In vitro inhalation bioaccessibility for particle-bound hydrophobic organic chemicals: Method development, effects of particle size and hydrophobicity, and risk assessment

Shan-Yi Xie, Jia-Yong Lao, Chen-Chou Wu, Lian-Jun Bao⁎, Eddy Y. Zeng

School of Environment and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 511443, China

ABSTRACT

Bioaccessibility of particle-bound hydrophobic organic contaminants and related particle size effects are significant for assessing the potential human health risk via inhalation exposure, but have not been clearly evaluated. To fill this knowledge gap, the present study developed an in vitro method to estimate the inhalation bioaccessibility of particulate polycyclic aromatic hydrocarbons (PAHs) using simulated human lung fluids, i.e., a modified Gamble’s solution (MGS) and artificial lysosomal fluid (ALF) with Tenax as the adsorption media. Assay parameters, namely incubation time (10 d) and influence of filter use, were optimized for establishing the in vitro method. The results showed that the bioaccessibility of PAHs increased with increasing particle size, but other factors, such as total organic carbon and chemical hydrophobicity, also played a large role in the fate of these compounds. The results from this portion of the present study were then used to evaluate human health risk, which showed that the risk of these particle-bound PAHs by incorporating size-dependent PAHs bioaccessibility and deposition efficiency in the human respiratory tract into inhalation exposure risk calculations was reduced by >90% when compared to using total concentration. This suggested that the inhalation bioaccessibility and deposition efficiency of hydrophobic organic chemicals should be included in human health risk assessment.

1. Introduction

Air pollution has become one of the most important topics of research and governance in China over two decades (Beelen et al., 2014). Epidemiological studies have consistently shown that atmospheric particulate pollution increases the morbidity and mortality of exposed individuals due to respiratory diseases, e.g., asthma, chronic obstructive pulmonary disease, and lung cancer (Balakrishnan et al., 2014; Beelen et al., 2014; Beelen et al., 2005; Zhang et al., 2017). Atmospheric particulate matter is a complex matrix of a variety of inorganic and organic compounds such as heavy metals and potentially a suite of organic contaminants (Choi et al., 2017; Kioumourtzoglou et al., 2015; Martins et al., 2016). Thus, in addition to the harmful effects of particulate matter itself, the harmful effects of contaminants absorbed on particulate matter cannot be overlooked.

However, not all contaminants can be completely released from particulate matter and absorbed into the bloodstream, as only a fraction of contaminants can release into interstitial fluids and an even smaller amount can pass through the cell membrane. Those that do, however, can accumulate in human organs and reach the body’s circulatory system, resulting in risk to human health (Collins et al., 2015; Semple et al., 2004). The fractions which can dissolve in human interstitial fluids are defined as being bioaccessible, while those that can cross the cell membrane to enter into the capillaries and reach blood circulation are commonly referred to as being bioavailable (Collins et al., 2015; Semple et al., 2004). As assessments solely on total concentrations of toxic chemicals would greatly overestimate risk, researchers have gradually converted from using total concentration to using the bioaccessible and/or bioavailable fractions in risk assessments (Ruby and Lowney, 2012; Shen et al., 2016). The bioavailable fraction can be determined by evaluating the contents of toxic chemicals inside human blood or by exposures with animals (e.g., mice, pigs, or monkeys), wherein concentrations can be evaluated in a variety of tissues (including blood, organs, etc.) (Cui et al., 2016; Kastury et al., 2017). Unfortunately, the use of human or animals as test subjects has limitations as sample collection can be difficult, expensive, and constrained by ethical concerns (Beriro et al., 2016). Thus, many researchers have chosen to evaluate the bioaccessible fraction, which can be obtained...
through an in vitro simulation method rather than using the bioavailable fraction obtained through the more difficult in vivo method (Collins et al., 2015; Zhang et al., 2016).

To date, the in vitro methods for evaluating the inhalation bioaccessibility of heavy metals and hydrophobic organic chemicals (HOCs) varied with assay parameters such as simulated lung fluids and incubation times. Most researchers have adopted simulated lung fluids (such as Gamble solution (GS) and artificial lysosomal fluid (ALF)) (Li et al., 2015; Wiseman and Zereini, 2014; Witt et al., 2014; Zereini et al., 2012). The GS simulates extracellular interstitial fluid within the deep lung (at a pH of 7.4–7.6), while the ALF mimics the intracellular fluid of alveolar and macrophages after phagocytosis of particulate matter (at a pH of 4.5–5.0) (Pelfrène et al., 2017; Wiseman, 2015). Although some researchers have used unmodified GS, other researchers have also started modifying the GS to mimic the pulmonary environment more closely (Boisa et al., 2014; Julien et al., 2011). Previous studies found that the epithelial type II cells secrete lung surfactant, which is highly enriched with dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), into the alveolar space to lower the surface tension at the air-liquid interface (Bernhard, 2016; Veldhuizen and Haagsman, 2000). Thereby, one commonly used modification is the addition of DPPC. This surfactant has been shown to significantly increase the bioaccessibility of Pb by 5.6% to 18% (Li et al., 2016), and its use is considered to be more biologically relevant (and as such was used in the present study as well).

However, the simulated lung fluids such as GS or ALF have largely been used to assess the bioaccessibility of heavy metals on particulate pollutants (Dartry et al., 2014; Wiseman, 2015), with little information regarding inhalation bioaccessibility of organic pollutants (Wei et al., 2018). A recent review (Wei et al., 2018) found only five articles on measuring the bioaccessible fractions of HOCs using in vitro methods. Different artificial lung fluids (water, phospholipid vesicles, 1-octanol, and saline containing DPPC) (Bevan and Yonda, 1985; Born et al., 2005; Gerde et al., 2001) were used and these in vitro methods were developed without carefully examining the effects of bioassay parameters, such as incubation time and temperature. Although Kademoglou et al. (2018) recently applied the GS and ALF to evaluate the inhalation bioaccessibility of phthalate esters and alternative plasticizers in indoor dusts, they also pointed out the necessity to develop a unified and biologically relevant in vitro method for inhalation bioaccessibility. In addition, particulate matter ranges over four orders of magnitude in diameter, from nanometers to microns. The effects of particle size and chemical hydrophobicity on inhalation bioaccessibility of HOCs remain limited, even unknown. Therefore, the need for research in this area is highly warranted.

The objectives of the present study were to (1) develop an in vitro method using sediment particles in modified GS or ALF, optimizing for incubation time and evaluating the effects of filter use; (2) examine the effects of particle size and hydrophobicity on inhalation bioaccessibility of particle-bound polycyclic aromatic hydrocarbons (PAHs); and (3) assess the associated health risks based on total concentration, deposition efficiency, and a combination of deposition efficiency and inhalation bioaccessible fraction of PAHs with e-waste burning particles as a case study.

2. Materials and methods

2.1. Materials and method optimization – sample preparation

Twenty-five individual PAHs (including BP, 2,6-DNAP, AC, ACE, 2,3,5-TNAP, FL, PHE, ANT, 2-MPHE, 1-MPHE, 2,6-DMPHE, FLU, PYR, 11-BBF, BaA, CHR, B(b)F, B(k)F, BeP, BaP, PER, 9,10-DPHE, IcdP, Daha, and BghiP) were selected as target compounds. The physical-chemical properties and full names of individual PAHs, and detailed purity of standards and reagents are presented in Table S1 and Text S1 of Supplementary data; “S” designates text, tables, and figures in the Supplementary data afterwards.

Before e-waste burning particle samples could be evaluated, the in vitro method needed to be optimized for both fluids being used (i.e., to optimize incubation time and to determine if filter use would affect bioaccessibility) by using spiked sediment particles. Sediment samples were collected from Dongjiang River in Dongguan, Guangdong Province, China with a stainless steel grab. The sediment was dried at 60 °C in an oven, ground with a mortar and pestle, and sieved through 187 μm sieve to obtain sediment particle samples. Sediment particles (aerodynamic diameter: 5.9 ± 5.0 μm; Zeiss Microscopy) were then spiked with a mixture of the target PAHs in 5 mL dichloromethane for 20 mg of particles to achieve individual PAH concentrations of 10 μg g⁻¹. Spiked sediment samples were aged for one month before use. Prior to each incubation, the concentrations of spiked sediment PAHs were confirmed analytically. In short, 20 mg of the spiked sediment was placed into a Teflon tube, spiked with the surrogate standards (0.1 μg) in hexane, and sonicated three times (30 min each) with a mixed solvent of hexane, dichloromethane, and acetone (2:2:1 in volume). These three extracts were combined, solvent-exchanged to hexane, and concentrated to 1 mL under a gentle stream of N₂. The extract was purified on a glass column packed with neutral silica gel (12 cm) and anhydrous sodium sulfate granular (1 cm) from bottom to top, and again concentrated to 0.1 mL under N₂. All samples were spiked with the internal standards (0.1 μg) before instrumental analysis.

Although the sediment particles were not completely representative of inhaled particles, they were comparable surrogates for developing the in vitro method as the effects of assay parameters on the releases of HOCs from sediment and inhaled particles into lung fluids were deemed similar.

2.2. Method optimization – bioaccessibility experiments

Bioaccessibility assays were undertaken to evaluate incubation time and filter use with both artificial lung fluids (ALF and GS). Only the GS was modified (referred to as MGS afterwards) by adding 100 mg of DPPC into every 1000 mL of the original GS. The final chemical compositions of ALF (pH = 4.5) without bio-surfactant and MGS (pH = 7.4) are presented in Table S2. Prior to experiments, Tenax (1 g) was wrapped with two layers of metallic sieve (mesh size of 13 μm) and stainless steel wire to produce a small ‘stick-like’ structure (Fig. S1) wherein both ends were blocked with a Teflon plug. The small ‘stick-like’ structure looks like a column with the radius of 1 cm and length of 10 cm. This Tenax, 200 mL of either ALF or MGS, and 20 mg of spiked particulate sample were mixed in a glass bottle, incubated at 37 °C, and shaken at 150 rpm for 0.5, 1, 2, 3, 7, 10, and 14 d. Although the total human lung fluid volume is around 20–60 mL, the solid/liquid ratio (1/10000; g/mL) was taken account into the mass of particle matter deposited in the lung and volume of lung liquid (Kastury et al., 2017). Another mixture was prepared using the same amounts and procedure to evaluate the role of the 47 mm diameter glass microfiber filter (Whatman International, Maidstone, England). The filter was added at the same time as the Tenax, and the mixture was allowed to incubate for 10 d. Additionally, there was one more mixture without Tenax and glass microfiber filter to incubate for 3 d. At each time point, Tenax was carefully removed, rinsed thoroughly with deionized water, and placed in a Teflon tube. All rinsing waters were also collected and combined with leftover simulated lung fluid solution (referred to as ‘simulated lung fluid’ solution moving forward) and filtered through a mixing fibro membrane (0.2 μm) to separate particles from the solution. This solution and the particles obtained from filtering were kept for future chemical analysis. Five replicates were performed during each bioaccessibility assay.

All samples were spiked with the surrogate standards before extraction. ‘Simulated lung fluid’ solution (approximately 230 mL) was liquid-liquid extracted three times, each with 40 mL of dichloromethane in a 1000-mL separatory funnel. The Tenax insert was
sonicated three times (30 min each) with 25 mL of a mixture of hexane and acetone (1:1 in volume). Particulate matter was sonicated three times, each with 15 mL of a mixture of hexane, dichloromethane, and acetone (2:2:1 in volume). All extracts were dried with anhydrous sodium sulfate, concentrated (using N2), solvent-exchanged to hexane, and concentrated again to 1 mL. Each of these was purified on a glass column packed with neutral silica gel (5 cm for ‘simulated lung fluid’ solution and 12 cm for Tenax and particulate residues) and anhydrous sodium sulfate (1 cm for all samples) from bottom to top. Samples were concentrated to 0.1 mL under N2, and finally spiked with internal standards (50 ng for ‘simulated lung fluid’ samples and 100 ng for particulate and Tenax samples) before instrumental analysis. The procedures and parameters of instrumental analysis are detailed in Text S1.

2.3. Effects of particle size experiments

To represent open-burned e-waste, a TV casing (front) was collected from an e-waste collection factory in Foshan, Guangdong Province, China. The TV casing was crushed and sieved to yield fractions between 840 µm to 2000 µm. A 2-g sample of this crushed matter was burned with a spray gun for 5 min within a stainless steel rectangular box (0.5 × 0.5 × 1.5 m). The air being introduced into the box (during collection of particles) was cleaned with a glass microfiber filter and a polyurethane foam. Size-fractionated airborne particle samples were collected with a Micro-Orifice Uniform Deposit Impactor (M110-R, MSP Corporation, Shoreview, MN, USA) during a 3-h sampling period. All particulate samples were collected on 47 mm diameter glass microfiber filters at a flow rate of 30 L min⁻¹, and separated initially into 11 size fractions (from 0.056 to larger than 18.0 µm). However, as some fractions contained non-detectable target analytes (Fig. S2), the number of fractions evaluated was reduced to six (0.056–0.18, 0.18–0.32, 0.32–0.56, 0.56–1.0, 1.0–1.8, and 1.8–5.6 µm), each of which was extracted with the optimized procedures discussed above. Three replicates were conducted for open-burned e-waste. All airborne particle samples were incubated in ALF or MGS with Tenax for 10 d. All airborne particle samples were incubated in ALF or MGS with Tenax for 10 d. In addition, total organic carbon (TOC) in each airborne e-waste particle sample was measured with an elemental analyzer (Vario EL III Elementar, Element, Germany), which are detailed in Text S1.

2.4. Quality assurance and quality control

One procedural blank with solvents only or glass microfiber filter without particles was analyzed with every 10 samples and concentrations of detected target analytes had blank samples concentrations deducted. The recoveries of the surrogate standards, i.e., naphthalene-d12,acenaphthylene-d10,phenanthrene-d10, chrysene-d12, perylene-d12, and benzo[ghi]perylene-d12, were 71 ± 13%, 76 ± 14%, 89 ± 17%, 92 ± 17%, 82 ± 20%, and 85 ± 17% in extracted liquid fractions, 69 ± 11%, 73 ± 18%, 71 ± 16%, 82 ± 20%, 82 ± 23, and 83 ± 21% in extracted Tenax samples, 64 ± 9%, 69 ± 11%, 73 ± 10%, 87 ± 14%, 82 ± 17%, and 80 ± 15% in particle samples after incubation, and 73 ± 15%, 80 ± 15%, 81 ± 14%, 99 ± 17%, 101 ± 21, and 103 ± 20% in particle samples. The sum of the masses of PAHs in Tenax, artificial lung fluid, and residual particulates was 99 ± 24% of the initial mass. The lowest calibration concentration divided by the actual sample volume/weight was defined as the reporting limit for a target compound, which was 0.09 ng m⁻³ for each of PAHs evaluated for the e-waste burning particle samples.

2.5. Data analysis

Inhalation bioaccessibility fraction (IBAF; %) was the chosen end-point in the present study and is defined as:

\[
\text{IBAF} = \frac{M_T + TA}{M_T + TA + M_R} \times 100\%
\]  

where \(M_T\) refers to the mass of the target compound released into the simulated lung fluid; \(M_R\) is the residual fraction in the e-waste airborne particle or spiked sediment particle of the target compound after incubation; and \(TA\) is the mass of the target compound absorbed onto the Tenax. The calculated IBAF was used to evaluate the potential inhalation health risk from open e-waste burning, with the use of RiskO, RiskDE, and RiskDE+BA, which are defined as the inhalation health risk based on the total concentration of contaminant, the deposited fraction in the lung, and the deposited fraction that can dissolve into lung fluid (or bioaccessible fraction), respectively. As such, the overall concentration and therefore the risk are refined with each step, with RiskDE+BA providing the most accurate assessment. It should be noted that the relative potential inhalation health risks with different estimated methods were assessed and have not been compared with the actual exposure risk. The health risk assessed in the present study is calculated based on concentrations of PAHs in the e-waste airborne particles (Table S3), with the procedures detailed in Text S2.
Fig. 1. Inhalation bioaccessibility fraction (IBAF; %) for PAHs during incubation with Tenax and those without in both artificial lysosomal fluid (ALF; a) and modified Gamble solution (MGS; b).

Fig. 2. Inhalation bioaccessibility fraction (IBAF) of a subset of PAHs analyzed after various incubation times in artificial lysosomal fluid (ALF; a–d) and in modified Gamble solution (MGS; e–h). Figs. (a) and (e) are 2-ring PAHs, (b) and (f) are 3-ring PAHs, (c) and (g) are 4-ring PAHs, and (d) and (h) are 5-ring PAHs. Additional results can be found in Figs. S3 and S4.
S3–S4). For instance, 2-ring PAHs approached equilibrium within 3 d in ALF, while 3-ring and 4 to higher-ring PAHs required 7 d and between 7 and 10 d, respectively. These same target PAHs, especially those with fewer rings, required more time to reach equilibrium in MGS, as 2-ring PAHs approached equilibrium within 7 d, while 3-ring and 4 to higher-ring PAHs required 10 d. However, no significant differences between IBAFs of individual PAHs (for each individual fluid) were noted between 10 and 14 d (independent-samples t-test, p > 0.05). As a result, a 10-d incubation time was chosen for all future work.

Another hindrance with the development of an in vitro method was the need of filter materials for the collection of air particles. In reality, particles enter the human respiratory system and make contact directly with pulmonary fluids, but in the routine experiments particles would first need to be loaded onto a glass microfiber membrane (via air samplers). During the sampling process some particles were more deeply embedded than those in the glass microfiber membranes, and as such not all particles could be entirely removed from the membrane material. Therefore it was important to evaluate the use of these glass microfiber filters on IBAF before a reliable in vitro method could be established.

The results of the 10-d incubation experiment using both simulated lung fluids were also used to evaluate the presence and absence of glass fiber filters. This portion of the present study revealed no significant differences of IBAFs of the target analytes (with the exception of 2,3,5-TNAP in MGS; Fig. 3) between the presence and absence of filters. These results suggested that the use of glass microfiber filters to collect field samples would not affect the outcome of the in vitro method.

Outside of validating the incubation time as well as the use of filters, it was also important to maintain mass balance in the system. The absolute recovery of 10-d incubated samples, which was the sum of the PAH masses in the Tenax, artificial lung fluid, and residual particle divided by the initial mass, ranged from 92% to 112% and 75% to 99% for ALF and MGS, respectively (Table S4). With mass balance achieved in the test system and incubation time and filter use optimized, e-waste burning particle samples could be analyzed with confidence.

3.2. Bioaccessibility of PAHs in e-waste particles in ALF and MGS lung solutions

The IBAF values were quite different between the two lung solutions, and greater significantly in ALF for 4-ring PAHs (AVOVA, p < 0.05) (which was similar to the sediment particle results). For instance, IBAF values of 4-ring PAHs were all above 60% (regardless of particle size) in ALF, but were in most circumstances below 50% in MGS (Table S1). However, Kademoglou et al. (2018) found that there were no statistically significant differences for IBAF values of phthalates and alternative plasticizers (except dimethyl phthalate) in the two simulated lung fluids (ALF and GS without DPPC). In addition, the apparent bioaccessibility of most PAHs during the small intestine stage increased with increasing pH values due to the reduction in the aggregation number of dihydroxy bile acid micelles (Zhang et al., 2015). In the present study, MGS-containing DPPC is neutral (pH = 7), while ALF is more acidic (pH = 4). The DPPC contains a hydrophilic side with a negative charge on the phosphate group and a positive charge on the choline group. The differences on IBAF values of PAHs between the two simulation fluids may be attributed to the formation of DPPC micelle concentrations in MGS, which may affect the bioaccessibility of low hydrophobicity PAHs and needs to be further investigated. These results suggest that different lung solutions may behave differently and result in different estimates of risk. The results from the present study also indicate that PAHs may uptake more in the intracellular lung environment (as represented by ALF), rather than via the extracellular matrix (as represented by MGS).

Although the IBAFs were higher in ALF than in MGS, many trends were similar between the two fluids. The first trend is that in both solutions, only a subset of PAHs were detected at high concentrations for accurate quantification. Concentrations of 2- and 3-ring PAHs were too low to accurately quantify IBAF and as such were not evaluated further in this portion of the present study. The reason for this was most likely due to the tendency of lower-ring PAHs to partition more easily to the gas phase than to the particulate phase (Zhang et al., 2012). Another interesting finding was that PER and IcdP were not detected in MGS, but were detected in ALF. It may be due to the smaller amount of particles used in MGS than ALF (Table 1) and requires further investigation.

3.3. Role of hydrophobicity

Another trend that was similar for both solutions was that IBAFs were higher for lower-ring PAHs (i.e., 4-ring > 5-ring > 6-ring) (independent-samples t-test, p < 0.05; Table 1). This trend was also apparent in the spiked sediment particles. As the lower-ring PAHs are more hydrophilic than the higher-ring ones, this result was not surprising, as the ability of chemicals to transit from particles to interstitial fluid is highly dependent on hydrophobicity (Collins et al., 2015; Fang and Stapleton, 2014; McLachlan, 1994). The PAHs in the present study have a wide range of log KOW values (from 4.02 to 6.70), and their IBAF values, following a sigmoidal distribution, decreased with increasing hydrophobicity (or higher log KOW values) for e-waste burning particles (Figs. 4 and 5).

The coefficients of determination (R²) for the IBAF and log KOW correlations ranged from 0.50 to 0.93 in ALF and 0.46 to 0.83 in MGS. These R² values are quite high, especially considering that these correlations include all differing size fractions, showing the importance of hydrophobicity in assessing inhalation health risk.

These same trends have been reported by other researchers as well, e.g., Fang and Stapleton (2014) showed similar results in flame

![Fig. 3. Comparing inhalation bioaccessibility fraction (IBAF; %) for PAHs on sediment particles processed during incubation with and without a glass fiber filter in both artificial lysosomal fluid (ALF; a) and modified Gamble solution (MGS; b). The symbol ** represents the significant level (p < 0.05) between groups.](image-url)
tract, but act differently in the organism and are both highly dependent on the properties of the contaminant. The organic film is the dominant ‘resister’ for adsorption when $K_{OW}$ is small and behaves independently of $log K_{OW}$. However, the water film becomes the primary ‘resister’ of adsorption when log $K_{OW}$ is high, and this film is highly depend on the log $K_{OW}$ of the compound. Thus in theory, the diffusive transport of chemicals with high log $K_{OW}$ values (such as a large number of PAHs in the present study) through the water barrier is quite low, resulting in low IBAFs for these compounds. The results from the present study also suggest that this two-way resistance model may occur in the lungs as well.

Table 1

Inhalation bioaccessibility fraction (average ± standard deviation; %) of polycyclic aromatic hydrocarbons in burned e-waste samples as a function of particle diameter (µm) in artificial lysosomal fluid (ALF) and modified Gamble solution (MGS). In the ALF and MGS solution, 13 and 11 PAHs, respectively, were detected out of 25 evaluated.

<table>
<thead>
<tr>
<th>Fluid solution</th>
<th>Cyclic rings</th>
<th>PAH name</th>
<th>Size fraction (µm)</th>
<th>0.056–0.18</th>
<th>0.18–0.32</th>
<th>0.32–0.56</th>
<th>0.56–1.0</th>
<th>1.0–1.8</th>
<th>1.8–5.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% TOC</td>
<td>Particle mass (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>35</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>4 FLU</td>
<td></td>
<td></td>
<td>93.0 ± 14a</td>
<td>1.47</td>
<td>31</td>
<td>35</td>
<td>38</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>11-BbF</td>
<td></td>
<td></td>
<td>89.6 ± 3.8a</td>
<td>1.85</td>
<td>35</td>
<td>38</td>
<td>30</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>BaA</td>
<td></td>
<td></td>
<td>94.8 ± 2.7a</td>
<td>3.89</td>
<td>38</td>
<td>30</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>CHR</td>
<td></td>
<td></td>
<td>92.4 ± 0.5a</td>
<td>2.89</td>
<td>30</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>MGS</td>
<td></td>
<td></td>
<td>1.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 FLU</td>
<td></td>
<td></td>
<td>54.7 ± 8.8a</td>
<td>0.77</td>
<td>31</td>
<td>35</td>
<td>38</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>11-BbF</td>
<td></td>
<td></td>
<td>62.1 ± 6.0ab</td>
<td>1.11</td>
<td>35</td>
<td>38</td>
<td>30</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>BaA</td>
<td></td>
<td></td>
<td>63.1 ± 13.0ab</td>
<td>1.24</td>
<td>38</td>
<td>30</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>CHR</td>
<td></td>
<td></td>
<td>66.9 ± 8.8ab</td>
<td>0.89</td>
<td>30</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>MGS</td>
<td></td>
<td></td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different superscript letters indicate the significant differences between groups ($p < 0.05$).

Fig. 4. Relationship between inhalation bioaccessibility fraction of PAHs present on various sizes of e-waste burning particle matter and their associated log $K_{OW}$ values in artificial lysosomal fluid (ALF). Dp is the aerodynamic diameter of particle.

retardants found in dust samples in the digestive system, as bioaccessibility decreased in a sigmoidal distribution with increasing hydrophobicity. Kademoglu et al. (2018) found that the IBAF of phthalate esters and alternative plasticisers in indoor dusts and SRM 2585 decreased with increasing hydrophobicity. To explain this relationship, McLachlan (1994) proposed a two-way resistance model, i.e., two films, a water and an organic film, control the absorption in the digestive tract, but act differently in the organism and are both highly dependent on the properties of the contaminant. The organic film is the dominant ‘resister’ for adsorption when $K_{OW}$ is small and behaves independently of log $K_{OW}$. However, the water film becomes the primary ‘resister’ of adsorption when log $K_{OW}$ is high, and this film is highly depend on the log $K_{OW}$ of the compound. Thus in theory, the diffusive transport of chemicals with high log $K_{OW}$ values (such as a large number of PAHs in the present study) through the water barrier is quite low, resulting in low IBAFs for these compounds. The results from the present study also suggest that this two-way resistance model may occur in the lungs as well.

![Fig. 4. Relationship between inhalation bioaccessibility fraction of PAHs present on various sizes of e-waste burning particle matter and their associated log $K_{OW}$ values in artificial lysosomal fluid (ALF). Dp is the aerodynamic diameter of particle.](image)
Dieds have shown that ALF and MGS, respectively. These results were expected as many substances with particle size exerting little influence on bioaccessibility. Outside of this one exception, IBAFs were consistently higher in large size fractions, up to 9.1 and 8.7 folds from the smallest to the largest fraction for ALF and MGS, respectively. These results were expected as many studies have shown that finer particles contain greater surface areas allowing for stronger sorption of chemicals onto organic matter (Cornelissen et al., 1997; Mehler et al., 2011; Sun et al., 2008).

Interestingly, previous studies using more traditional fluids (deionized water, acetate buffer, and nitric acid) have shown that particle size effects may be contaminant class specific, as some metals seem to behave differently than PAHs in the present study. For instance, Birmili et al. (2006) found that some heavy metals (Pb, Co, and Cd) in air particulate matter, using water as the exposure media, have high solubility in the fine particle size range, whereas other metals (Zn and Ba) showed the opposite trend. They speculated that finer particles containing Pb, Co, and Cd originated from high-temperature combustion sources which emit a higher fraction of water-soluble particles than coarse particles resulting in a more soluble ion phase in this fraction. Another study evaluated the association of the properties of Cu with different size fractions of airborne particulate matter in two exposure media, acetate buffer and nitric acid. Copper bioaccessibility was different dependent on which solution was used, i.e., increased bioaccessibility was noted with increasing particle size for nitric acid, but no correlation was found between bioaccessibility and particle size for acetate buffer (Canepari et al., 2010). Although the results of the previous studies may differ from those of the present study, the mechanism on the influence of particle size on the inhalation bioaccessibility of chemicals must be carefully considered.

3.5. Role of total organic carbon

To date, no studies have been conducted to assess the effects of particle size on HOCs in regards to inhalation risk, but the effects of particle size on bioaccessibility in sediments have been evaluated. In most cases, these studies have obtained similar findings as in the present study that smaller size fractions result in lower bioaccessibility (Cornelissen et al., 1999; Sun et al., 2008). However, a subset of previous studies have shown the opposite trend, i.e., larger-sized sediment particles with higher TOC possess lower bioaccessibility than finer particles with lower TOC (Li et al., 1996; Wu et al., 2016). This trend is believed to be driven by TOC rather than particle size (Amweg et al., 2006). These results are significant in demonstrating the importance of chemical hydrophobicity and particle size, as well as TOC, in influencing the bioaccessibility of chemicals.

In general, higher bioaccessibility was found in lower TOC sediments (Amweg et al., 2006). The ramifications of different TOCs for differing particles, however, were not observed in the present study as each size fraction of particles contained almost the same TOC (Table 1). The similarities of TOC (31% to 38% in ALF and 33% to 48% in MGS) were attributed to the fact that these samples are from the same burning source. Although this was a drawback of the present study as it did not allow for adequate representation of e-waste particulates, it was also a strength as TOC did not affect the results, thereby allowing for a more accurate investigation of the relationship between particle size and IBAF.

It should be noted, however, that particles from burning e-waste contained quite high levels of TOC than particles from other systems. For instance, TOC contents in soil particles (Li et al., 1996) and air particles (Zhang et al., 2009) are below 5%, which is an order of magnitude lower than what was observed in the present study (up to 50%). Although the role of TOC in IBAF determination and ultimately risk characterization was not the focus of this study, the information provided here shows that further work on this topic is warranted and would be a of extreme importance when assessing inhalation health risk of HOCs associated with airborne particulate matter.

3.6. Inhalation health risk assessment based on bioaccessibility

The combined health risks for all PAHs and all size fractions (Table 2) illustrate the overall importance of evaluating these additional parameters, i.e., IBAF and deposition fraction in the lung. E-waste burning particle inhalation health risk based on the total concentration (Risk0) for an adult was $2.56 \times 10^{-5}$ and $6.2 \times 10^{-5}$, respectively, without the incubation in ALF and MGS. This risk decreases dramatically when deposition efficiency (RiskDE) is also considered ($3.06 \times 10^{-5}$ for ALF and $7.3 \times 10^{-6}$ for MGS) and further decreases when both deposition efficiency and bioaccessibility are considered ($1.2 \times 10^{-5}$ for ALF and $2.2 \times 10^{-6}$ for MGS). When each of the particle size fractions is examined separately, the more refined risks

![Fig. 5. Relationship between inhalation bioaccessibility fraction of PAHs present on various sizes of e-waste burning particle matter and their associated log $K_{ow}$ values in modified Gamble solution (MGS). Dp is the aerodynamic diameter of particle.](image-url)
several particle diameters. LDE/O (%) and LDE+BA/O (%) show the level of re-

Finally, the present study suggested that the deposition efficiency and bioaccessibility of particle-bound HOCs were significant factors in the human inhalation health risk assessment.

Acknowledgements

The present study was financially supported by the National Natural Science Foundation of China (Nos. 41390240 and 21722701). We would also like to thank W. Tyler Mehler for assistance with manuscript editing.

<table>
<thead>
<tr>
<th>Particulate size diameter (µm)</th>
<th>ALF</th>
<th>MGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RiskO</td>
<td>RiskE</td>
</tr>
<tr>
<td>Combined</td>
<td>255.8</td>
<td>30.6</td>
</tr>
<tr>
<td>0.056–0.18</td>
<td>45.8</td>
<td>8.7</td>
</tr>
<tr>
<td>0.18–0.32</td>
<td>65.0</td>
<td>4.6</td>
</tr>
<tr>
<td>0.32–0.56</td>
<td>63.7</td>
<td>5.3</td>
</tr>
<tr>
<td>0.56–1.0</td>
<td>366.3</td>
<td>4.7</td>
</tr>
<tr>
<td>1.0–1.8</td>
<td>25.4</td>
<td>4.4</td>
</tr>
<tr>
<td>1.8–5.6</td>
<td>19.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

(RiskE and RiskE+BA) are still much lower than RiskO (Table 2 and Fig. S5). Additionally, the IBAF trends discussed above for differing size particles, in general, hold the same pattern when assessing risk, i.e., inhalation risk increased with increasing particle size. Consequently the ratio between RiskE+BA and RiskO increased from the smallest to the largest fraction by up to 5.9 and 6.6 fold for ALF and MGS, respectively.

Duan et al. (2015) recently proposed that the risk-based management of PAHs should consider bioaccessibility. Our results echo this sentiment, suggesting bioaccessibility should always be favored over other possible organics or metals which would also be present in e-

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.08.015.

References


Bernard, W., 2016. Lung surfactant: function and composition in the context of develop-


Oberdorster, G., Schins, R.P.F., 2005. Formation of PAH-DNA adducts after in vivo exposure of rats and lung cells to di-


Canepari, S., Astolfi, M.I., Moretti, S., Curini, R., 2010. Comparison of extracting solu-

tions for elemental fractionation in airborne particulate matter. Talanta 82, 834–844.


pendence of slow adsorption and desorption kinetics of organic compounds in sedi-
