELF. The mass of $PM_{2.5}$ on each sampling filter ranged from 34.1 to 274 mg, and therefore the solid to liquid (S/L) ratio ranged from 1:600 to 1:4000. This was comparable to a range of 1:500 to 1:5000 in which inhalation bioaccessibility