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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

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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 absorption 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; Delfino 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

* Corresponding author.
E-mail address: baolianjun@jnu.edu.cn (L.-J. Bao).

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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) (Pelfrêne et al., 2017; Wiseman, 2015). Although some researchers have used unmodified GS, other researchers have also started modifying the GS to mimics 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 (Dartey 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; Borm 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-DPHA, 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 evaluate 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 N_2. 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 N_2. 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 fibroid 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