Novel *in vitro* method for measuring the mass fraction of bioaccessible atmospheric polycyclic aromatic hydrocarbons using simulated human lung fluids[☆]

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ABSTRACT

The bioaccessibility of organic pollutants is a key factor in human health risk assessments. We developed a novel *in vitro* method for determining the mass fraction of bioaccessible atmospheric polycyclic aromatic hydrocarbons (PAHs) using an air-washing device containing simulated human lung fluid. The experimental parameters were optimized based on the deposition fractions (DFs) of PAHs in human lung fluids. The DFs were measured for PAHs based on the mass of compounds in the mainstream and exhaled cigarette smoke. The mass fractions of bioaccessible PAHs were measured by passing the mainstream cigarette smoke through the air-washing device, and they were calculated via a simple mass balance equation based on the PAHs in the fluid and mainstream cigarette smoke. The DFs of individual PAHs ranged from 20.5% to 78.1%, and the bioaccessible mass fractions varied between 45.5% and 99.8%. The octanol-water partition coefficients (K_{OW}) significantly influenced both the DFs and bioaccessible mass fractions of PAHs, and the optimized *in vitro* method could be used to estimate the bioavailable atmospheric PAHs. This *in vitro* method can potentially be used to measure the mass fraction of bioaccessible atmospheric PAHs and to assess the health risk related to human exposure to airborne PAHs.

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1. Introduction

In order to assess human exposure to atmospheric pollutants, the external exposure dose is usually estimated based on the chemical concentration in the matrix, the mass of the matrix taken in, the intake frequency, and the bioavailability of the chemical (Yu et al., 2012c; Jamhari et al., 2014). In general, bioavailability describes the extent and rate of absorption for a xenobiotic that enters the systemic circulation in an unaltered form from the site of application. Thus, the bioavailability reflects the extent to which a chemical absorbed by a living organism can cause adverse physiological or toxicological responses. It is generally calculated as a percentage of the fraction absorbed relative to the total applied based on *in vivo* experiments using animals or humans. Determining the bioavailability of a toxic chemical in the human body is very difficult. Therefore, the bioavailability determined in animals is generally extrapolated to the human body and used to assess the human health risk (Wragg and Cave, 2002). However, many limiting factors affect the bioavailability measurements obtained in animal experiments, such as their high cost, the time required, and ethical issues related to the use of animal (Pu et al., 2006; Budinsky et al., 2008; Li et al., 2015). In addition, the significant interspecies and intraspecies differences between humans and animals make the data difficult to interpret.

Thus, *in vitro* methods for testing bioaccessibility have been developed in previous studies in order to evaluate the bioavailability of pollutants in the human body (Ruby et al., 1999; Wang et al., 2010; Man et al., 2010). Bioaccessibility refers to the percentage of a chemical released into the body fluid from its matrix

that is available for absorption in an organism, where it reflects the maximum extent to which a chemical can be absorbed (Yu et al., 2012a, 2012b). In recent years, many studies have determined the oral bioaccessibility of environmental pollutants by using an *in vitro* gastrointestinal digestion model for oral ingestion contaminants (Tao et al., 2009; Yu et al., 2009, 2012c; Zhang et al., 2011a; Cui et al., 2016). By contrast, very few investigations have considered the bioaccessibility of atmospheric pollutants after inhalation. Some studies have determined the bioaccessibility of inorganic substances from particulate matters using simulated lung fluids, including artificial lysosomal fluid, a lung epithelial lining fluid simulant (Gamble's solution), and an artificial lung lining fluid simulant (Hatch's solution) (Berlinger et al., 2008; Zerenini et al., 2012; Guney et al., 2016; Li et al., 2014; Kastury et al., 2017).

However, according to Guney et al. (2016) and Kastury et al. (2017), the existing methods for measuring the bioaccessibility of substances via inhalation have several shortcomings. First, the existing methods have generally been applied to only a few inorganic contaminants, especially heavy metals such as the platinum group elements in airborne particulate matter, and some organic contaminants, but not polycyclic aromatic hydrocarbons (PAHs) (Zereini et al., 2012; Kademoglou et al., 2018). The test parameters applied in these approaches have not been fully investigated, such as the agitation method and speed, exposure time, solid to liquid ratio, chemical composition of simulated lung fluids, and the use of static or dynamic assays, and a limited number of methods have been employed for analyzing the trace elements in air particles or organic compounds in house dust (Guney et al., 2016; Kastury et al., 2017; Kademoglou et al., 2018). In addition, comparisons of *in vitro* tests and *in vivo* results have been conducted rarely.

The available methods based on simulated lung fluids generally measure the bioaccessibility of contaminants by considering the release of chemicals from matrices into fluids over a long exposure time ranging from 24 h to 30 days (Zereini et al., 2012). In fact, particles (especially fine particles) may be deposited deep in the lungs and they might be phagocytosed by macrophages to cause adverse effects on human health. Therefore, the mass fraction deposited in the human lungs proposed by the European Committee for Standardization for occupational exposure assessments might be more important than the fraction released from the deposited particles (i.e., bioaccessibility) tested using *in vitro* simulation methods, although a very long incubation time is used to simulate the release of chemicals from matrices. In a recent review, Wei et al. (2018) noted that the gas and particle phase chemicals deposited in the respiratory tract are bioaccessible, but the chemical released into the fluid is still used to assess the bioaccessibility of a compound in particulate matter from the air. Many studies have evaluated the deposition fraction (DF) for particulate matter in the lungs using animals and humans, or based on calculations with mathematical models (Wei et al., 2018). We consider that the contaminants in particles deposited in the lung should be treated as bioaccessible, as suggested by Wei et al. (2018) and as applied by the European Committee for Standardization. To the best of our knowledge, no previous studies have investigated bioaccessible atmospheric organic pollutants using simulated lung fluid by considering the DF. In general, the available methods are applicable to released contaminants, especially for inorganic substances. Thus, it is very important to develop a novel physiological *in vitro* method for measuring bioaccessible atmospheric organic contaminants.

Therefore, in this study, we developed a novel *in vitro* method based on simulated lung fluid to assess bioaccessible atmospheric organic contaminants according to the mass fraction deposited in simulated lung fluid, where we investigated PAHs as an example. The testing parameters were optimized based on the DF of PAHs in

humans measured for a volunteer smoker. Considering the low concentrations of PAHs in the atmospheric environment, cigarette smoke was used as the source of PAHs in the present study to conduct our experiments.

2. Materials and methods

2.1. Reagents and materials

Standards of 15 PAHs (acenaphthylene [ACY], acenaphthene [ACE], fluorene [FL], phenanthrene [PHE], anthracene [ANT], fluoranthene [FLU], pyrene [PYR], benz[a]anthracene [BaA], chrysene [CHR], benzo[a]pyrene [BaP], benzo[b]fluoranthene [BbF], benzo[k] fluoranthene [BkF], indeno[1,2,3-c,d]pyrene [IcdP], dibenzo[a,h] anthracene [DahA], and benzo[g,h,i]perylene [BghiP]) in a mixed solution, four surrogate standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{10}, and perylene-d_{12}), and an internal standard (hexamethylbenzene) were purchased from Dr. Ehrenstorfer (Germany).

Gamble's solution was used as the simulated lung fluid and it was prepared by adding 0.095 g $MgCl_2$, 6.019 g NaCl, 0.0298 g KCl, 0.126 g Na_2HPO_4, 0.063 g Na_2SO_4, 0.368 g $CaCl_2 \cdot 2H_2O$, 2.604 g Na_2CO_3, 0.0574 g $C_2H_3O_2Na$ (sodium acetate), and 0.097 g $C_6H_5Na_3O_7 \cdot 2H_2O$ (sodium citrate dihydrate) to 1 L of water (Coombo et al., 2008). Hexane, dichloromethane, and acetone were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and redistilled using a glass system before use. Neutral silica gel (80–100 mesh) was purchased from Qingdao Haiyang Chemical Co., Ltd (Qingdao, China), and aluminum oxide (100–200 mesh) was purchased from Sinopharm Chemical Reagent Co., Ltd. Sodium sulfate was heated at 450 °C and stored in sealed containers before use. Tenax-TA (GEEQ-000919, 20–35 mesh) for gas phase PAHs was purchased from CNW technologies (China) and quartz microfiber filters (Cat No. 1851–865) for particle phase PAHs were purchased from Whatman (UK) (Vu et al., 2011; Magnusson et al., 2016). The cigarette brand was HS (the exact name is not given to avoid any conflict of interest) and they were manufactured in Anhui Province, China. The mainstream cigarette smoke was collected with a home-made stainless steel smoke collection device, as shown in Figure S1.

2.2. Breakthrough experiment

To test the absorption efficiency of Tenax-TA and the influence of the quartz microfiber filters on the detection of PAHs in the gas phase, two quartz microfiber filters (diameter = 50 mm) were used and another Tenax-TA column (length = 5 cm) was connected to the air outlet of the smoke collection device in series. Two groups of breakthrough experiments were conducted with a high concentration PAHs at a low collection rate and a low concentration of PAHs at a high collection rate. In the first experiment, the mainstream cigarette smoke was collected at flow rates of 0.1 and 0.2 L/min after lighting the cigarette (Figure S2). The first cigarette was replaced when it had burned down close to the filter tip, i.e., two cigarettes were used in the same experiment. The quartz microfiber filters and Tenax-TA were collected, extracted with acetone, and treated as described in the sample treatment protocols given in the following. In addition, the air inlet of the smoke collection device was washed with acetone. The acetone containing PAHs washed from the device was added to the extract from the quartz microfiber filters. In the second experiment, the mainstream cigarette smoke (from one, two, and four cigarettes) was collected in a 100-L Teflon sample bag, as shown in Figure S3. The mainstream smoke was then collected as described above at flow rates of 1, 2, and 4 L/min. PAHs in the particulates and gas phase were collected and treated

as described above. Each experiment was repeated independently three times.

2.3. PAHs in mainstream cigarette smoke

PAHs in the mainstream cigarette smoke were measured using a similar method to that employed in the breakthrough experiment for the high concentration PAHs at low collection rates of 0.1 and 0.2 L/min (Figure S2). Only one quartz microfiber filter was used in this experiment and the 5 cm Tenax-TA column was also removed. The mainstream cigarette smoke was collected according to the method recommended by the US Federal Trade Commission (Randolph, 1974). Briefly, after lighting the cigarette, the smoke was passed through the home-made collection device using a syringe. Each time, 35 mL of air was pumped for 2 s at an interval of 58 s to simulate human smoking and the cycle was repeated until the cigarette burned down close to the filter tip. Two cigarettes were used in each experiment and after completing all of the processes, the quartz microfiber filters and Tenax-TA were collected and treated according to the protocols described in the following. The experiments were repeated independently five times.

2.4. DF test

In the present study, the volunteer was a smoker who generally smoked four cigarettes per day. Before the experiment, the volunteer did not smoke cigarettes for at least 8 h. The volunteer smoked cigarettes according to his daily habits, and the exhaled gas was collected by the smoke collection device until the cigarette burned down to the filter tip (Figure S4). In general, the gas volume was only one-seventh of the exhaled gas in the human lung that can be replaced by fresh air, i.e., 15 breaths can exchange 90.1% of the gas in the human lung. Therefore, after smoking, the volunteer took 15 breaths and the exhaled gas was collected using the device, and treated as the residual smoke in the lung. Finally, the quartz microfiber filters and Tenax-TA were collected and treated as described in the following, where the experiment was repeated five times on different days.

2.5. Bioaccessible mass fraction test

Two concentrations of PAHs (high and low concentrations) were tested in the experiment. In the high concentration experiment, a cigarette was lit and connected directly to the air inlet (Figure S5). The mainstream smoke then passed through an air-washing device containing simulated lung liquid (with the liquid level heights of 12, 24, and 42 cm) at a flow rate of 0.1 L/min. The PAHs that were not absorbed by the simulated lung fluid were also collected by the home-made collection device. Two cigarettes were used in each experiment. However, in the low concentration experiment, the mainstream cigarette smoke from two cigarettes was first mixed with nitrogen in a 100-L Teflon sampling bag (Figure S5), before it passed through the simulated lung liquid (with liquid level heights of 8, 12, and 24 cm) and the home-made collection device at a flow rate of 4 L/min, in a similar manner to the respiratory rate of adults under resting and sitting conditions, i.e., 3.82–4.58 L/min (Exposure Factors Handbook of Chinese Population, 2013). Finally, the PAHs in the simulated lung liquid, and the quartz microfiber filters and Tenax-TA in the collection device were collected and treated according to the following method. Each experiment was repeated independently three times.

2.6. Sample treatment protocols and PAH analysis

The PAHs in the quartz microfiber filters, Tenax-TA from the smoke collection device, and the polyether polyurethane sponge in the air-washing device were extracted ultrasonically, and the PAHs in the simulated lung liquid were extracted by liquid–liquid extraction using a similar method to that described in our previous study, with minor modifications (Yu et al., 2012a; Chen et al., 2016). Briefly, the quartz microfiber filters were cut into pieces and placed into a 40-mL brown glass bottle. The surrogate standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{10}, and perylene-d_{12}) were added and extracted ultrasonically with 35 mL n-hexane/acetone (1:1, v/v) for 10 min. The extract was collected and re-extracted two times, before combining the three extracts together. The method used for extracting PAHs from the Tenax-TA was the same as that employed for the quartz microfiber filters. The polyether polyurethane sponge (used to adsorb chemicals from the simulated lung fluid and to disperse gas bubbles as the smoke passed through the fluid) was extracted ultrasonically using 40 mL of acetone for 10 min and this process was repeated three times. This extract was added to the extract from the simulated lung fluid as described in the following.

PAHs were extracted from the simulated lung liquid by liquid-liquid extraction using a similar method to that employed in our previous studies for extracting PAHs from simulated gastrointestinal fluid (Yu et al., 2012a). The surrogate standards were added to the simulated lung fluid before extraction. Each 100 mL of lung fluid was mixed with 20 mL of acetone and then shaken. Next, 30 mL n-hexane/dichloromethane (1:3, v/v) was added to the extract and the extraction process was repeated three times, where the extracts were combined with those from the polyether polyurethane sponge. Finally, all of the extracts were treated using a similar method to that employed in our previous study, with minor modifications (Yu et al., 2012a). Briefly, the extracts were concentrated to approximately 1 mL and the solvent was exchanged with n-hexane. The solution was further purified with a multilayer silica:alumina (12 cm:6 cm) column using 70 mL of a mixture of n-hexane:dichloromethane (1:1, v/v) as the mobile phase. The fraction containing PAHs was collected and concentrated, and the internal standard (hexamethylbenzene) was added (Wang et al., 2012; Zhang et al., 2011b, 2017). The eluent was stored in 50 μL of n-hexane at 4 °C until instrumental analysis. The PAHs were quantified using an Agilent 6890 N gas chromatograph coupled to an Agilent 5975 mass spectrometer in the electron ionization mode, as described in our previous studies (Yu et al., 2012a; Zhang et al., 2017).

2.7. Quality assurance and quality control

A procedural blank was included in each batch of samples. The procedural blank was used to monitor interference peaks and to correct the sample values. The calibration plots had satisfactory linear regression coefficients ($R^2 = 0.99$) for all of the PAHs. The reported concentrations were not corrected based on the recovery rates of the surrogate standards for acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{10}, and perylene-d_{12} because the recoveries were satisfactory, i.e., 84.9% ± 15.4%, 88.4% ± 26.6%, 91.4% ± 17.1%, and 86.3% ± 17.4%, respectively. The limits of quantification ranged from 9.8 to 15.8 pg/cigarette for individual PAHs, which were calculated based on 3.36 times the standard deviation values obtained from six separate analyses of the standard solution with signal to noise ratios of 10. The concentrations of two PAHs comprising BbF and BkF were reported as the summed concentrations of B[b+k]F because they were not separated in the present study.

2.8. Calculations

In the present study, the DF of a PAH refers to the fraction of a PAH in the smoke retained in the respiratory system. DF was calculated according to the following equation:

$$DF\ (\%) = \frac{M_{MS} - M_{ES}}{M_{MS}} \tag{1}$$

where M_{MS} (ng) is the mass of a PAH in the mainstream smoke of a cigarette and M_{ES} (ng) is the mass of a PAH in the exhaled cigarette smoke collected by the home-made air collection device.

The mass fraction (MF%) of a bioaccessible PAH refers to the fraction of a PAH in the smoke retained in the simulated lung fluid when the smoke passed through the home-made air-washing device. MF% was calculated as follows:

$$MF\% = \frac{M_{LL}}{M_{LL} + M_{CD}} \tag{2}$$

where M_{LL} and M_{CD} (ng) are the masses of a PAH in the simulated lung liquid and in the home-made air collection device, respectively.

3. Results and discussion

3.1. Breakthrough experiment

Due to the lack of a suitable commercial test device, we designed and built the smoke collection device ourselves. Therefore, the breakthrough experiments tested whether the device containing Tenax-TA (an absorbent of semi-/volatile organic compounds) could completely absorb the gas phase PAHs in the smoke stream (Magnusson et al., 2016). Thus, a column packed using Tenax-TA with a length of 5 cm was connected to the end of the device in series. In addition, we tested the effect of the two filters on the PAH measurements.

PAHs were not detected in the first or second column when the mainstream cigarette smoke was collected at a flow rate of 0.1 L/min. However, PAHs were detected in the first column but not in the second column at a flow rate of 0.2 L/min (data not shown). These results demonstrate that PAHs in the gas phase could be absorbed on the particulates because of the tar in the cigarette mainstream smoke. Thus, the PAHs in the gas phase were intercepted by the quartz microfiber filters at a lower flow rate of 0.1 L/min. However, the PAHs overcame the absorption by tar and volatilized from the filters at a higher flow rate of 0.2 L/min, so they could be detected in the first column with Tenax-TA. Therefore, we found that the Tenax-TA in the self-made smoke collection device could effectively absorb PAHs in the gas phase when the collection rate was lower than 0.2 L/min.

We also investigated the influence of the quartz microfiber filter on PAH detection because previous reports have suggested that quartz microfiber filters might affect the determination of organic compounds in the gas phase (Sangiorgi et al., 2014; Xie et al., 2014). In general, the effects were grouped into two types. The absorption of organic compounds onto the filter would lead to a positive error when detecting the chemical in the particulate phase, whereas a negative error would occur for measurements of the chemical in the gas phase. In the present study, most of the PAHs were detected on the first filter (Figure S6). At a collection rate of 0.1 L/min, the individual PAHs collected on the first filter comprised more than 90% of the total, except for DahA (Figure S6A). Similar results were obtained at a rate of 0.2 L/min (Figure S6B). The PAHs on the first filter and the total PAHs on the first and second filters comprised more than 90% of the PAHs when the smoke collection rate was 0.2 L/min. We analyzed the ratios of the PAHs absorbed on the second quartz microfiber filter relative to those on the Tenax-TA to determine whether the quartz microfiber filter influenced the detection of the gas phase PAHs on the Tenax-TA. We only calculated the ratios at a collection rate of 0.2 L/min because the gas phase PAHs were not detected on the Tenax-TA at a flow rate of 0.1 L/min. The PAHs collected on the Tenax-TA accounted comprised more than 90% of the total masses of each PAH collected on the second quartz microfiber filter and Tenax-TA (Figure S6C), thereby demonstrating that the quartz microfiber filter did not influence the detection of PAHs in both the particulates and the gas phase.

Similarly, in the breakthrough experiment conducted using low concentrations of PAHs with a high collection rate, the mainstream cigarette smoke (one, two, and four cigarettes) was collected in a 100-L Teflon sample bag. The smoke was then collected at flow rates of 1, 2, and 4 L/min. In all cases, the PAHs collected on the first filter and the total PAHs on the first and second filters comprised more than 90% of the PAHs in these experiments (data not shown). Our results demonstrate that the Tenax-TA in the smoke collection device could effectively absorb the PAHs in the gas phase, and the quartz microfiber filter did not influence the detection of PAHs in both the particulate and gas phases.

3.2. PAHs in mainstream cigarette smoke

Fifteen PAHs (B[b+k]F as the sum of BbF and BkF) were determined in the mainstream cigarette smoke. We found that most of the gas phase PAHs could not be detected on the Tenax-TA. Therefore, in the following, we focus on the PAHs in particles, unless specified otherwise. The masses of the PAHs in the mainstream cigarette smoke are shown in Fig. 1. The average levels of individual PAHs in the mainstream cigarette smoke ranged from 1.23 to 153 ng/cigarette. The highest mass was determined for FL (mean: 153 ± 21.0 ng/cigarette), followed by PHE (mean: 151 ± 38.8 ng/cigarette). ACE and PYR were at the same levels (Table S1). The lowest mass was determined for DahA with 1.23 ng/cigarette. FL and PHE were the main PAHs, where they accounted for 26.7% and 26.3% of the total, respectively. The masses of the 3–4 ring PAHs (85.3%) were much higher than those of the 5–6 ring PAHs (3.8%).

Several previous studies have investigated PAHs in mainstream cigarette smoke (Moldoveanu et al., 2008; Wang et al., 2015). The masses of ACY (30.2 ng/cigarette), ACE (32.8 ng/cigarette), ANT (49.1 ng/cigarette), FLU (55.0 ng/cigarette), PYR (34.4 ng/cigarette), and DahA (1.23 ng/cigarette) determined in the mainstream cigarette smoke in the present study were lower than those reported in the mainstream smoke of six types of cigarettes by Wang et al. (2015), but the concentrations of the other PAHs were in the same ranges (Table S1). Our results are also consistent with those reported by Moldoveanu et al. (2008) estimated based on nicotine, although the concentrations of the PAHs clearly differed from those in our study, i.e., ACY, FL, PYR, CHR, and IcdP comprised 69.9, 170, 45.0, 16.6, and 1.49 ng/cigarette, respectively (Table S1). In general, several factors can influence the levels of PAHs in the mainstream cigarette smoke, such as the lighting rate of cigarettes, the brand of cigarettes according to the variable the usage of tobacco, different tobacco blends, ingredients, and differences in cigarette design (Moldoveanu et al., 2008).

3.3. PAHs in exhaled cigarette smoke and DFs of PAHs

The levels of different PAHs in the exhaled cigarette smoke varied from 0.69 to 69.9 ng/cigarette, where PHE had the highest level (Fig. 1 and Table S2). PAHs with 5–6 rings such as BaP, IcdP,
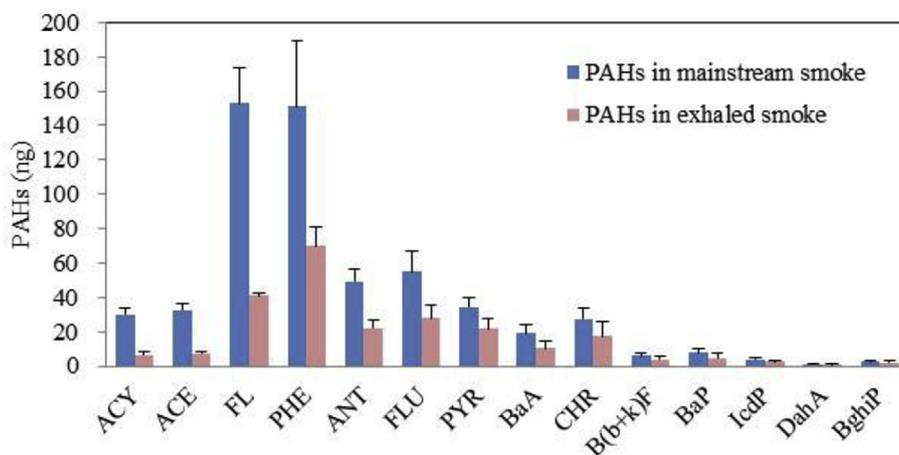
Fig. 1. Masses of PAHs in mainstream cigarette smoke and exhaled cigarette smoke.

DahA, and BghiP generally comprised less than 10 ng/cigarette, which was much lower than that for the PAHs with 3–4 rings. This was consistent with the distribution profile of PAHs in the mainstream cigarette smoke, as discussed above. We found that nine PAHs comprising ACE, FL, PHE, ANT, FLU, PYR, BaA, CHR, and IcdP had levels of 7.39, 40.91, 69.9, 22.0, 28.0, 21.7, 10.4, 17.3, and 2.39 ng/cigarette, respectively, in the exhaled smoke, which were greater than the maximum results of 3.56, 12.0, 18.9, 4.88, 7.70, 12.8, 5.24, 5.74, and 1.78 ng/cigarette reported by Moldoveanu et al. (2008), although the results for ACY, BaP, DahA, B[b+k]F, and BghiP were consistent with those obtained in their study (Table S2). These different results might be explained by a number of factors, such as the different types of cigarettes tested, smoking habits of the volunteers, and the frequency of cigarette smoking, which affects the burning rate of cigarettes. In addition, the residual smoke in the lungs was collected from 15 additional breaths in the present study, whereas it was not considered by Moldoveanu et al. (2008).

The DF of each PAH compound was calculated according to its mass in the exhaled cigarette smoke and mainstream cigarette smoke. The average DFs for the individual PAHs ranged between 20.5% and 78.1%, with the highest value for ACY and the lowest for BghiP (Fig. 2A). In general, PAHs with small molecules had relatively higher DFs than the larger molecules. In organisms, the absorption of chemicals is usually correlated with the properties of chemicals, such as $LogK_{OW}$. Therefore, we also analyzed the relationship between the DF and $LogK_{OW}$ for the PAHs. The $LogK_{OW}$ value of B[b+k]F was estimated using the mean value for BbF ($LogK_{OW} = 5.8$) and BkF ($LogK_{OW} = 6.0$). There was a significant negative linear relationship between DF and $LogK_{OW}$ (Fig. 2B), which is consistent with the rule that chemicals with higher lipophilicity are generally more difficult to transport through cells (Yu et al., 2017) and they are considered bioavailable chemicals. In addition, PAHs with higher $LogK_{OW}$ values might be less soluble in human lung fluid. Thus, PAHs with higher $LogK_{OW}$ values had lower DFs.

In the present study, the DFs of most PAHs ranged between 40% and 80%, which are lower than the retention rates of PAHs in the human lung determined by Moldoveanu et al. (2008), who found that the retention rates of individual PAHs ranged from 36.8% for BghiP to 98.0% for FL (Table S3). In addition, previous studies have investigated the retention of nicotine and particulate matter by cigarette smokers. For example, Sahu et al. (2012) evaluated the retention rate of mainstream cigarette smoke by using the multiple path particle dosimetry method and found that the retention rates were 16.3%, 15.2%, and 29.8% in the oral cavity, bronchi, and lungs,
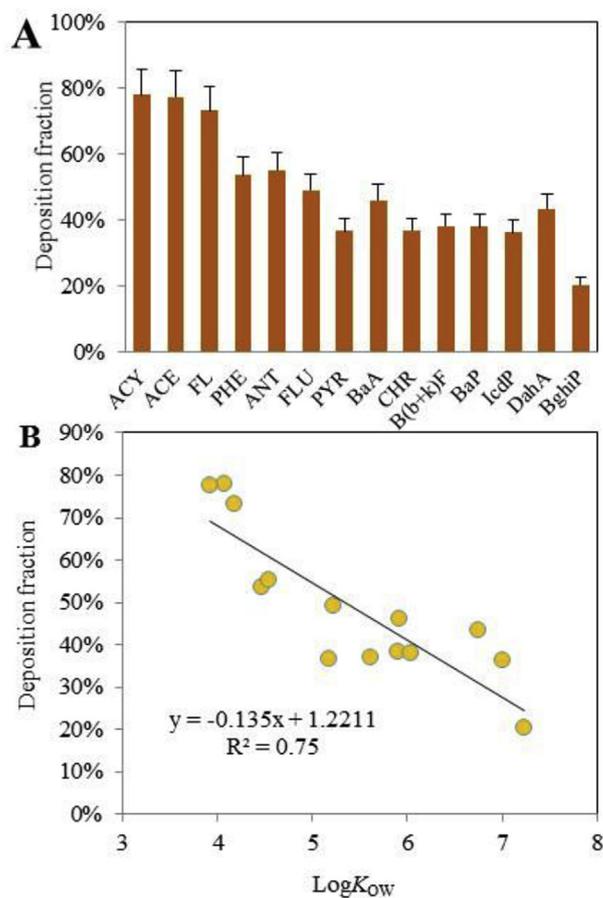

Fig. 2. Deposition fraction (DF) of PAHs in mainstream cigarette smoke via human inhalation (A) and the relationship between DF and $LogK_{OW}$ for PAHs (B).

respectively. Baker and Dixon (2006) reviewed nearly 100 years of research and found that 60%–80% of the particles in mainstream cigarette smoke were retained in the lungs after inhalation. As mentioned earlier, the PAHs detected in the present study were in particles. The DFs of most PAHs ranged from 40% to 80% in the present study, thereby agreeing with the retention rate of mainstream cigarette smoke determined in other studies.

3.4. Mass fractions of bioaccessible PAHs in mainstream cigarette smoke

The concentrations of atmospheric PAHs are generally much lower than those in mainstream cigarette smoke. Thus, a large volume air must be collected to obtain sufficient PAHs and high collection rates are also needed to effectively measure the bioaccessible mass fractions of PAHs. Therefore, in the present study, we investigated the bioaccessible mass fractions of PAHs in cigarette smoke at high concentrations (undiluted cigarette smoke) and a low flow rate (0.1 L/min), as well as at low concentrations (diluted cigarette smoke, Table S4) and a high flow rate (4 L/min). The mass balance was also studied to accurately measure the bioaccessible mass fractions. The summed masses of a given PAH in the simulated lung fluid and the smoke collection device were comparable to those in the mainstream cigarette smoke, except for ACY and DahA (Figure S7). The ratios were approximately 100%, thereby demonstrating that the losses of PAHs within the system were very low.

The bioaccessible mass fractions of PAHs were measured using an air-washing device containing simulated lung fluid with different fluid heights under two conditions, i.e., high PAH concentrations at a low flow rate (0.1 L/min) and low PAH concentrations at a high flow rate (4 L/min), as shown in Figure S8. The bioaccessible mass fractions of the PAHs ranged from 45.5% to 99.8%. The liquid level height was an important parameter that affected the bioaccessible mass fraction of PAHs, where the bioaccessible mass fraction increased as the liquid height level increased. Clearly, smoke passing through a longer route in the fluid would lead to more matter in the solution, thereby resulting in higher bioaccessible mass fractions for PAHs. In addition, similar to the DF results, there was a negative relationship between the bioaccessible mass fraction and $\log K_{OW}$ for the PAHs (Fig. 3), probably because the simulated lung fluid was an aqueous solution containing inorganic compounds. Therefore, the solubility of the PAHs in the fluid decreased as the $\log K_{OW}$ increased, and thus the bioaccessible mass fraction decreased. This also applied to the results of the DF measurements, as mentioned earlier.

3.5. Relationship between DF and bioaccessible mass fraction of PAHs

As shown in Fig. 4, there was a significant linear correlation ($R^2 = 0.94$) between DF and the bioaccessible mass fraction for the high concentration PAHs at a flow rate of 0.1 L/min in the air-washing device with a liquid level height of 12 cm (Fig. 4A). The slope was determined as 0.997 and the intercept as 0.191, thereby indicating that the bioaccessible mass fractions of the PAHs generally exceeded the DF by about 19%. For the low concentration cigarette smoke at a flow rate of 4 L/min (Fig. 4B), similar linear correlations were observed between DF and the bioaccessible mass fractions of PAHs in the simulated lung fluid with liquid level heights of 8 cm ($R^2 = 0.82$) or 12 cm ($R^2 = 0.91$), where the slopes were determined as 1.036 and 0.924, respectively, and the intercepts as 0.087 (i.e., 8.7%) and 0.246 (i.e., 24.6%).

In order to use the oral bioaccessibility measurement obtained using an *in vitro* method based on the simulated gastrointestinal tract to predict the oral bioavailability of a contaminant, it is assumed that the relationship between the oral bioavailability and bioaccessibility should meet the following criteria: (1) the coefficient (R^2) of the linear fitting curve should be greater than 0.6; (2) the slope of the fitting curve should be between 0.8 and 1.2; and (3) the intercept of the fitted curve must be close to zero (Wragg et al., 2011). Unfortunately, no similar criteria are available for applying inhalation bioaccessibility data to predict the inhalation bioavailability. In a review by Wei et al. (2018), they clearly stated that all of
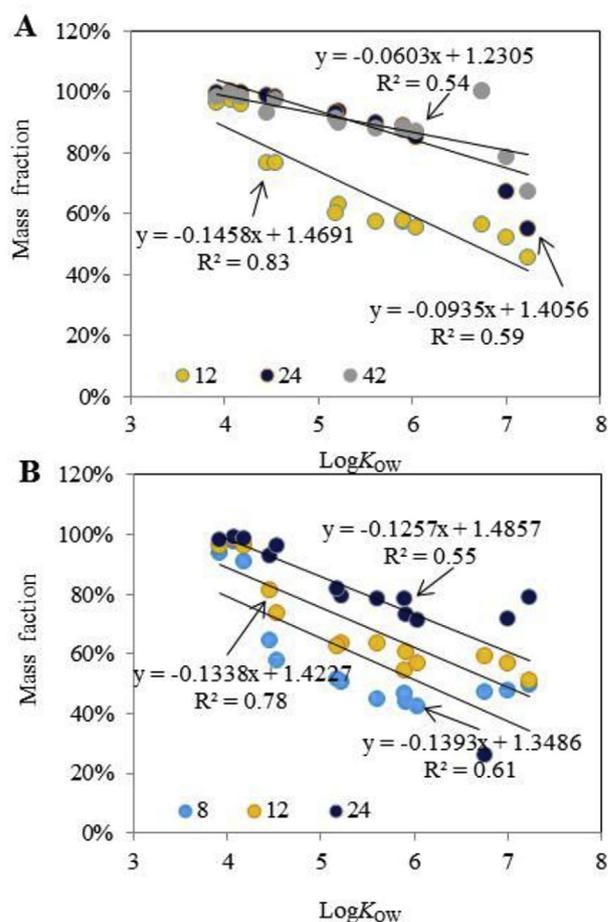

Fig. 3. Relationships between the bioaccessible mass fraction and $\log K_{OW}$ for PAHs (A: high PAH concentrations at a low flow rate of 0.1 L/min; B: low PAH concentrations at a high flow rate of 4 L/min).

the compounds in the deposited particles and the compounds that evaporate from inhaled particle phase compounds are bioaccessible compounds, and thus they are the maximum bioavailable compounds in particles (Figure S9). In the present study, DF was the maximal bioavailable fraction of PAHs in human lungs because it included the PAHs in deposited particles and the PAHs that evaporated from inhaled particles in the lung or respiratory tract. We assessed the relationships between the bioaccessible mass fraction and DF of PAHs, and similar criteria were used to evaluate whether the bioaccessible mass fraction can be used to estimate the inhalation bioavailability of PAHs.

We found that the bioaccessible mass fraction tested in the simulated lung fluid at a liquid level height of 12 cm confirmed the criteria, thereby indicating that the mass fraction tested by passing cigarette smoke through liquid with a level height of 12 cm at a flow rate of 0.1 L/min can be used to estimate the inhalation bioavailability at high PAH concentrations. However, the actual atmospheric PAHs are present at much lower concentrations and the human respiratory rate is much higher than 0.1 L/min. According to our results, the bioaccessible mass fractions of PAHs with relatively lower concentrations (although they were still higher than air) measured based on the simulated lung fluid with liquid level heights of 8 cm and 12 cm at a flow rate of 4 L/min also met the criteria. Thus, these two conditions can be used to measure the bioaccessible mass fraction of PAHs and estimate the bioavailability when the concentrations of atmospheric PAHs are low. From a
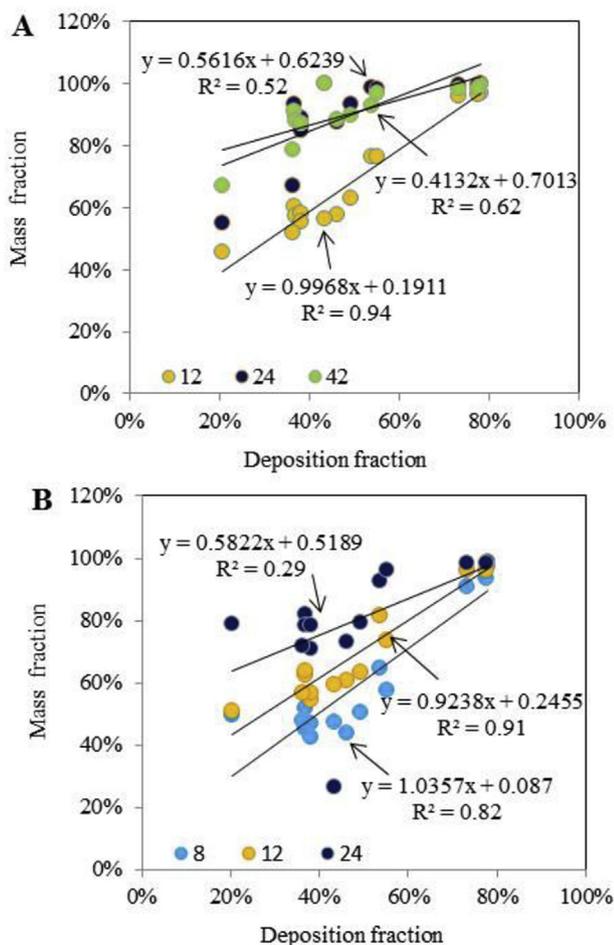
Fig. 4. Relationship between the DF and bioaccessible mass fractions of PAHs (A: high PAH concentrations at a low flow rate of 0.1 L/min; B: low PAH concentrations at a high flow rate of 4 L/min).

public health perspective, a relatively higher bioaccessible mass fraction is more suitable for predicting the bioavailability. Therefore, the simulated lung fluid with a liquid level height of 12 cm is recommended for testing the bioaccessible mass fraction of atmospheric PAHs. Furthermore, the bioaccessible mass fraction of PAHs determined using the novel *in vitro* method can be used to estimate the bioavailability of PAHs in the air.

3.6. Limitations of the in vitro method

We should note that the present study had many limitations. First, a method proposed for measuring bioaccessible chemicals in the air in order to predict the chemical bioavailability must address many challenges, and even using bioaccessibility as a surrogate for bioavailability in health risk assessments is open to debate. In contrast to oral bioaccessibility measurements that only consider the fractions of chemicals released from their matrices, measuring bioaccessible chemicals in the air requires the consideration of gas and particle phase chemicals. The chemicals can be deposited in the human respiratory tract via four mechanisms to become bioaccessible, as shown in Figure S9 (Wei et al., 2018). In addition, the bioavailability of inhaled pollutants depends on various biological variables, such as the alveolar clearance rate, lung expansion, contraction, respiratory rate, and the volume of exhaled versus inhaled air.

Different conditions are found in the human lung, with various pH values. It has been demonstrated that the pH value is the main determinant of the targets for inhalation bioaccessibility measurements of inorganic substances (Collier et al., 1992; Kastury et al., 2017). For example, the dissolution of CO from CO_3O_4 in bicarbonate and citrate is determined by the pH according to measurements conducted at three different pH values comprising 7.4 (to simulate extracellular fluid), 6.1 (to simulate macrophage cytoplasm), and 4.6 (to simulate macrophage lysosomes) (Collier et al., 1992). Li et al. (2016) found that the bioaccessibility of Pb in samples from the Youth Olympic Games (YOG) in Nanjing, China was lower than that in non-YOG samples tested in the artificial lysosomal fluid (ALF) (pH = 4.5), but higher than those in non-YOG samples based on Gamble's solution (pH = 7.4), where the difference were attributed to the lower pH and organic acids in ALF. However, we did not test simulated lung fluids with lower pH values such as ALF, although we previously demonstrated the higher bioaccessibility of polybrominated diphenyl ethers in simulated gastrointestinal digestion solution with a pH of 7.3 compared with an acid solution that had a pH of 5.9 (Yu et al., 2009). Moreover, Van de Wiele et al. reported no increase in the release of PAH from soil when the gastric pH of 2 was increased to the intestinal pH of 7, and this was different from the results obtained using inorganic substances. Therefore, the effects of pH on the bioaccessibility of organic substances in simulated lung fluid require further study.

We used a similar flow rate (4 L/min) to the respiratory rates (3.82—4.58 L/min) of adults at rest and sitting (Exposure Factors Handbook of Chinese Population, 2013), but we did not consider the retention time of air in the human lungs during the *in vitro* tests with various liquid height levels, although this is an important parameter that influences the bioaccessible mass fractions deposited in the simulated human lung fluid. A previous study found variations in PAH retention among different individuals (Moldoveanu et al., 2008), and the single volunteer used in the present *in vivo* assay may have differed from the overall population. The present study was not conducted at the human body temperature of 37 °C but instead we used room temperature in the same manner as Lima et al. (2013), although previous studies have not reported significant effects of temperature on the bioaccessibility of chemicals.

Finally, cigarette smoke was used to obtain PAHs, and thus the concentrations of the PAHs were much higher than those in the air. Therefore, the suitability of our novel *in vitro* method for use in public health risk assessments of PAHs requires further study. In fact, measuring bioaccessible contaminants in the air is a major challenge and great efforts have been made to solve this problem. Measuring inhalation bioaccessibility and associated risk assessments comprise a new field, especially for organic substances in the air whereas studies of inorganic substances have been conducted for over 20 years (Kastury et al., 2017; Wei et al., 2018). The development of real time analysis techniques such as single particle aerosol mass spectrometry (Ma et al., 2016; Rissler et al., 2017) should facilitate measurements of the bioaccessible mass fractions of contaminants in particles.

4. Conclusion

In this study, we developed and optimized a novel *in vitro* method for simulating human lung fluid to determine the bioaccessible mass fractions of atmospheric PAHs based on the DF using exhaled cigarette smoke. We collected 15 PAHs in mainstream cigarette smoke and exhaled cigarette smoke with a homemade smoke collection device and the DFs were calculated for the compounds. The bioaccessible mass fractions of PAHs in cigarette

smoke were measured using an air-washing device containing simulated lung liquid. The results suggested that the air-washing device containing simulated lung fluid with a liquid level height of 12 cm can be used to determine the bioaccessible mass fractions of atmospheric PAHs and to estimate the bioavailability in order to assess the human health risk of PAHs in air.

Notes

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.07.114.

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