Inhalation Bioaccessibility of Polycyclic Aromatic Hydrocarbons in PM$_{2.5}$ under Various Lung Environments: Implications for Air Pollution Control during Coronavirus Disease-19 Outbreak

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ABSTRACT: Global spread of coronavirus disease-19 (COVID-19) is placing an unprecedented pressure on the environment and health. In this study, a new perspective is proposed to assess the inhalation bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) in PM$_{2.5}$ for people with various lung health conditions. In vitro bioaccessibility (IVBA) was measured using modified epithelial lung fluids simulating the extracellular environment of patients with severe and mild lung inflammation. The average PAH IVBA in PM$_{2.5}$ of 24.5 ± 4.52% under healthy conditions increased ($p = 0.06$) to 28.6 ± 3.17% and significantly ($p < 0.05$) to 32.3 ± 5.32% under mild and severe lung inflammation conditions. A mechanistic study showed that lung inflammation decreased the critical micelle concentrations of main pulmonary surfactants (i.e., from 67.8 (for dipalmitoyl phosphatidylcholine) and 53.3 mg/L (for bovine serum albumin) to 44.5 mg/L) and promoted the formation of micelles, which enhanced the solubilization and competitive desorption of PAHs from PM$_{2.5}$ in the lung fluids. In addition, risk assessment considering different IVBA values suggested that PAH contamination levels in PM$_{2.5}$, which were safe for healthy people, may not be acceptable for patients with lung inflammation. Because of the large number of COVID-19 infections, and the fact that some survivors of COVID-19 were observed to still show symptoms of interstitial lung inflammation, the finding here can provide important implications for both the scientific community and policy makers in addressing health risk and air pollution control during the COVID-19 outbreak.

KEYWORDS: COVID-19, inhalation bioaccessibility, lung inflammation, health risk

INTRODUCTION

According to the recent analysis by the Lancet Commission, air pollution is responsible for 16% of global deaths. Ambient fine particulate matter (PM$_{2.5}$), as one major air contaminant, has attracted growing attention during the past decades. Some adverse effects of PM$_{2.5}$ can be attributed to the particle-associated contaminants, such as heavy metals and polycyclic aromatic hydrocarbons (PAHs). However, not all contaminants in PM$_{2.5}$ can be absorbed into the systemic circulation because of the binding of contaminants with PM$_{2.5}$ and their limited release in lung fluid. As a result, quantifying the released fraction of contaminants in the lung fluid (i.e., bioaccessibility) facilitates more accurate assessment of the exposure risk. In recent decades, several in vitro methods have been developed to measure inhalation bioaccessibility by use of artificial lung fluids, including artificial lysosomal fluid, Gamble’s solution, simulated epithelial lung fluid (SELF), Hatch’s solution, and phagolysosomal simulant fluid. Among these methods, SELF was recently developed based on Gamble’s solution with addition of lung surfactants (secreted by epithelial type II cells). For example, the average in vitro bioaccessibility (IVBA) of PM$_{2.5}$-bound PAHs ranged from 17.8% (dibenz(a,h)anthracene) to 67.9% (fluorene) when extracted by SELF.

However, the SELF formulation was developed based on the lung environment of healthy people. With the global outbreak of coronavirus 2019 (COVID-19), more than 93.6 million infected cases and 2 million deaths have been reported worldwide since January 18, 2021. In one recent study, 35 out of 837 (4.8%) COVID-19 survivors were observed to still show symptoms of interstitial lung inflammation and obvious lung functional deficits at 4 weeks after discharge. It therefore can be expected that many people may still suffer inflammatory sequelae even after recovery given the large number of COVID-19 infections worldwide. Notably, pneumonia and its sequelae were reported to affect the compositions of lung
fluid. Previous studies reported that lung inflammation induced unbalanced anabolism/catabolism of pulmonary surfactants and increased their concentrations in lung fluid. Similarly, Johnson et al. and Bell et al. found that the amount of hyaluronic acid (HA) detected in the lung tissues of inamed mice was approximately fourfold higher than that in healthy mice and was positively correlated with the severity of lung inflammation. Despite no data being available so far to describe the lung fluid compositions of COVID-19 survivors, it is possible that the lung fluid compositions may be altered compared with healthy people because the most common symptom of COVID-19 is lung inflammation. For example, Hellman et al. observed the elevation of HA in the lung tissues of three COVID-19 deceased patients, which was related to the high levels of inflammatory cytokines (IL-1β, IL-6, and TNFα). These changes in lung fluid compositions may affect the migration of contaminants from solid particles into the respiratory tract. For example, Zhao et al. observed that the adsorption of anthracene (Ant) on nanocarbon particles was notably inhibited by approximately 60% after adding 40 mg/L pulmonary surfactant into saline. Likewise, pulmonary surfactants (i.e., Curosurf) promoted desorption of phenanthrene from carbon nanotubes (CNTs), increasing the pulmonary toxicity of inhaled PAH-associated CNTs. Thus, it is important to consider the effects of pneumonia on lung fluid compositions, in which the bioaccessible concentrations of airborne pollutants in the lung environment may be increased, and extra adverse effects can therefore be anticipated.

In this study, PM2.5 samples were collected in both winter and spring from Nanjing during the COVID-19 outbreak in the early of 2021. The objectives of this study were to (1) determine PAH IVBA in PM2.5 under different health conditions by use of SELF methods with various levels of surfactants; (2) investigate the mechanisms through the characterization of lung surfactant micelles as well as the solubility and competitive adsorption between PAHs and surfactants; and (3) evaluate the health risks through exposure to PM2.5-bound PAHs for humans with different health conditions.

■ MATERIALS AND METHODS

Chemicals and PM2.5 Samples. The mixed standard of 16PAHs was purchased from J&K Scientific (Shanghai, China) with purity >98% (the detailed names of the 16 PAHs can be found in the Supporting Information as Text S1). The standards of phenanthrene (Phe) and pyrene (Pyr) were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity >99%. Dipalmitoyl phosphatidylcholine (DPPC) and bovine serum albumin (BSA) were purchased from Yuanye Bio-Technology (Shanghai, China) and Macklin (Shanghai, China). All other reagents used in this study were of high-performance liquid chromatography or analytical grade. The PM2.5 samples were collected on the building roof (about 25 m above ground) of the School of the Environment of Nanjing University on Xianlin campus. This campus is located in primarily university and residential area of Northeastern Nanjing. A total of 24 PM2.5 samples were collected from January 9, 2021 to March 30, 2021 (January 9 to February 2 for winter, and March 2 to March 30 for spring). The detailed sampling procedure and quantification of PAHs in PM2.5 are given in Text S1 and Table S1.

Design of IVBA Assays. In this study, the default SELF method was used as a control group to represent healthy individuals, and the compositions of SELF are given in Figure 1. Text S2 describes the reasons for choosing the SELF method. The concentrations of surfactants (i.e., DPPC and BSA) and HA were adjusted to simulate conditions of mild and severe lung inflammation according to previous results. For example, lung inflammation can decrease numbers of alveolar macrophages, resulting in the accumulation of lung lipids and surfactants (e.g., phosphatidylcholine, phospholipids, and proteins) in alveolar space approximately 1.1−3.5 times higher.
than those in healthy controls. In addition, loss of alveolar macrophages and inflammatory immune responses triggered by lung injury have been reported to induce a rapid increase in lung HA levels, which peaked with maximization of leukocyte infiltration and then declined as homeostasis was restored. For example, Zhao et al. observed that the in vivo concentrations of HA in rats significantly increased from 6.19 to 311 μg/L on day 3 of bleomycin-induced lung injury, peaked on day 7 with a value of 411 μg/L, and gradually decreased to 176 μg/L on day 14. To simulate the lung fluid of patients with mild lung inflammation (e.g., early stage of pneumonia infection, middle of treatment, and pneumonia sequelae), the concentrations of DPPC and BSA were set two times their default values in SELF, and HA concentration was set as 200 μg/L. Accordingly, the 4-times increase of DPPC and BSA together with the HA level of 400 μg/L was set to simulate severe lung inflammation. The modified SELF methods are described in detail in Figure 1.

**PAH IVBA in PM2.5.** Seven PM2.5 samples with PAH concentrations ranging from 10.9 to 58.1 ng/m3 were chosen to measure PAH IVBA by SELF extractions simulating various scenarios. These in vitro extractions include default SELF to represent health condition and SELF with elevated levels of DPPC (200 and 400 mg/L), BSA (520 and 1040 mg/L), and HA (200 and 400 μg/L) to simulate the mild and severe lung inflammation, respectively (Figure 1). In addition, levels of the three components in SELF were also altered separately for in vitro extraction to characterize the effect of single components on PAH IVBA. The IVBA experiment and extraction were conducted according to our previous study, and further details are provided in Text S3. The PAH IVBA in PM2.5 was calculated as follows:  

\[
\text{PAH IVBA (%) = } \frac{\text{in vitro PAHs}}{\text{total PAHs}} \times 100\%
\]

where in vitro PAHs are the mass of PAHs extracted in simulated lung fluids, and total PAHs are the total mass of PAHs on filters.

**Characterization of Micellar Systems.** In this study, the modification of the SELF method had a significant effect on PAH bioaccessibility, which may be presumably related to the micelle formation from surfactants (i.e., DPPC and BSA). First, the critical micelle concentration (CMC) of DPPC and BSA, as a key factor in micellar growth behavior, was investigated by the steady-state fluorescence probe method. Pyrene was chosen as a probe because of its intensity in the first (373 nm) and third (383 nm) bands (I₁ and I₃), which was highly sensitive to the polar properties of the surrounding medium. Briefly, DPPC and BSA were separately suspended in deionized (DI) water by ultrasonication (40 kHz, 100 W, and 20 ± 5 °C) for 30 min to achieve a series of concentrations, and solutions containing a mixture of DPPC, BSA, and HA were also prepared accordingly. The detailed concentrations of these solutions and measurement of CMC are summarized in Text S4 and Table S2. In addition, the number of micelles aggregated by DPPC in DPPC aqueous solution as well as solution containing a mixture of DPPC, BSA, and HA was measured by the steady-state fluorescence quenching method (Text S5).

**Solubilization Effect of Surfactants and Adsorption Experiments.** To further investigate and compare the effect of DPPC, BSA, and HA on PAH solubilization, their apparent enhancement coefficients \( f \) for the solubilization of typical PAHs (i.e., phenanthrene and pyrene) were determined under a series of concentrations of the three compounds (i.e., DPPC, BSA, and HA) according to previous studies, and further details are described in Text S6 and Table S3. Surface adsorption of components (i.e., DPPC, BSA, and HA) onto PM2.5 particles was studied using Fourier transform infrared spectroscopy (FTIR, NEXUS870, USA). Briefly, the 1/32 of sampling filters were added into DPPC, BSA, HA, and DPPC-BSA-HA solutions as mentioned above (Table S3) and shaken for 24 h in an incubator (100 rpm) at 37 °C in darkness. The filters with PM2.5 particles on their surface were then collected by centrifugation, washed with DI water, freeze-dried, and characterized by FTIR.

**Health Risk Assessment.** It has been reported that some patients of COVID-19 continue to show symptoms of interstitial lung inflammation and significant pulmonary deficits for 4 weeks to 2 months after discharge. In addition, some chronic lung diseases may last for more than 3 months and occur multiple times over 2 years. Considering the uncertainty of time for people living with lung inflammation, daily inhalation exposure (DIE) was chosen to evaluate the health risk through exposure to PM2.5-associated PAHs and calculated according to the study by Boiza et al.:  

\[
\text{DIE} = \frac{q_{\text{PAHs}} \times F_{\text{bio}} \times TR \times C_{\text{PM2.5}} \times V_{\text{resp}}}{BW}
\]

where \( q_{\text{PAHs}} \) represents the total PAH concentrations in PM2.5 (mg/kg), \( F_{\text{bio}} \) are the average PAH IVBA in PM2.5 extracted by simulated lung fluids with values of 24.5, 28.6, and 32.3% corresponding to healthy people and people with mild and severe lung inflammation. TR represents the tracheobronchial retention with a value of 75%; \( C_{\text{PM2.5}} \) is the concentration of PM2.5 in air (μg/m³); \( V_{\text{resp}} \) represents the inhalation rate (m³/day) with a value of 20 for adults; and BW (kg) means the body weight with a value of 60 kg for adults.

The acceptable daily inhalation exposure (ADIE) was calculated using the acceptable value of carcinogenicity risk (CR with a value of 10⁻⁶) for an individual carcinogen suggested by the U.S. EPA:  

\[
\text{ADIE} = \sum_{n=1}^{n=15} \frac{\text{IPF}_{B(a)P} \times \text{TEF}_{B(a)P}}{\text{CR}}
\]

where IPF\(_{B(a)P}\) represents the inhalation potency factor for benzo(a)pyrene (BaP) with a value of 3.9 × 10⁻⁵ (ng/kg/day)⁻¹ as reported by OEHHA; TEF values are the toxicity equivalent factors (Table S4). The ADIE for 15 PAHs (Nap was excluded because of its low recovery from artificial lung fluid) was calculated as 1.63 ng/kg/day.

**QA/QC and Statistics and Data Analysis.** All glassware and quartz microfilter fibers were heated at 500 °C for 4 h before use to avoid potential contamination. PAHs were not detected in procedural blanks. The recovery of PAHs in spiked “clean” filters and simulated lung fluids ranged from 63.8 ± 7.2% (Ace) to 109.6 ± 2.0% (BaA) with the exception of Nap (37.8 ± 3.4 and 29.9 ± 1.8%) because of volatility issues. Three replicates were adopted in all experiments, and the results were shown as means ± standard deviation. Significant differences between treatment groups were analyzed using the T-test, and the significance level was set as \( p < 0.05 \).
RESULTS AND DISCUSSION

Contamination Levels of PAHs in PM_{2.5}. The concentrations of PAHs in the 13 winter (January 9 to February 2, 2021) and 11 spring PM_{2.5} samples (March 3 to March 30, 2021) are shown in Figure S1, and the detailed concentrations of individual 16 PAHs are summarized in Table S5. The total concentrations of 16 PAHs in winter PM_{2.5} varied from 18.0 to 58.1 ng/m³ (mean = 34.0 ng/m³), which were much higher than those in spring PM_{2.5} (5.86−14.0 with a mean concentration of 9.47 ng/m³). A similar seasonal variation was also observed in other cities such as Zhengzhou, Guangzhou, and Shenzhen in China. Factors, such as more combustion sources, shorter daylight, and lower temperatures during winter, can explain the higher PAH emission and lower tendency of volatilization or photodegradation of PAHs in winter. Of note, the total PAH concentrations in this study (mean of 22.8 ng/m³) were lower than those reported for district Xianlin (mean of 38.0 ng/m³) and Luhe in Nanjing (mean of 50.0 ng/m³) in 2019. The lower PAH concentrations in PM_{2.5} may be ascribed to the improved air quality as a result of the source control measures implemented in China in recent years. Another explanation is maybe due to the impact of COVID-19, decreasing the travel, energy demand, and industrial output (e.g., domestic flights, coal-fired power plants, and steel industries) since 2020. Zhang et al. also observed that particulate PAH concentrations in western Japan decreased by 36.6−56.7% from February to April 2020 compared to the previous years, particularly for the control period of COVID-19. However, the mean concentrations of benzo(a)pyrene (BaP) were 1.91 ng/m³ and 0.51 ng/m³ in winter and spring. These values were still higher than the health-based guideline value of 0.1 ng/m³ proposed by Bostrom et al., indicating the potential health risks of PAH-associated PM_{2.5} even after the pollution emission reduction because of both the source control and COVID-19.

Inhalation Bioaccessibility of PAHs in PM_{2.5} Measured by In Vitro Lung Fluids Simulating Different Health Conditions. Seven out of 24 PM_{2.5} samples, with PAHs ranging from 10.9 to 58.1 ng/m³, were utilized to measure PAH inhalation bioaccessibility. As mentioned above, unmodified SELF was chosen as the control group to represent the extracellular lung environment of healthy humans. The concentrations of pulmonary surfactant components (i.e., DPPC and BSA) were modified to 2 and 4 times their default values with HA levels of 200 and 400 μg/L, which simulated the lung fluid of people with mild and severe lung inflammation (Figure 1), respectively. Nap was not included in the analysis because of its low extraction recoveries from lung fluid. As shown in Figure 2, the IVBA in the healthy group (extracted by unmodified SELF and reported based on the sum of 15 PAHs) varied from 18.6 to 30.9% (with an average of 24.5 ± 4.52%), which was comparable to those previously reported for 19 PAHs obtained by SELF in PM_{2.5} (3.55−35.3%). When the DPPC concentrations increased to 200 and 400 mg/L (simulating lung fluids with mild and severe lung inflammation), PM_{2.5}-bound PAHs were found to be more readily mobilized. The average bioaccessible fractions were 29.3 ± 6.08% (200 mg/L) and 33.9 ± 4.38% (400 mg/L), and the latter was significantly (p < 0.05) higher than 24.5 ± 4.52% in the healthy group (Figure 2). When looking into individual PAH congeners (Figure S2), the IVBA of higher molecular-weight PAHs (log K_{ow} > 5.5) was more pronounced affected by DPPC than...
PAHs with lower molecular weights. For example, the increase folds of benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), and benzo(k)fluoranthene (BkF) were 1.03, 0.57, and 0.59 in the presence of 400 mg/L DPPC compared to the healthy group, while other PAHs, such as Phe, Ant, and fluorene, were relatively slightly influenced with increase folds of 0.13, 0.06, and 0.06, respectively. A comparable result was observed for gastrointestinal solution, where the IVBA of PM$_{2.5}$-bound HOCs increased about 5–7 times with the increase of surfactant (i.e., bile salts) in small intestine fluid from 0.15 to 0.30%, and the enhancement was more pronounced for HOCs with log $K_{ow}$ higher than 5 because of their stronger hydrophilic interaction with bile salts.

Similar to DPPC, the PAH IVBA was also positively related to the concentration of BSA, averaging at 30.9 ± 8.98 and 33.1 ± 6.26% with BSA levels of 520 and 1040 mg/L. The IVBA values were approximately 1.26 and 1.35 times higher compared to those in the healthy group. It was reported that BSA can enhance PAH desorption from soil through van der Waals forces as well as forming hydrogen bonds, and the binding strength was observed to be positively dependent on BSA concentrations. By contrast, the IVBA was less influenced by addition of HA. The PAH IVBA increased only 3.61% under mild conditions (200 μg/L) and 6.00% under severe conditions (400 μg/L), respectively. This result is not unanticipated as HA concentration was approximately three orders of magnitude lower than that of DPPC and BSA (200 μg/L vs 200 mg/L, and 520 mg/L).

In addition, concentrations of DPPC, BSA, and HA were increased at corresponding levels simultaneously to investigate the combined effects, namely scenarios close to real conditions. The average PAH IVBA ($p = 0.06$) increased from 24.5 ± 4.52% under healthy conditions to 28.6 ± 3.17% and significantly ($p < 0.05$) to 32.3 ± 5.32% under mild and severe lung inflammation conditions, respectively. The combined effect was lower than the alteration from the individual components of DPPC and BSA. This result can be explained by the fact that the coexistence of DPPC and BSA led to a reduction in the binding sites of BSA or DPPC monomer molecules to PAHs, which has been demonstrated in the study by Zhao et al. that the sorption of phenanthrene on fluid was observed when the bile salt concentration was higher than its CMC. Apparently, more PM$_{2.5}$-associated PAHs can be mobilized in lung fluids of this study because their concentrations in unmodiﬁed lung ﬂuid. Note that a decrease of the CMC value for the mixture of DPPC and BSA was observed in the solution containing DPPC, BSA, and HA (44.5 ± 1.22 mg/L) compared with CMC values for their single surfactant (i.e., 67.8 ± 1.08 for DPPC and 53.3 ± 1.15 mg/L for BSA); this may be due to their cooperative binding process. For instance, BSA was reported to enhance the aggregation of DPPC vesicles, and interaction with HA altered the interfacial conformation of BSA, leading to a more compact protein layer. A previous study also implied that the adsorption of humic acids by amide molecules reduced the mutual repulsion of its polar groups (−COO$^-$) as well as the energy required for its micellization and in turn lowered the CMC values.

In addition, the DPPC micelle aggregation number ($N_{agg}$) in DPPC single solution and DPPC-BSA-HA solution was determined, which can describe the process of micelle growth in simulated lung fluid (Figure 3). The number of BSA micelle aggregation was not considered here because of the uncertainty of molecular weight of BSA. The growth of micelles were strongly dependent on DPPC concentration in solution. For example, at the DPPC single solution, the $N_{agg}$ increased significantly from 2 (at 100 mg/L of DPPC) to 13 and 21 (400 and 800 mg/L). A similar trend was found in DPPC-BSA-HA solution, increasing from 3 (at a DPPC concentration of 100 mg/L) to 12 and 18 (400 and 800 mg/L). Zhang et al. reported that the higher micelle numbers were induced by the increase of bile concentrations. Therefore, it can be concluded that DPPC and BSA micelles were present in various lung fluids of this study because their concentrations were well above their CMC values. The numbers of micelle aggregation were proportional to surfactant concentrations, which partly explained the significant increase of PAH inhalation bioaccessibility at higher DPPC and BSA concentrations.

**Figure 3.** Plot of ratio $I_1/I_2$ against the logarithm of surfactant concentrations. Insert: the relationship between the micellar formation and growth behaviors of surfactants (i.e., DPPC and BSA) on PAH IVBA in PM$_{2.5}$ samples, the micelle formation and growth behaviors were investigated, including CMC values of the two surfactants and micelle aggregation number ($N_{agg}$) at different surfactant concentrations. The CMC values for both DPPC and BSA in aqueous solution were 67.8 ± 1.08 and 53.3 ± 1.15 mg/L, respectively (Figure 3), which were much lower than their default values in SELF fluids being 100 mg/L for DPPC and 260 mg/L for BSA. This indicated that monomers of both surfactants can form micelles consisting of a hydrophilic shell and a hydrophobic core in unmodified lung fluid. Note that a decrease of the CMC value for the mixture of DPPC and BSA was observed in the solution containing DPPC, BSA, and HA (44.5 ± 1.22 mg/L) compared with CMC values for their single surfactant (i.e., 67.8 ± 1.08 for DPPC and 53.3 ± 1.15 mg/L for BSA); this may be due to their cooperative binding process. For instance, BSA was reported to enhance the aggregation of DPPC vesicles, and interaction with HA altered the interfacial conformation of BSA, leading to a more compact protein layer. A previous study also implied that the adsorption of humic acids by amide molecules reduced the mutual repulsion of its polar groups (−COO$^-$) as well as the energy required for its micellization and in turn lowered the CMC values.

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Mechanism Exploration: Effect of Micelles on PAH Solubility as Well as Competitive Adsorption between PAHs and Surfactants. In the previous section, the surfactant concentration-dependent behavior of micelles was confirmed in various lung fluids. Therefore, the mechanism of micelle effects on PAH inhalation bioaccessibility was further explored. The solubilization capacity is one of the main factors influencing the desorption of hydrophobic compounds from solid phase. As detailed by Zhao et al., high surfactant concentrations likely favored partitioning of PAHs from the aqueous phase into micelles, thus increasing their solubility. To test this hypothesis, the solubilization of typical PAHs (i.e., Phe and Pyr) in different surfactants’ aqueous solution (i.e., DPPC, BSA, and mixture of DPPC-BSA-HA) was measured (Tables S6 and S7). The solubilities of Phe and Pyr in DI water were 0.83 ± 0.01 and 0.10 ± 0.01 mg/L. As shown in Figure 4, the PAH solubility increased linearly with DPPC and BSA concentrations; for example, the f values (enhancement coefficient of solubility) of Phe were up to 1.20 ± 0.27 and 1.66 ± 0.04 at 200 mg/L DPPC and BSA, respectively. With further increasing concentration of DPPC and BSA to 400 mg/L, the f values continued to reach 2.07 ± 0.18 and 2.98 ± 0.11. This was also in agreement with that in DPPC-BSA-HA solution, where the f values of Phe were 1.49 ± 0.28 when solution contained 100 mg/L DPPC, BSA, and 100 μg/L HA and increased to 2.05 ± 0.44 and 2.59 ± 0.18 as the three-component concentrations increased by 2 or 4 times. The results were comparable to trends of micelle aggregation numbers, where the $N_{agg}$ increased by about 5- and 3-times as the DPPC concentration increased from 100 to 400 mg/L at DPPC single solution and DPPC-BSA-HA solution, respectively. Furthermore, no significant change of Phe and Pyr solubility was observed with increasing HA concentration, suggesting that the change of PAH IVBA may be mainly attributable to interaction with DPPC and BSA micelles (e.g., decrease DPPC and BSA CMC values). Notably, a more pronounced effect on the solubility of Pyr was observed compared to Phe. The f values of Pyr significantly increased to 6.60 ± 0.45 and 4.32 ± 0.08 at 400 mg/L DPPC and BSA, which were 3.19 and 1.45 times higher than those of Phe under the same conditions. This was consistent with IVBA results that higher increases were observed in PAHs with stronger hydrophobicity. Accordingly, it was reported that octanol/water distribution coefficients ($K_{ow}$) of PAHs were critical for micelle–PAH interaction.

In addition to solubilization enhancement, the competitive adsorption between PAHs and surfactants onto the PM$_{2.5}$ surface may also contribute to the elevated PAH IVBA. In other words, the sorption sites of PAHs on the PM$_{2.5}$ surface may be replaced by surfactants and in turn induced the release of PAHs from solid particles into lung fluids. FTIR spectroscopy was chosen to characterize the PM$_{2.5}$ surface sorption of surfactants (Figure 4). The C–H stretching peaks at 2913 and 2845 cm$^{-1}$ were observed for DPPC-treated PM$_{2.5}$ samples, which may be attributed to the alkane of stearic acid. The adsorption of DPPC on the PM$_{2.5}$ surface can be
Figure 5. Risks of PM$_{2.5}$-bound PAHs as functions of atmospheric PM$_{2.5}$ concentrations and total 15 PAH concentrations in PM$_{2.5}$, the DIE value calculated using bioaccessibility of healthy group (A), mild lung inflammation (B), and severe lung inflammation (C). The risk tolerance lines (i.e., purple curve) represent ADIE of total PAHs.

Further demonstrated by the presence of a characteristic peak at the same wave numbers (2913 and 2845 cm$^{-1}$) in DPPC-BSA-HA solution, and transmittance exhibited an obvious increase along with the concentrations of DPPC. In contrast, no new transmission peak was observed for BSA and HA-treated samples. A similar result was observed in which DPPC showed higher adsorption capacity on the surface of silica particles compared to BSA, with lower Freundlich affinity coefficients values of Phe in DPPC solution (1.73) than BSA solution (2.32). Consequently, it can be suggested that PM$_{2.5}$-PAHs may be released into respiratory tracts mainly by micelle solubilization, and in the presence of DPPC, can also be desorbed because of competitive adsorption between PAHs and DPPC on PM$_{2.5}$ particles.

**Estimation of Inhalation Cancer Risk.** The health risk of exposure to PM$_{2.5}$-associated PAHs was assessed by comparing DIE with ADIE, which were calculated based on carcinogenic effect levels. According to the average concentration of PM$_{2.5}$ (215 μg/m$^3$) and PAHs (117 mg/kg in PM$_{2.5}$ samples) measured in our experiment, the concentration range of PM$_{2.5}$ and PAHs was chosen as 1–250 μg/m$^3$ and 1–150 mg/kg to draw the contour plots of risk assessment. In addition, DIE values were calculated using PAH IVBA under healthy and mild and severe lung inflammation conditions. The IVBA values were measured by in vitro methods with adjusting surfactant concentrations simultaneously, which was more representative of the conditions of patients with lung inflammation. Figure 5 showed the estimated risk of PM$_{2.5}$-associated PAHs as a function of PM$_{2.5}$ and PAH concentrations. The risk tolerance line (purple curve) on each graph represented the maximum acceptable inhalation exposure dose to PAHs; that is, it was considered as unacceptable when the calculated DIE value was above the risk tolerance line. A remarkable increase in the unacceptable risk area was observed in humans with mild and severe lung inflammation, with the percentage of the unacceptable area from 4.74% in health people increasing to 9.05% (for mild condition) and 13.02% (severe). For example, exposure of healthy people to PM$_{2.5}$ at an air concentration of 200 μg/m$^3$ and PAH concentration of 120 mg/kg (i.e., point A in Figure 5A) induced a cancer risk with a DIE value of 1.47 ng/kg/day, which was lower than the ADIE of 1.63 ng/kg/day. However, under the same PM$_{2.5}$ contamination level (i.e., PM$_{2.5}$ at an air concentration of 200 μg/m$^3$ and PAH concentration of 120 mg/kg), for mild and severe lung inflammation patients (i.e., points B and C in Figure 5B,C), the cancer risk for both groups exceeded the acceptable risk tolerance (the purple curves) with DIE values of 1.72 and 1.93 ng/kg/day, respectively. This indicated that PM$_{2.5}$ exposure, which is safe for healthy humans, may not be acceptable for people with lung inflammation. Consequently, it is highly recommendable that the risk assessment and regulation for air pollution control need to consider not only healthy people, but also the people with pneumonia or its sequelae, particularly when facing the large and increasing number of COVID-19 infection rate all over the world. It should be noted that there are limits about the risk assessment based on IVBA data, because the in vitro methods need to be validated by in vivo data. The lack of validation with in vivo results makes it difficult to determine which IVBA assays are more appropriate, because there are variations present in major IVBA assays, such as Gamble’s solution (simulating extracellular fluid in the deep lung) and Hatch’s solution (simulating the combination of lung fluid and mucus layer). At the current stage, it is still challenging to perform in vivo tests to measure inhalation bioavailability of contaminants in PM$_{2.5}$. For the most in vivo tests, it usually requires a lot of work to maintain the good condition of animals, because the number of particles in each delivered fluid and the frequency of delivery need to be carefully controlled to prevent pulmonary edema in animals. Even with the immature in vivo tests, some preliminary studies reported that Pb inhalation IVBA in fine soil particles measured by Gamble’s solution showed good correlation with in vivo results ($R^2 = 0.73$) by mouse tests. In our previous study, bioaccessible PAH concentrations measured by SELF assay were better at predicting dioxin toxicity of PM$_{2.5}$-associated PAHs when compared with total PAH concentrations in PM$_{2.5}$. Both the studies indicated the potential of IVBA in the aspects of risk assessment and toxicity prediction. On the other hand, the selection of in vitro methods may be out of the main scope of this study, because bioaccessibility and risk assessment were compared among different pulmonary surfactant levels in one single in vitro method instead of different in vitro methods. Nevertheless, developing standard in vitro methods needs to be conducted in future by establishing correlation between in vivo and in vitro results, which is highly beneficial for refinement of risk assessment.

**Environmental Implications.** As the COVID-19 pandemic continues, the number of patients with pneumonia and its sequelae is expected to increase worldwide. In this study, the SELF method was modified to mimic the extracellular lung environment of patients with lung inflammation. Our results...
demonstrated that lung inflammation, particularly under severe conditions, can significantly increase the inhalation bioaccessibility of PAHs in PM$_{2.5}$. Health risk assessments indicated that the inhalation exposure of PM$_{2.5}$-bound PAHs can pose unacceptable carcinogenic risks for patients with lung inflammation, even when the contamination level is safe for people in healthy condition. Our result here also implies that various lung conditions need to be considered when developing in vitro methods for inhalation IVBA measurement. On the other hand, the implications provided by this study can also be broadened to other scenarios exacerbating lung inflammation, such as on-street vehicle exhaust exposure and household air pollution due to solid fuel cooking.

**ASSOCIATED CONTENT**

*Supporting Information*

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c08052.

Chemicals and PM$_{2.5}$ samples; reasons for choosing the SELF method; IVBA experiment and extraction methods; CMC and micelle aggregation number determination; pyrene or phenanthrene solubilization determination; detailed information about PM$_{2.5}$ samples; surfactants’ aqueous solution concentration gradients for CMC measurement; solution concentrations of HA and surfactants for solubilization effect experiments; toxicity equivalence factors of 16 PAHs; solubilization and $f$ values of Phe and Pyr solubilization in different surfactants’ aqueous solutions; 16 PAH concentrations in PM$_{2.5}$ samples; a range of individual PAH IVBA in modified SELF (PDF)

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors appreciate the financial support from the National Natural Science Foundation of China (Nos. 41922058 and 21876084).

**REFERENCES**

(1) Landrigan, P.; Fuller, R.; Acosta, N. J. R.; Adeyi, O.; Arnold, R.; Basu, N.; Balde, A. B.; Bertollini, R.; Bose-O’Reilly, S.; Boufford, J. I.; Bressey, P. N.; Chiles, T.; Mahidol, C.; Coll-Seck, A. M.; Cropper, M. L.; Fobil, J.; Fuster, V.; Greenstone, M.; Haines, A.; Hanrahan, D.; Hunter, D.; Khare, M.; Krupnick, A.; Lanphear, B.; Lohani, B.; Martin, K.; Mathiasen, K. V.; McTeer, M. A.; Murray, C. J. L.; Ndhahiminanjara, J. D.; Perera, F.; Potocnik, J.; Preker, A. S.; Ramesh, J.; Rockstrom, J.; Salinas, C.; Samson, L. D.; Sandiliya, K.; Sly, P. D.; Smith, K. R.; Steiner, A.; Stewart, R. B.; Suk, W. A.; Van Schayck, O. C. P.; Yadam, G. N.; Yumkella, K.; Zhong, M. The lancet commission on pollution and health. *Lancet* 2018, 391, 462–512.

(2) Liu, C.; Hsu, P. C.; Lee, H. W.; Ye, M.; Zheng, G. Y.; Liu, N. A.; Li, W. Y.; Cui, Y. Transparent air filter for high-efficiency PM$_{2.5}$ capture. *Nat. Commun.* 2015, 6, 6205.

(3) Antinolo, M.; Willis, M. D.; Zhou, S. M.; Abbatt, J. P. D. Connecting the oxidation of soot to its redox cycling abilities. *Nat. Commun.* 2015, 6, 6812.

(4) Li, Y. Z.; Juhász, A. L.; Ma, L. Q.; Cui, X. Y. Inhalation bioaccessibility of PAHs in PM$_{2.5}$: Implications for risk assessment and toxicity prediction. *Sci. Total Environ.* 2019, 650, 56–64.

(5) Tao, S.; Zhang, D. Y.; Lu, Y.; Li, L.; Ding, J. N.; Yang, Y.; Yang, Y. F.; Wang, X. L.; Liu, W. X.; Xing, B. S. Mobility of polycyclic aromatic hydrocarbons in the gastrointestinal tract assessed using an in vitro digestion model with sorption rectification. *Environ. Sci. Technol.* 2010, 44, 5608–5612.

(6) Herting, G.; Wallinder, I. O.; Leygraf, C. Factors that influence the release of metals from stainless steels exposed to physiological media. *Corros. Sci.* 2006, 48, 2120–2132.

(7) Metzger, R.; Wichers, D.; Vaselin, J.; Velasquez, P. Solubility characterization of airborne uranium from an in situ uranium processing plant. *Health Phys.* 1997, 72, 418–422.

(8) Schaidt, L. A.; Senn, D. B.; Brabander, D. J.; McCarthy, K. D.; Shine, J. P. Characterization of zinc, lead, and cadmium in mine waste: Implications for transport, exposure, and bioavailability. *Environ. Sci. Technol.* 2007, 41, 4164–4171.

(9) Berlinger, B.; Ellingsen, D. G.; Náräy, M.; Zaráy, G.; Thomassen, Y. A study of the bio-accessibility of welding fumes. *J. Environ. Monit.* 2008, 10, 1448–1453.

(10) Boisa, N.; Elom, N.; Dean, J. R.; Deary, M. E.; Bird, G.; Entwistle, J. A. Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM$_{10}$ size fraction of soil. *Environ. Int.* 2014, 70, 132–142.

(11) Zeng, H.; Zhang, L.; Sun, F. Z.; Liu, J. J.; Fang, B.; Yang, W. Q.; Meng, C. Y.; Wang, M. M.; Wang, Q.; Hao, Y. L. Inhalation bioaccessibility, health risk assessment, and source appointment of ambient PM$_{2.5}$-bound polycyclic aromatic hydrocarbons (PAHs) in Caofeidian, China. *Environ. Sci. Pollut. Res.* 2021, 28, 47574–47587.

(12) Li, Z. G.; Song, G. F.; Bi, Y. H.; Gao, W.; He, A.; Lu, Y.; Wang, Y. W.; Jiang, G. B. Occurrence and Distribution of Disinfection Byproducts in Domestic Wastewater Effluent, Tap Water, and Surface Water during the SARS-CoV-2 Pandemic in China. *Environ. Sci. Technol. 2021, 55, 4103–4114.

(13) Myall, K. J.; Mukherjee, B.; Castanheira, A. M.; Lam, J. L.; Benedetti, G.; Mak, S. M.; Preston, R.; Thillai, M.; Dewar, A.; Monreal, P. L.; West, A. G. Persistent post–COVID-19 interstitial lung disease an observational study of corticosteroid treatment. *Ann. Am. Thorac. Soc.* 2021, 18, 799–806.

(14) D’Arongco, S.; Simonato, M.; Vedovelli, L.; Baritussio, A.; Verlato, G.; Nobile, S.; Giorgetti, C.; Nespeca, M.; Carnielli, V. P.; Cogo, P. E. Surfactant protein B and A concentrations are increased in neonatal pneumonia. *Pediatr. Res.* 2015, 78, 401–406.

(15) Johnson, P.; Arif, A. A.; Lee-Sayer, S. S. M.; Dong, Y. F. Hyaluronan and its interactions with immune cells in the healthy and inflamed lung. *Front. Immunol.* 2018, 9, 2787.

(16) Bell, T. J.; Brand, O. J.; Morgan, D. J.; Salek-Ardakani, S.; Jagger, C.; Fujimori, T.; Cholewa, L.; Tilakaratna, V.; Ostling, J.; Thomas, M.; Day, A. J.; Snelgrove, R. J.; Russell, T. Defective lung function following influenza virus is due to prolonged, reversible hyaluronan synthesis. *Matrix Biol.* 2019, 80, 14–28.
(17) Hang, W. J.; Chen, C.; Zhang, X. A.; Wang, D. W. Endothelial dysfunction in COVID-19 calls for immediate attention: the emerging roles of the endothelium in inflammation caused by SARS-CoV-2. *Front. Med.* 2020, 15, 638–643.

(18) Hellman, U.; Karlsson, M. G.; Engstrom-Laurent, A.; Cajander, S.; Doroanse, I.; Ahlm, C.; Laurent, C.; Blomberg, A. Presence of hyaluronan in lung alveoli in severe Covid-19: An opening for new treatment options? *J. Biol. Chem.* 2020, 295, 15418–15422.

(19) Zhao, Q.; Li, Y. J.; Chai, X. L.; Geng, Y. X.; Cao, Y. Z.; Xue, Y. Z.; Zhang, L. F.; Huang, J. H.; Ning, P.; Tian, S. L. Interaction of nano carbon particles and anthracene with pulmonary surfactant: The potential hazards of inhaled nanoparticles. *Chemosphere* 2019, 215, 746–752.

(20) Zhao, J.; Wang, Z. Y.; Mashayekhi, H.; Mayer, P.; Cheftetz, B.; Xing, B. S. Pulmonary surfactant suppressed phenonadine adsorption on carbon nanotubes through solubilization and competition as examined by passive dosing technique. *Environ. Sci. Technol.* 2012, 46, 5369–5377.

(21) Dong, Y. F.; Arif, A. A.; Guo, J.; Ha, Z. L.; Lee-Sayer, S. S. M.; Poon, G. F. T.; Dosanjh, M.; Roskelley, C. D.; Huan, T. J.; Johnson, P. CD44 loss disrupts lung lipid surfactant homeostasis and exacerbates oxidized lipid-induced lung inflammation. *Front. Immunol.* 2020, 11, 29.

(22) Zhao, H. W.; Lu, C. J.; Yu, R. J.; Hou, X. M. An increase in hyaluronan by lung fibroblasts: a biomarker for intensity and activity of interstitial pulmonary fibrosis? *Respirology* 1999, 4, 131–138.

(23) Gerola, A. F.; Wanderlind, E. H.; Idrees, M.; Sangaletti, P.; Zaramello, L.; Nome, R. A.; Silva, G. T. M.; Quina, F. H.; Tachiya, M.; Nome, F.; Fiedler, H. D. Anion binding to surfactant aggregates: AuCl4− in cationic, anionic and zwitterionic micelles. *J. Mol. Liq.* 2020, 314, No. 113607.

(24) Wilk, K. A.; Laska, U.; Zielinska, K.; Olszowski, A. Fluorescence probe studies upon microenvironment characteristics and aggregation properties of gemini sugar surfactants in an aquatic environment. *J. Photochem. Photobiol. A* 2011, 219, 204–210.

(25) Zhu, L. Z.; Chiou, C. T. Water solubility enhancements of carbon particles and anthracene with pulmonary surfactant: The on carbon nanotubes through solubilization and competition as examined by passive dosing technique. *Environ. Sci. Technol.* 2012, 46, 5369–5377.
M. H.; Tao, S. Pollutant emissions from improved coal- and wood-fuelled cookstoves in rural households. *Environ. Sci. Technol.* 2015, 49, 6590−6598.