Impact of particle size on distribution, bioaccessibility, and cytotoxicity of polycyclic aromatic hydrocarbons in indoor dust

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A B S T R A C T
Contaminants, such as polycyclic aromatic hydrocarbons (PAHs), can be absorbed on the particles of indoor dust, which may pose potential health risks. In this study, indoor dust samples were collected and sieved into 6 size fractions (i.e., < 43 μm, 43–63 μm, 63–100 μm, 100–150 μm, 150–200 μm, and 200–2000 μm). Ingestion bioaccessibility of PAHs was measured by physiologically based extraction test. Bioaccessibility in fractions of 200–2000 μm was generally higher than those in other particle sizes. Daily uptake doses based on benzo(a)pyrene toxic equivalency quantity were 1.09–15.0 ng/d/kg, and peaked at fractions of < 43 μm, while doses considering bioaccessibility ranged from 0.02 to 0.21 ng/d/kg, and peaked at fractions with relatively larger particle size. Cell toxicity was also investigated by human normal liver cell line viability through exposure to organic extracts of indoor dust fractions with various particle sizes. Our results indicated that it is crucial to consider dust particle size and bioaccessibility during risk assessment.

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds containing two or more benzene rings in structure. Convention on long-range transboundary air pollution (CLRTAP, 1998) ranked PAHs as Persistent Organic Pollutants (POPs). PAHs can be produced by incomplete combustion of biofuels such as crop straw [1]. It has been reported that PAHs are widely distributed in human habitat, including atmosphere, soil, sediment and dust [2–4]. The health risk associated with PAH exposure has been paid a lot of attention to due to their toxicity, mutagenicity, and (possible) carcinogenicity [5–7].

PAHs can be released into environment by vehicle emission, fossil fuel combustion and other human activities, leading to their accumulation in indoor dust [5]. Growing evidence showed that indoor dust plays a significant role in human exposure to PAHs [8]. As a complex mixture with particles from multiple sources, dust particle size varies from nanometer to millimeter level [9,10]. Organic contaminant distribution among different sized indoor dust fractions is complicated and may be a function of many factors, such as total organic carbon in dust, surface area of dust particles, or the way of the contaminants go into dust [11,12]. For example, more polybrominated diphenyl ethers (PBDEs) were present in finer fraction of < 63 μm in dust [12]. On the other hand, Cao et al. found that in dormitory dust, hexabromocyclododecane (HBCDs) were mainly enriched in rough particles (> 300 μm) [13]. However, the distributions of PAHs in different particle size fractions of indoor dust have not been fully understood.

However, not all the contaminants can be transferred into human bodies, which makes bioaccessibility adopted as an adjusted method to assess actual intake of contaminants. In recent decades, some physiologically-based in vitro methods have been used to measure the bioaccessibility of PAHs in soil and sediment samples [14]. However, information about the bioaccessibility of PAHs in indoor dust is still rare. Their bioaccessibility in dust may also depend on particle size (USEPA, 1995), and has been investigated for some organic contaminants, e.g., phthalate esters (PAEs) [15]. In our previous study, bioaccessibility of organic phosphorus flame retardants (OPFRs) was found highest in the larger particle fractions (200–2000 μm) [16]. To our knowledge, no information is available about the effect of particle size on PAH bioaccessibility in dust so far.

Cytotoxicity of the dust extracts based on human cell line has been widely used as a bioassay to characterize the toxicity of indoor dust [17,18]. As human liver is of great importance in metabolism and detoxification of organic compounds, human liver hepatocellular cancer (Hep-G2) cells were commonly used to evaluate the potential adverse effects on human health via dust [17,19]. However, Hep-G2 cell line
has weaker capability to metabolize the compounds and lower anti- 
tumor activities compared with human normal liver cell line (HL-7702) 
[20], which is more suitable to investigate the cytotoxicity on organic 
extracts exposure. However, toxicity of PAHs in indoor dust, especially 
in various sized dust particles, by use of human normal liver cell line 
(HL-7702) has yet to be investigated.

In the current study, different types of indoor dust samples were 
collected from Nanjing, China. Nanjing is a megacity in eastern China 
with an area of 6587 km² and population of 8.2 million [21]. There are 
several large chemical plants located in this city, such as Yangzi Petro-
chemical, Jinling Petrochemical, and Nanjing Chemical. According to 
the local government, the electricity consumption was around 4.0–5.9 
billion kwh per month during year 2017. More than 60% of electricity 
was generated by coal combustion, while non-fossil energy was less 
than 14%. For the traffic condition, it is estimated that more than 
35,000 vehicles pass through one of the four main avenues of the city 
per day. It can be expected that relatively higher levels of PAHs may be 
detected in dust samples due to the chemical plants, coal combustion 
for energy, and busy traffic in the city. Therefore, the major objectives 
of this study were (1) to investigate the PAH levels in different sized 
indoor dust samples collected from different microenvironments in 
Nanjing; (2) to estimate the health risk of PAH exposure based on their 
bioaccessibility concentrations; (3) to study the cytotoxicity of different 
sized indoor dust particles by use of normal human liver cell (HL-7702).

2. Materials and methods

2.1. Chemicals and reagents

The standards of 16 EPA priority PAHs were obtained from Aladdin 
Industrial Corporation (Shanghai, China) and J&K Scientific (Shanghai, 
China) with purity > 98%. Cell culture plates and dishes were pur-
brished from Coring Inc. (NY, USA). Cell culture medium and relevant 
reagents were acquired from Thermo Fisher Scientific Inc. (GIBCO, 
USA). CCK-8 cell viability assay kit was purchased from Nanjing 
Jiancheng Bioengineering Institute (Nanjing, China). Pepsin, bile salts, 
and pancreatin were obtained from Tokyo Chemical Industry Co. 
(Tokyo, Japan). Malic acid disodium salt, trisodium citrate dehydrate, 
acetic acid glacial, and lactic acid were acquired from Sinopharm 
Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Indoor dust collection

All the dust samples, including office (n = 12), public micro-
environments (PME) (n = 7, 3 laboratories, 1 classroom, 1 lobby, 1 
hotel and 1 supermarket) and a car wash station (mixture dust samples 
from more than 100 cars) were collected in Nanjing, China. Dust 
samples from office and PME were collected by brushing the air-condi-
tioner filters. Brush was cleaned by water between every sample to 
avoid cross contamination. Vacuum cleaners were used to collect the 
car dust from carpet surface. For the office sampling sites, 9 offices 
belong to local government and are open to people for civil service. The 
rest 3 offices have area of about 15 m², where the same people (1–2 
people) work with computers for paper work. There is 1 air conditioner 
in each office. For all the PME sampling sites, there are 2 air condi-
tioners in all the places except the supermarket. The dust sample were 
collected from both the 2 air conditioners for each PME sampling site. 
For the supermarket, the dust was collected from the central air con-
ditioner. The 3 laboratories (with area of about 80 m² for each lab) and 
the classroom (~130 m²) are used for teaching and open to students. The 
3 laboratories, used for chemical experiment, are located on 
Xianlin campus of Nanjing University, Pukou campus of Nanjing 
University, and Xinzhuang campus of Nanjing Forestry University. The 
three campus are close to highway or road with busy traffic. Lobby and 
supermarket are located in downtown of Jianye District (N32°0’, 
E118°42’) with no factories and highway nearby. The dust samples 
were mixed as three composited dust samples (i.e., office, PME, and 
car). Briefly, aliquots of 2 g dusts from all the 12 office sampling sites 
were mixed thoroughly by spoon. Similarly, composited PME dust was 
also obtained in the same way. Car dust sample was collected on carpet 
surface from more 100 cars, and this dust was considered as a com-
posited sample. These composited dust samples were homogenized and 
sieved through nylon sieve to collect particles less than 2000 μm. For 
进一步 analysis, samples were separated into six fractions via sieving, 
including F1 (200–2000 μm), F2 (150–200 μm), F3 (100–150 μm), F4 
(63–100 μm), F5 (43–63 μm), and F6 (< 43 μm). The detailed sieving 
process was as follows: sieves with 10 mesh were firstly used to retain 
all the dust with particle size less than 2000 μm. The retained dust 
samples then go through 75-mesh sieves, and 200–2000 μm fractions 
were retained on these sieves. Similarly, dust fractions with smaller 
sizes was then successively got by going through sieves with higher 
mesh values. To measure the total organic carbon (TOC) contents, 
50 mg dust samples were firstly washed by 0.5 M hydrochloric acid 
solution at room temperature to remove the inorganic carbon. This 
process last till no bubbles formed. Then the dust was washed with 
Milli-Q water, and dried under 90 °C overnight. An aliquot of 20 mg 
of the treated dust sample was analyzed by element analyzer (vario TOC 
select, Elementar, Germany) with working temperature of 950 °C. 
The specific surface area of dust particles in each sized fraction was de-
temined by N2 sorption using a Micromeritics ASAP 2020 automated 
system. About 0.1–0.2 g of dust sample was used for surface area 
measurement under a relative vapor pressure of 0.01–0.3 at −196 °C.

2.3. PAH extraction, bioaccessibility and analysis

PAH extraction method followed the protocol used in our previous 
study [22]. Aliquots of samples (~0.2 g, dry weight) were extracted 
three times via ultrasonic bath with 20 mL n-hexane for 30 min. The 
supernatants after each extraction were collected by centrifugation at 
3000 rpm for 5 min. The combined extracts were concentrated until 
near dryness by a rotary evaporator (IKA® RV10, Germany) working 
at 40 °C with vacuum of 326 mbar, and the near dry residue was re-
dissolved with 2 mL n-hexane. The solution was then transferred to 
2 mL amber vials by filtration through 0.45 μm Teflon filter, which were 
stored at −20 °C until analysis.

Bioaccessibility assessment was conducted with physiologically 
based extraction test (PBET) method [22]. About 0.2 g dust sample was 
added to 20 mL gastric fluid (The gastric fluid consisted of 0.5 g stomach 
malate, 0.5 g sodium citrate, 1.25 g pepsin, 420 g lactic acid, and 
500 gL glacial acetic acid in 1L Milli-Q water at pH 2.5). After shaking 
at 37 °C in an incubator (HZP–250, China) with speed of 150 rpm for 
1 h, sodium hydroxide was added to convert the solution to intestinal 
fluid (pH = 7) with supplement of 0.035 g bile salts and 0.01 g pancreatin. The intestinal solution was then shaken at 37 °C, for 4 h 
(150 rpm), and centrifuged at 3000 rpm for 5 min to collect the super-
natant. After filtrated through a 0.45 μm PTFE filter (SCAA–113, 
China), 10 mL supernatant was extracted three times with 10 mL n-
hexane for 30 min in ultrasonic bath. The extracts were transferred to 
150 mL flask bottle through a funnel with anhydrous sodium sulfate, and 
concentrated using a rotary evaporator at 40°C to near dryness. The 
condensed residue was reconstituted with 2 mL n-hexane, and fil-
trated through 0.45 μm PTFE filter (ANPEL, China) into 2 mL amber 
vial for further analysis. The bioaccessibility was calculated by the 
following equation:

\[
\text{Bioaccessibility} = \left( \frac{\text{Extracted PAHs by PBET}}{\text{Total PAHs in dust}} \right) \times 100%
\]

PAHs analysis was conducted on GC–MS (Agilent Technologies 
7890A–5977 A) in Selective Ion Monitoring (SIM) mode. All the samples 
were separated on a 30 m × 250 μm × 0.25 μm Agilent HP–5 ms 
column with helium as carrier gas flowing at a rate of 1 mL/min. 
Operating conditions were as follows: the oven temperature was
programmed from 45 °C (2-min initiation) to 260 °C at 10 °C/min, then to 290 °C at 2 °C/min. Injector and transfer line temperatures were maintained at 300 °C and 250 °C.

2.4. Risk assessment

The daily uptake dose based on benzo(a)pyrene toxic equivalency quantity (TEQ) was calculated by the following equation:

\[
\text{Dose} = \frac{\text{Σ(C}_{\text{PAHs}} \times \text{TEF}) \times \text{IngR}}{\text{BW}}
\]

While dose based on TEQ considering bioaccessibility was calculated by the following equation,

\[
\text{Dose} = \frac{\text{Σ(C}_{\text{PAHs}} \times \text{bioaccessibility}\% \times \text{TEF}) \times \text{IngR}}{\text{BW}}
\]

Where \(\text{C}_{\text{PAHs}}\) represents the total concentration of PAHs in dust samples. \(\text{TEF} = \text{Toxic Equivalency Factor, which is used to express the potency of individual PAH in terms of benzo(a)pyrene (Table S1). IngR refers to the ingestion rate of indoor dust, which is 0.11 g/d for adults. BW refers to body weight (kg), which is 61.5 kg for adults [22].

2.5. Cell viability

Human normal liver cell line (HL7702), obtained from the Cell Bank of the Chinese Academy of Science, was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% Penicillin, and Streptomycin. Cells was incubated at 37 °C in a humidified atmosphere containing 5% CO₂. For further experiments, cells were seeded into 96-well plates at a concentration of \(1 \times 10^4\) cells per well and were exposed after 24 h attachment. To study the effect of particle size and dust category on the cell viability, the three types of dust samples (office, car, and PME) at size of < 43 μm, 43–63 μm, and 200–2000 μm was exposed to cells with concentration of 16, 48, 144, 432, 1296 μg/100 μL. The cell concentrations were selected based on our previous study to make sure that LC50 values can be derived [23]. For the size of < 43 μm and 200–2000 μm, they are the finest and most coarse fraction among all the size fractions, while the most abundant accumulation of PAHs was observed in dust samples with particle size of 43–63 μm, which may be more toxic as well. Therefore, the three sized particles (i.e., < 43 μm, 43–63 μm, and 200–2000 μm) were used for toxicity test. After 24 h exposure, cell viability was measured by CCK–8 viability assay kit.

2.6. QA/QC

For all the experiments, only glassware was used. Glassware was firstly washed by detergent and Milli-Q water. After being heated at 450 °C for 4 h, all the glassware was rinsed by acetone before use. Method blanks and procedural blanks were analyzed, and solvent blanks were also measured every 8 samples on GC-MS. The PAHs were not detectable in these blanks. To test the recovery of PAHs, samples were spiked with 16 PAH standard solution, and the recovery efficiencies, except relatively lower values for naphthalene, acenaphthylene, and acenaphthene due to their stronger volatility, were 65.8–91.8% and 77.4–126% for dust extraction and PBET digested supernatant extraction. The detailed recovery values for all the PAHs are listed in Table S2. The limits of detection (LOD) for PAHs were defined as three times background noise level (S/N ≥ 3) by running 7 solvent blanks. The LODs of 16 PAHs were 0.30 ± 0.036 μg/kg.

2.7. Statistical and data analysis

All experiments were carried out in triplicate. Statistical analyses were conducted using one-way ANOVA and Tukey’s post hoc test by PASW Statistics Version 18 (SPSS Inc., USA). Significant differences were set at p-value < 0.05. Correlations between surface area of dust particles and PAH concentrations were conducted using Origin 2016. To assess the effective concentration resulting in the median lethal concentrations (LC50), four-parameter concentration-response curves were fit to mean fold change data of cell viability using log-transformed contaminants concentrations via GraphPad Prism Version 6.

3. Results and discussion

3.1. Concentrations and profiles of PAHs in indoor dust

The concentrations of total PAHs, low molecular weight (LMW, 2–3 ringed), and high molecular weight (HMW, 4–6 rings) PAHs in different size fractions of all the three dust types are shown in Fig. 1. Total PAH concentrations varied from 10.6 to 42.2 μg/g, while LMW PAHs were from 2.22 to 10.6 μg/g, and HMW PAHs were from 7.08 to 35.1 μg/g. HMW PAHs made up the most of total PAHs (63.0% to 85.5%), which was comparable with that in dust samples from Hong Kong (84.1% on average) [17]. This may be due to their relatively lower volatility, which makes HMW PAHs more inclined to stay in solid phase and be absorbed by indoor dust. According to Brown and Soclo, LMW/HMW
ratio usually represents the origins of PAHs [24,25]. The LMW/HMW ratio lower than 1 (0.17–0.59) indicated that PAHs in dust samples in the current work mainly derived from incomplete combustion of fossil fuels [25].

Comparison among different dust types was conducted on the basis of PAH concentrations in all the 6 fractions (Fig. 1). Total PAHs in PME dust samples were significantly higher than those in dust samples collected from cars and offices (p < 0.01). For example, the PAH concentrations were three times in PME dusts as much as in the car and office dusts at size 200–2000 μm, while the car dusts were slightly higher than office dusts. The much higher PAH levels in PME dust may be attributed to the multiple sources of PAHs. As shown in Fig. 2, the ratio of Anthracene (Ant)/(Ant + Phenanthrene (Phe)) for all the size fractions of the three types of dust was > 0.1, indicating that PAHs came from the combustion sources, i.e., a mixture of biomass, coal and petroleum emissions [26]. The ratios of Benz(a)anthracene (BaA)/(BaA + Chrysene (Chr)) and Indeno(1,2,3-cd)pyrene (Ind)/ (Ind + Benzo(g,h,i)perylene (BghiP)) in car dust were 0.31–0.35 and 0.36–0.42, indicating that PAHs mainly came from the vehicular sources [1]. Ratios for office dusts were in 0.50 to 0.78 in BaA/ (BaA + Chr) and 0.72 to 0.78 in Ind/(Ind + BghiP), which revealed biomass combustion sources [5,27]. For PME dusts, the ratios of BaA/ (BaA + Chr) were 0.81–0.83, indicating releases from biomass and coal combustion, while the ratios of Ind/(Ind + BghiP) were 0.43–0.47, indicating traffic emissions [17,28]. In summary, there are more contamination sources for PAHs in PME dust (i.e., both combustion sources and traffic emission), partially explaining the higher levels of PAHs observed in PME dust. In addition, it should be more open to outdoor environment for PME than car and office, the deposition of outdoor air particle may also contribute to the elevated levels of PAHs in PME dust.

The PAHs distribution in six sized dust fractions in different microenvironment was shown in Fig. 1 with detailed concentrations listed in Tables S3–S5. PAHs concentrations fluctuated with the particle size and peaked at F4 (63–100 μm) in car dust, while concentrations reached a summit at the finest fraction (< 43 μm) in office and PME dusts. In order to find out the factor influencing PAH distribution, correlations between surface areas of dust particles and PAHs concentrations were conducted. For car and office dust, dust particle surface area was positively, even not significantly, correlated with total PAHs (R² = 0.64, p = 0.06, n = 6 and R² = 0.65, p = 0.05, n = 6), LMW PAHs (R² = 0.75, p = 0.03, n = 6 and R² = 0.77, p = 0.02, n = 6) and HMW PAHs (R² = 0.59, p = 0.07, n = 6 and R² = 0.60, p = 0.07, n = 6) (Fig. 3). The positive correlations between dust particle surface area and PAH concentrations are consistent with previous reports where distribution of PAHs and PAEs in dust was generally dependent on dust surface area [11,15]. The positive correlation here may partially suggest that PAHs were probably sorbed on the particle surfaces instead of being inside the particles [11]. For PME dust, a positive correlation between dust particle surface area and PAH concentrations was only observed for LMW PAHs (R² = 0.69, p = 0.04, n = 6), but not for total PAHs (R² = 0.16, p = 0.43, n = 6) and HMW PAHs (R² = 0.0, p = 0.95, n = 6). This may be due to the multiple origins of PAHs in PME dusts. For example, the PAHs may enter the indoor dust together with some particles instead of surface sorption, which may blur the correlation between surface area and distribution among different particle sizes.

3.2. PAHs bioaccessibility and human risk

Data in Table S6 suggested that PAHs showed diverse bioaccessibility among six fractions of the three types of dust. The bioaccessibility in fractions of 200–2000 μm was generally higher than those in other particle sizes, and no significant difference was observed among the other five size particles (p > 0.05). For example, the BaA bioaccessibility of 200–2000 μm in car dusts was 57.6%, while in other size particles was 20.1%–29.0%. This was different from the results in former study on PAEs, which reached the highest bioaccessibility at finest fractions of < 63 μm [15].

In order to assess the risk through ingestion of indoor dust, TEQ was used to calculate the daily uptake doses [29]. As shown in Table 2, the average daily doses based on TEQ were in the order of car (1.97 ng/kg/d) < office (3.05 ng/kg/d) < PME (9.22 ng/kg/d) with significant difference (p < 0.01). According to USEPA, the reference dose (RfD) of Bap was 300 ng/kg/day for oral exposure. The average daily uptake doses for different types of dust in the current study were much lower than the RfD for oral Bap exposure. The trend of daily uptake doses among different types of dust was consistent with the PAH levels in the three types of dust. For example, TEQ concentrations of PME dusts (mean = 5.16 μg/g) were much higher than car dusts (mean = 1.10 μg/g) and office dusts (mean = 1.70 μg/g) (Table 1). This also agreed with many previous studies that PAH-associated risk was mainly dependent on PAH levels in dust irrespective of dust type [30,31]. However, the trend was different if bioaccessibility was taken into consideration. With the adjustment of bioaccessibility, the average daily doses of TEQ were in the order of office (0.03 ng/kg/d) < car (0.08 ng/kg/d) < PME (0.15 ng/kg/d).

Similar to previous studies, more risk was expected for fine particles, since more PAHs would be attached in particles with smaller size if
only total PAH concentrations were used for calculation [32,33]. The highest dose among all the 6 fractions was at 63–100 μm (2.39 ± 0.10 ng/kg/d) for car dust, < 43 μm (4.72 ± 0.68 ng/kg/d) for office dust, and < 43 μm (15.0 ± 2.38 ng/kg/d) for PME dusts. However, more risk was observed for relatively larger particles when bioaccessibility was considered. Daily dose of bioaccessible TEQ reached a peak at 200–2000 μm fractions with 0.14 ± 0.03 and 0.04 ± 0.005 ng/kg/day for car and office, and in 150–200 μm fractions with 0.21 ± 0.03 ng/kg/day for PME dusts (Table 2). Our results were consistent to previous study [34] that bioaccessible daily dose fluctuated and peaked at relative larger particle size due to the higher bioaccessibility observed for coarse particles. The observation here indicated that dust particle size can affect the bioaccessibility, which made the assessment more complicated. It would be more precise to adopt bioaccessibility concept for human risk assessment.

### 3.3. HL-7702 cytotoxicity of different particle sized dust fractions

Human liver is an important organ for metabolizing and detoxifying organic contaminants, and liver cancer cell, Hep-G2, is the most commonly used model for cytotoxicity study. However, Hep-G2 cell line has weaker capability to metabolize the compounds and lower antitumor activities if compared with human normal liver cell line HL-7702 [20].

In this study, the normal human liver cell, HL-7702 cell line, was therefore used for cytotoxicity test. The cell viability curves for exposure to organic extracts from six particle size fractions of the three types of dust are depicted in Fig. 4 with lethal concentration 50 (LC50) values being listed in Table S7. Generally, the dose-response curves showed an increase for cell mortality with the concentration of dust extracts. Among all of the particle sizes, the highest toxicity was observed generally in fine particle fractions, i.e., < 43 μm fraction for office and car dust extracts with LC50 values of 1082 and 988 μg/100 μL. While 150–200 μm fraction for PME dust extracts has the lowest LC50 value of 1091 μg/100 μL (Table S7). The results here were consistent with previous study that the LC50 of fine dust particle (i.e., < 63 and 63–100 μm) were significantly lower (p < 0.05) than coarse particles (i.e., 100–280 and 280–2000 μm), indicating the decreased toxicity with the increase of particle size [15].

The LC50 values here represented cytotoxicity induced by organic extracts of indoor dust fractions, and the organic extracts were mixture of organic contaminants in indoor dust. It therefore can be hypothesized that the different cytotoxicity among different size fractions may be related with the distribution of contaminants. Though Kang et al observed a moderate negative correlation between the total PAH concentrations and LC50 for both Hep-G2 (r = −0.44, p < 0.01) and human skin keratinocyte cell lines (KERTr) (r = −0.51, p < 0.01) [17], there was no correlation between total PAHs and LC50 for HL-7702 in the current study (Fig. 5A). Since there were many other pollutants, besides PAHs, in the dusts organic extracts, the cytotoxicity may be the synergetic or antagonistic effect from all the different types of pollutants. Therefore, several other types of pollutants were taken into the correlation, including organophosphate flame retardants, novel brominated flame retardants, PBDEs, and PAEs. Flame retardants (FRs) concentrations at the 6 size fractions can be found in our previous work [16]. Concentrations of PAEs in 6 fractions were also measured and are listed as unpublished data in Tables S3–5. In addition, the total concentrations of all the compounds mentioned above are also listed in Fig. S1. As shown in Fig. S5B, a significantly negative correlation (R2 = 0.70, p < 0.0001, n = 18) was observed between LC50 and total concentrations of all the compounds mentioned above (i.e., FRs, PAEs, and PAHs). In other words, the FRs, PAHs and PAEs in indoor dust

### Table 1

<table>
<thead>
<tr>
<th>Particle size (μm)</th>
<th>TEQ (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>car</td>
</tr>
<tr>
<td>200–2000</td>
<td>0.61</td>
</tr>
<tr>
<td>150–200</td>
<td>1.09</td>
</tr>
<tr>
<td>100–150</td>
<td>1.16</td>
</tr>
<tr>
<td>63–100</td>
<td>1.34</td>
</tr>
<tr>
<td>43–63</td>
<td>1.17</td>
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<td>&lt; 43</td>
<td>1.24</td>
</tr>
<tr>
<td>mean</td>
<td>1.10</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Particle size (μm)</th>
<th>Daily dose based on TEQ (mg/kg/d)</th>
<th>Daily dose based on bioaccessible TEQ (ng/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>car</td>
<td>office</td>
</tr>
<tr>
<td>200–2000</td>
<td>1.09 ± 0.24</td>
<td>1.19 ± 0.28</td>
</tr>
<tr>
<td>150–200</td>
<td>1.95 ± 0.23</td>
<td>2.26 ± 0.25</td>
</tr>
<tr>
<td>100–150</td>
<td>2.07 ± 0.07</td>
<td>3.65 ± 0.21</td>
</tr>
<tr>
<td>63–100</td>
<td>2.39 ± 0.10</td>
<td>3.71 ± 0.71</td>
</tr>
<tr>
<td>43–63</td>
<td>2.09 ± 0.17</td>
<td>2.75 ± 0.23</td>
</tr>
<tr>
<td>&lt; 43</td>
<td>2.21 ± 0.21</td>
<td>4.72 ± 0.68</td>
</tr>
<tr>
<td>mean</td>
<td>1.97 ± 0.17</td>
<td>3.05 ± 0.39</td>
</tr>
</tbody>
</table>

Fig. 3. Correlations between particle surface areas and PAH concentrations in (A) car, (B) office and (C) PME dusts.
contributed to majority cytotoxicity (70%) observed here. Many emerging organic contaminants (such as FRs, PAEs, or chemicals used in personal care products) have been added into daily used products in physical other than chemical ways. These contaminants therefore can be released from these products into indoor dust [35]. Indoor dust is composed of a large amount of organic material (e.g., food particles, exfoliated skin cells, hair, and small organisms) [36]. The relatively more organic materials can make indoor dust as a major sink for many organic contaminants from both internal (FRs and PAEs from consumer goods) and outside sources (e.g., PAHs in suspended particles). Based on our result that 70% of toxicity can be explained by PAHs, FRs, and PAEs in dust, higher toxicity might be expected for indoor dust since many other types of organic contaminants can also be detected in indoor dust [37]. Ingestion of dust is an important exposure pathway, especially for children with higher hand-mouth frequency. For example, daily dust mass of ingestion was 100 mg for adults and 200 mg for children [38]. Therefore, frequent house clean and hand wash would be beneficial to reduce the dust attachment to hands, which can reduce the risk associated with dust ingestion.

4. Conclusion

In the present study, we investigated the PAH distribution, bioaccessibility and cytotoxicity among different size fractions of three indoor dust. Different from most previous studies, bioaccessibility of PAHs in dust samples was measured and incorporated into risk assessment in the current study. The results here suggested that contaminant bioaccessibility can be potentially applied in exposure refinement, which is complimentary to those assessments based on total concentrations. In addition, our results indicated that selection of dust size can result in variations in exposure risk assessment due to the different distribution and bioaccessibility of PAHs in various sizes fractions. This complication due to the dust particle size and bioaccessibility suggested that dust sampling process should be standardized to avoid the large variation in dust particle size, and bioaccessibility should be measured when conducting exposure assessment.

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Appendix A. Supplementary data

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