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Bioaccessibilities of metal(loid)s and organic contaminants in particulates measured in simulated human lung fluids: A critical review☆

Helong Ren [a], Yingxin Yu [a, b, *], Taicheng An [a, b]

[a] Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Guangzhou Key Laboratory Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, China

[b] Synergy Innovation Institute of GDUT, Shantou, 515041, China

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ABSTRACT

Particle-bound pollutants can pose a health risk to humans. Inhalation exposure evaluated by total contaminant concentrations significantly overestimates the potential risk. To assess the risk more accurately, bioavailability, which is the fraction that enters into the systemic circulation, should be considered. Researchers have replaced bioavailability by bioaccessibility due to the rapid and cost-efficient measurement for the latter, especially for assessment by oral ingestion. However, contaminants in particulates have different behavior when inhaled than when orally ingested. Some of the contaminants are exhaled along with exhalation, and others are deposited in the lung with the particulates. In addition, a fraction of the contaminants is released into the lung fluid and absorbed by the lung, and another fraction enters systemic circulation under the action of cell phagocytosis on particulates. Even if the release fraction, i.e., release bioaccessibility, is considered, the measurement faces many challenges. The present study highlights the factors influencing release bioaccessibility and the incorporation of inhalation bioaccessibility into the risk assessment of inhaled contaminants. Currently, there are three types of extraction techniques for simulated human lung fluids, including simple chemical solutions, sequential extraction techniques, and physiologically based techniques. The last technique generally uses three kinds of solution: Gamble's solution, Hatch's solution, and artificial lysosomal fluid, which are the most widely used physiologically based simulated human lung fluids. External factors such as simulated lung fluid composition, pH, extraction time, and sorption sinks can affect release bioaccessibility, whereas particle size and contaminant properties are important internal factors. Overall, release bioaccessibility is less used than bioaccessibility considering the deposition fraction when assessing the risk of contaminants in inhaled particulates. The release bioaccessibility measurement poses two main challenges: developing a unified, accurate, stable, simple, and systematic biologically based method, and validating the method through *in-vivo* assays.

1. Introduction

Atmospheric particulate pollution, especially fine particulate matter with aerodynamic diameter less than 2.5 μm ($PM_{2.5}$), is one of the most hazardous factors influencing human health worldwide. According to epidemiological studies, atmospheric particulate pollution is consistently associated with morbidity and mortality from respiratory diseases, e.g., chronic obstructive pulmonary disease, pneumonia, and lung cancer (Pope et al., 2002; Wei et al., 2018). A great variety of substances, including metal(loid)s, organic chemicals, and pathogens, can be carried by particulate matter into the respiratory system through inhalation (Choi et al., 2017; Liu et al., 2019b; Yu et al., 2014). Their presence in particulates has been of great concern because of the toxic properties of

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* Corresponding author. Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Guangzhou Key Laboratory Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, China.
E-mail address: yuyingxin@gdut.edu.cn (Y. Yu).

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some substances, such as heavy metals and hydrophobic organic contaminants (HOCs). Hence, the harmful effects of contaminants in particulate matter are very important in evaluating human health risk by inhalation (Xie et al., 2018; Zhang et al., 2016).

To assess the human health risks of metal(loid)s or organic contaminants in inhaled particulates, many studies in the earlier literature have used total contaminant concentrations (Desboeufs et al., 2005; Gerde et al., 2001; Monn, 2001), and this is the case even in more recent studies (Liu et al., 2019b; Pelfrêne et al., 2017; Scheffler et al., 2018). However, evaluating the inhalation health risk of particle-bound contaminants using total concentrations overestimates the potential risk because only a part of the particulates can be deposited in the lungs by inhalation. Furthermore, only those contaminants in the deposited particulates can be effectively absorbed and enter into the systemic circulation (Kastury et al., 2018b; Li et al., 2016c). Hence, the deposition fraction and the release fraction into the lung fluid, which can be absorbed into the systemic circulation, of atmospheric particulate matter are important factors in evaluating the human health risk from particulate-based contaminants.

The deposition fraction of atmospheric particulate matter refers to the percentage of inhaled particulates that are deposited in the respiratory tract (Fig. 1). All the contaminants in the deposited particulates should be considered in the bioaccessible fraction of the total, although some contaminants (mainly volatile organic compounds) may be exhaled from the lungs because of volatilization from the deposited particulates (Wei et al., 2018). In the present review, the deposition fraction is called deposition bioaccessibility. Furthermore, some studies have indicated that contaminants released into human lung fluids can be described as the bioaccessible fraction (Zereini et al., 2012; Pelfrêne et al., 2017), similarly to oral bioaccessibility measurement using simulated human gastrointestinal digestion solution (Rostami and Juhasz, 2011; Yu et al., 2012b; Zhang et al., 2017). Therefore, the release fraction of contaminants from particulates in the simulated human lung fluid is called release bioaccessibility. These two kinds of bioaccessibility are both referred to here as inhalation bioaccessibility.

It should be noted that conditions are very different between the human gastrointestinal tract and the lungs because only the pollutants released into intestinal fluid can be absorbed by the small intestine in the gastrointestinal tract; the pollutants that cannot be released will be excreted along with feces (Dean and Ma, 2007; Deshommes et al., 2012; Yu et al., 2010). However, the outcome is very different for pollutants in particulate matter in the lungs. Once particulates are deposited in the lungs, whether or not the pollutants in the particulates release into the lung fluid, they are very difficult to get out of the lungs. The pollutants will either be released into the lung fluids for absorption or enter the human body under the action of cell phagocytosis on particulates. In other words, these pollutants are all bioaccessible. The fraction of contaminants crossing the cell membrane into the capillaries and reaching the systemic circulation is referred to as bioavailability, or the fraction of a contaminant that is bioavailable (Collins et al., 2015; Semple et al., 2004). Although inhalation bioavailability by *in-vivo* methods remains the most appropriate for assessing human health risk, its measurement is very difficult. Therefore, inhalation bioaccessibility, especially for release bioaccessibility, is predominately evaluated by *in-vitro* methods as a rapid, cost-efficient approach with few ethical concerns (Mukhtar and Limbeck, 2013b). Moreover, inhalation bioaccessibility has been proposed to replace inhalation bioavailability for human exposure assessment (Lu et al., 2018).

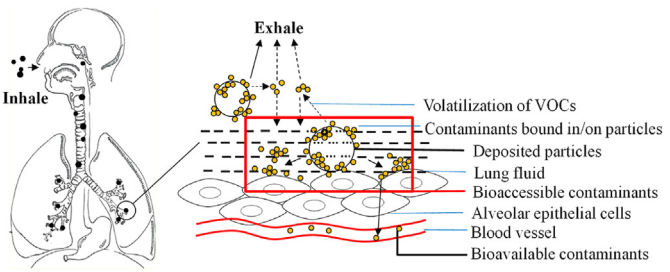

Fig. 1. Model schematics for inhalation bioaccessibility.

Compared with *in-vitro* methods using simulated gastrointestinal tract for oral bioaccessibility determination, research on release bioaccessibility using simulated lung fluids started much later (Dean and Ma, 2007; Wei et al., 2018; Wragg and Cave, 2002). Existing methods for release bioaccessibility do not have a unified protocol (Tables 1 and 2). The physiological parameters used in the available literature have not been fully explored, which poses many challenges for data interpretation and comparison among methodologies (Kademoglou et al., 2018; Wei et al., 2018). Therefore, the main objective of the present work is to review the development and progress of the release bioaccessibility measurement of pollutants in atmospheric particulate matter. The present review highlights and provides a comprehensive insight into the development of simulated lung fluids, the influencing factors of *in-vitro* methods of release bioaccessibility measurement, the challenges faced, and possibly a unified protocol.

2. Methodology and overview

The present study used “simulated lung fluids” and “particulate” as keywords for literature search. A total of 388 articles were found in *Web of Science* available on 10 October, 2019. The search was limited to references to contaminants released into simulated lung fluids from their matrix in the title or abstract. In addition, a manual search of the references of relevant publications was also conducted. Ultimately, 78 articles were found on released metal(loid)s or organic contaminants from particulates into simulated lung fluids. Fig. 2 shows the available reports on release bioaccessibility of particulate-bound contaminants by *in-vitro* methods from 2003 to 2019. It shows that more and more studies have been conducted in recent years and that studies on metal(loid)s have been much more numerous than studies of organic contaminants. Because no information is available on hydrophilic organic contaminants, metal(loid)s and HOCs are the focus of the present review.

Currently, there is no unified *in-vitro* method for measuring release bioaccessibilities of contaminants in particulates by inhalation, and many factors can affect the results. For example, different particulates including $PM_{2.5}$, woodstove particulates, black carbon, diesel soot, e-waste burning particulates, indoor dust, and biochar have been reported in the literature, and release bioaccessibility would be affected by the different physiochemical properties of the particulates. In addition, different simulated lung fluids, such as water, phospholipid vesicles, 1-octanol, dipalmitoylphosphatidylcholine (DPPC), Gamble’s solution, and artificial lysosomal fluid (ALF), were used to simulate the fluids lining the lung epithelium or the intracellular fluid in phagocytes after particulate deposition. Other factors, such as extraction time and particle size, were also of concern. Therefore, a comprehensive review of these factors would be helpful for developing a unified *in-vitro* method (Tables 1 and 2).

Furthermore, the properties of substances also have an important influence on release bioaccessibility. For instance, the bioaccessibilities of benzo[a]pyrene and benzo[k]fluoranthene range

Table 1
Measurements of the release bioaccessibilities of metal(loid)s.

Simulated lung fluids	Samples	Metal(loid)s	Particle sizes	Extraction time	S/L ratio (g/mL)	Agitation	References
Simple chemical solutions							
Water	APM	Fe, Mn	–	30 min	–	–	Desboeufs et al. (2005)
Water	APM, black smoke	Fe, Ni, Cu, Zn, Ti, V, Cr, Mn, As, Cd, Pb	PM_{10}, $PM_{2.5}$	1 h	–	Ultrasonic	Heal et al. (2005)
Water	APM	Cu, Fe, Mg, Mn, Al, Ba, Ca, Cr, Pb, Se, Ti, Zn	PM_{10}, $PM_{2.5}$	1 h	1:23866 –1:70922	Ultrasonic	Santos et al. (2009)
Water	NIST 1648a, BCR 038 , NIES 8, NIST 2584	Co, Cu, La, Mn, Pb, Ba, Cd, Ce, Mo, Ni, Pb, Sb, Zn	–	24 h	1:20000	Orbital shaker	Julien et al. (2011)
Water	Urban APM	Co, Cu, Mn, As, Ba, Cd, Ni, Pb, Zn	PM_{10}	1 h	–	Ultrasonic	Mukhtar and Limbeck (2013a)
Water	Nickel substances	Ni	100 μm	1 h, 24 h	1:500	Shaken	Oller et al. (2009)
Citric acid							
Citrate	Cobalt oodde	Co	1.7 μm	7 d	–	Shaken	Collier et al. (1992)
Ammonium citrate	Nickel substances	Ni	100 μm	1 h, 24 h	1:500	Shaken	Oller et al. (2009)
Bicarbonate	Cobalt oodde	Co	1.7 μm	7 d	–	Shaken	Collier et al. (1992)
Ammonium citrate Sodium chloride Ammonium acetate	Urban APM	Co, Cu, Mn, As, Ba, Cd, Ni, Pb, Zn	PM_{10}	1 h	–	Ultrasonic bath	Mukhtar and Limbeck (2013a)
Physiologically based techniques							
Gamble's solution	Slag dust	Ni, Cd, Zn, Mn	–	12h	1:1000	_	Lima et al. (2013)
Gamble's solution	Urban APM	Pt, Pd, Rh	PM_{10}, $PM_{2.5}$, PM_1	24h, 30d	–	Shaken	Zereini et al. (2012)
Gamble's solution	Urban APM	Co, Ni, Ce, Pb, Ti, V, Cr, Mn, Cu, As, Sb	PM_{10}, $PM_{2.5}$, PM_1	24 h	1:1163	Shaken	Wiseman and Zereini (2014)
Gamble's solution	Welsh mine waste	Pb	PM_{10}	630 h	1:44	Shaken	Wragg and Klinck (2007)
Modified Gamble's solution	Mine waste calcine	Hg	150 μm	24 h	1:20	Rotated	Gray et al. (2010)
Synthetic serum	Urban APM, Dustrial sites APM	Ni, Pb, Cd, Cu, Mn, Zn	7.2 μm	60 min	–	–	Voutsa and Samara (2002)
Lung simulating serum	Household APM	Cu, Zn, As, Cd, Cr, Mn, Sn, Sb, Ni, Hg, Pb	$PM_{2.5}$	24 h	–	Shaken	Huang et al. (2014)
Simulated epithelial lung fluid	Smelter, topsoil, tailing	Pb	PM_{10}	24 h	1:66.7	Rotated	Boisa et al. (2014)
Simulated lung fluid	Coal fly ash	Cu, Ni, Pb, Al, Cr III, Cr IV, Th, U, V, Zn	PM_{10}	144 h	1:20	Rotated	Twining et al. (2005)
Hatch's solution	Indoor PM	Hg, Mn, Ni, As, Cd, Cr, Pb, Zn	25, 37, 63 μm	16 h	1:100	Shaken	Sysalová et al. (2014)
Phagolysosomal simulant fluid	Bulk mine waste	Zn, Pb, Cd	37 μm	5 d	1:100	–	Schaider et al. (2007)
Artificial lysosomal fluid	Urban APM	Pt, Pd, Rh	PM_{10}, $PM_{2.5}$, PM_1	24h, 30d	–	Shaken	Zereini et al. (2012)

NIST: National Institute of Standards and Technology, NIES: National Institute for Environmental Studies, BCR: European Community Bureau of Reference, APM: airborne particulate matter.

from 25% to >85%, but the bioaccessibilities of the other PAHs are pretty low, with values less than 1.3% (Bevan and Yonda, 1985; Borm et al., 2005; Ewing et al., 2006; Gerde et al., 2001). Dimethyl phthalate (DMP) and diethyl phthalate (DEP) are >75% bioaccessible, whereas bis(2-ethylhexyl) phthalate (DEHP), di-isononyl-cyclohexane-1,2-dicarboxylate (DINCH), and bis(2-ethylhexyl) terephthalate (DEHT) have relatively low bioaccessibility (<5%) (Kademoglou et al., 2018). Similar differences were generally observed for metals. In samples from different sites, not detected (ND)–95% of Zn was found to be bioaccessible and <1%–96% of Pb was observed to be bioaccessible in different particulates (Huang et al., 2014; Julien et al., 2011; Lima et al., 2013; Mukhtar and Limbeck, 2013a; Sysalová et al., 2014; Voutsa and Samara, 2002; Wiseman and Zereini, 2014). The literature release bioaccessibilities of metal(loid)s were summarized and are shown in Fig. 3.

3. Simulated lung fluids

During development of a release bioaccessibility measurement, many simulated human lung fluids have been used (Fig. 4). Extraction techniques using simple chemical solution-based methods, sequential extraction techniques, and physiologically based techniques are widely used to simulate the extracellular fluids lining the lung epithelium or the intracellular fluid in phagocytes. These techniques use a variety of leaching agents as extraction solutions to maintain conditions as close as possible to the respiratory system. These simulated lung fluids and extraction techniques have been reviewed briefly.

3.1. Simple chemical solutions and sequential extraction techniques

In early times, many simple chemical solutions, such as water and solutions of sodium chloride, sodium chloride, ammonium acetate, and ammonium citrate (Fig. 4), have been generally used as simulated lung fluids to measure the release of metals from atmospheric particulate matter (such as $PM_{2.5}$, PM_{10}, and black smoke), welding fumes, and standard reference materials (SRMs) (Heal et al., 2005; Julien et al., 2011; Oller et al., 2009). Because of their practicality, cost-efficiency, and lack of damage to the instrument during testing, these solutions were widely used. In addition, it was believed that pollutants dissolved in simple chemical solutions could easily dissolve into natural human lung fluids and result in adverse effects on human health. However, the release of metals in particulates in natural human lung fluids might exhibit different behaviors than in simple chemical solutions because of the complex compositions of natural human lung fluids with different pH levels and ionic strengths (Mukhtar and Limbeck,

Table 2
Measurements of the release bioaccessibilities of HOCs.

Simulated lung fluids	Sample types	HOCs	Particle size	Extraction time	Sorption sinks	Sample amounts	solution amounts	S/L ratio (g/mL)	Agitation	Bioaccessibilities	References
Simple chemical solutions											
Phospholipid vesicles	Woodstove particle	Bap BkF	NA	18 h	–	100 mg	70 mg	–	Bath-type sonicator	25% 68%	Bevan and Yonda (1985)
1-octanol	Diesel soot	Bap	1.3 ± 0.2 μm	20 s	–	0.12 mg	17 mL	1:150000	Two-bladed impeller	36%	Gerde et al. (2001)
1-octanol	Silica	Bap	3.5 μm	5 min	–	100 mg	17 mL	1:170	Stirring	>85%	Ewing et al. (2006)
Dipalmitoylphosphatidylcholine	Diesel or carbon black	Phe Pyr Ant Chr Flu	NA	24 h	–	3–60 mg	3 mL	1:50 –1:1000	Shaking water bath	<1.2% <0.4% <1% <1.3% <1.3%	Borm et al. (2005)
Physiologically based techniques											
Simulated epithelial lung fluid	APM	19 PAHs	2.5 μm	24 h	–	–	20 mL	1:600 –1:4000	Shaking	3.21%–44.2%	Li et al. (2019)
Modified Gamble's solution	E-waste burning particles	PAHs	5.6 μm	10 d	Tenax	20 mg	200 mL	1:10000	–	3%–96.8%	Xie et al. (2018)
Artificial lysosomal fluid											
Gamble's solution	APM	9 PAHs	2.5 μm	24 h	–	–	25 mL	–	Shaking	>13.7%	Gao et al. (2019)
Artificial lysosomal fluid											
Gamble's solution	Indoor dust	DMP DEP DEHP DEHT DINCH	63 μm	96 h	–	200 mg	20 mL	1:100	–	>75% >75% <5% <5% <5%	Kademoglou et al. (2018)
Artificial lysosomal fluid											
Gamble's solution	Biochar	Phe Pyr	3 μm	30 min	–	4 mg	20 mL	1:5000	Shaking	0.47%–0.75%	Liu et al. (2019b)
Artificial lysosomal fluid											
Gamble's solution	Indoor dust	8 PAHs	–	24 h	–	–	25 mL	–	Shaking	ND–31.7%	Liu et al. (2019c)
Artificial lysosomal fluid											
Gamble's solution	APM	HFRs OPFRs PAHs	2.5 μm	1 d, 15 d	–	70 mg	70 mL	1:1000	Shaking	0.7%–24.5%	Zeng et, al. 2019
Artificial lysosomal fluid											

BaP: benzo[a]pyrene, BkF: benzo[k]fluoranthene, Phe: phenanthrene, Pyr: pyrene, Ant: anthracene, Chr: chrysene, Flu: fluoranthene, DMP: dimethyl phthalate, DEP: diethyl phthalate, DEHP: bis(2-ethylhexyl) phthalate, DEHT: bis(2-ethylhexyl) terephthalate, DINCH: di-isononyl-cyclohexane-1,2-dicarboxylate, PAHs: polycyclic aromatic hydrocarbons, APM: airborne particulate matter, HFRs: halogenated flame retardants, OPFRs: organophosphorus flame retardants, NA: not available, ND: not detected.

2013a). The pH of simple chemical solutions (especially involving water) is highly susceptible to change under the influence of sample variation. Therefore, these simple chemical solutions can influence the release efficiency of substances from their matrices, such as atmospheric particulate matter, and result in high inter-sample variability. Hence, different simulated lung fluids can affect the release bioaccessibilities of contaminants in *in-vitro* methods using simple chemical solutions. Therefore, more complex chemical solutions simulating human lung fluids for *in-vitro* methods should be developed and used.

Consequently, sequential extraction techniques were developed to leach metals with different chemical speciation by increasingly aggressive solutions to understand the chemical conditions of their mobilization from atmospheric particulate matter. Target metals were separated into different fractions, such as water-soluble and exchangeable metals, carbonates, oxides, and reducible metals. Knowledge of the chemical speciation of metals is vital in evaluating their release in natural human lung fluids (Santos et al., 2009; Schaider et al., 2007). For example, Schaider et al. (2007) found that refractory metal sulfides were shifted into relatively labile and desortable components in sequential extractions after physical and chemical weathering. About 50%–65% of Zn, Pb, and cadmium (Cd) in <37 μm mine waste particulates were exchangeable, as well as carbonate sequential extraction fractions. However, extraction solutions with strong acid reagents cannot represent biologically relevant human lung fluids because of differences in composition and physiological processes (Julien et al., 2011). Consequently, the bioaccessible fraction cannot be evaluated accurately in terms of composition and physiochemical properties using sequential extraction techniques or simple chemical solutions. To solve this problem, physiologically based simulated human lung fluid came into being.

3.2. Physiologically based techniques

3.2.1. Gamble's solution

As early as the 1940s, physiologically based solutions were used in tentative attempts to mimic the composition of human lung fluid. The earliest compositions of Gamble's solution, created by Gamble in 1942, had compositions similar to the extracellular fluid in the skeletal muscle (Gamble, 1942). The basic chemicals used in Gamble's solution are cations (magnesium, sodium, calcium, and potassium) and anions (proteins, bicarbonate, sulfate, organic acids, chloride, and monohydrogen phosphate) with a pH of approximately 7.4. Non-electrolytes (glucose, amino acid, and waste products of protein metabolism) and carbonic acid were also included in this simulated lung fluid (Gamble, 1942). To estimate the most likely exposure materials among twenty-two process

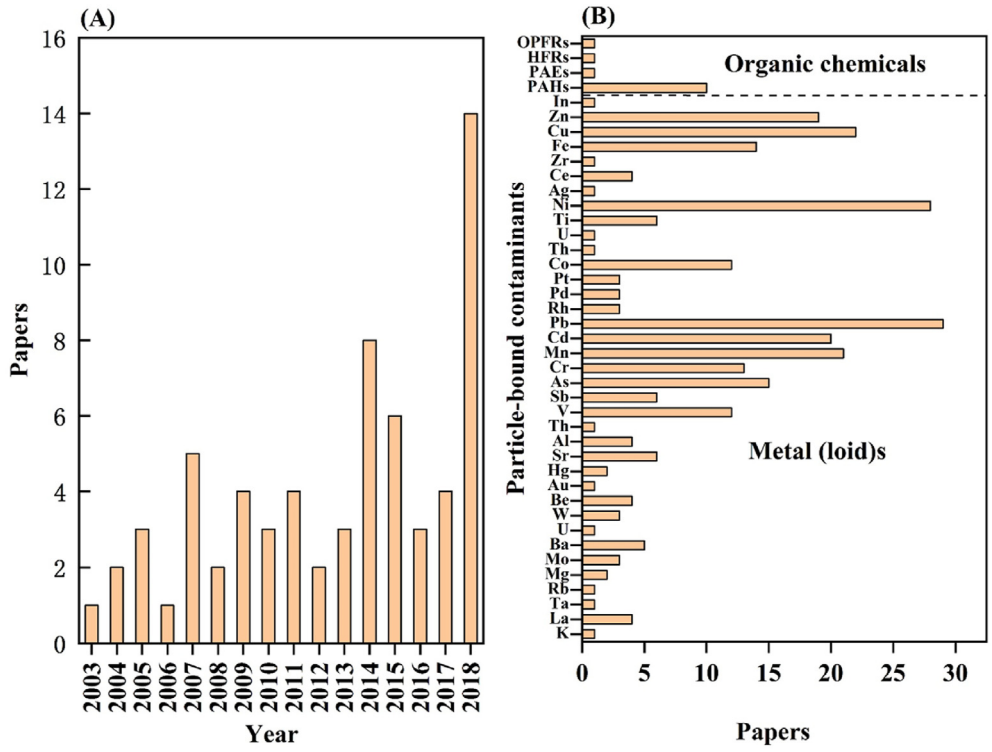

Fig. 2. Published articles in journals from 2003 to 2019 (A) and contaminants reported in the literature (B).

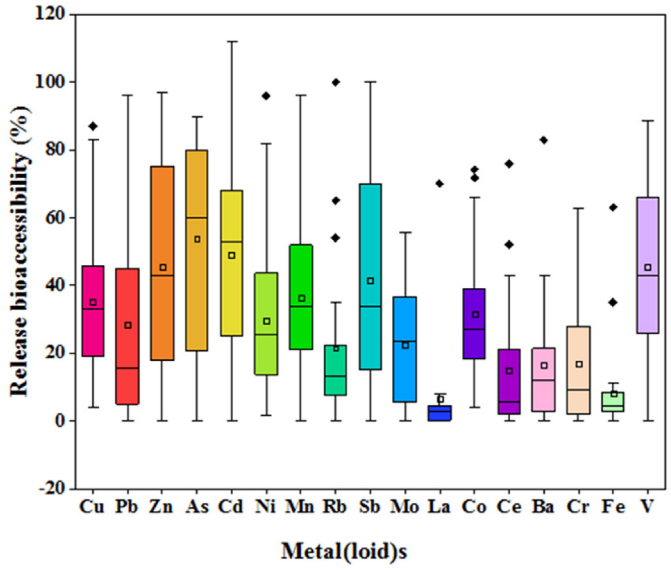

Fig. 3. Release bioaccessibilities of metal(loid)s in simulated human lung fluids (Boisa et al., 2014; da Silva et al., 2015; Ettler et al., 2014; Fernandez et al., 2002; Graney et al., 2004; Huang et al., 2014; Julien et al., 2011; Kyotani and Iwatsuki, 1998; Lima et al., 2013; Mukhtar and Limbeck, 2013a; Mukhtar and Limbeck, 2013b; Mustafa et al., 2007; Niu et al., 2010; Potgieter-Vermaak et al., 2012; Qureshi et al., 2006; Sysalová et al., 2014; Twining et al., 2005; von Schneidemesser et al., 2010; Voutsa and Samara, 2002; Wiseman and Zereini, 2014; Wragg and Klinck, 2007). Horizontal lines with in the box and the lower, upper limit of the bar indicate median and 25%, 75% values, and the squares represent mean values. The whisker extends to the last observation within 1.5 times the interquartile range. The diamonds outside the whiskers represent the outers.

materials in a Y-12 enriched uranium area, Steckel and West (1966) used the simulated lung fluid originally called Gamble's solution to conduct a uranium release study for 16 weeks. In 1979, the compositions and concentrations of the original Gamble's solution were presented in detail by Moss (1979).

More recently, the original Gamble's solution with modifications has been given different names in subsequent studies and has been widely used by many researchers (Mukhtar and Limbeck, 2013a; Wiseman and Zereini, 2014). With the development of *in-vitro* methods to measure contaminant release from particulates in simulated human lung fluids, Gamble's solution has continuously been modified in various ways (Fig. 4). Currently, the generally used Gamble's solution can be divided into four major subclasses (Kastury et al., 2017): the original Gamble's solution (Moss, 1979), Gamble's solution modified with proteins and amino acids, Gamble's solution modified with serum simulant (Kanapilly et al., 1973), and Gamble's solution modified with lung surfactants (Boisa et al., 2014), which are presented in Table 3.

3.2.2. *Modified Gamble's solution*

Early studies mainly focused on heavy metals. The proteins and amino acids in the original Gamble's solution significantly influence the chelation of heavy metals, although the organic components are only a small fraction of the total anions in the interstitial fluid. Modified Gamble's solutions with proteins and amino acids were therefore developed. For example, glycine (Kanapilly et al., 1973), protein (Morrow et al., 1968), bovine serum albumin (Twining et al., 2005), and mucin (Boisa et al., 2014) were added to the original Gamble's solution. For example, Kanapilly et al. (1973) added amino acids such as glycine to Gamble's solution to replace proteins and thus prevent clogging of filter pores. The release of particulate-bound contaminants, including Pb and Zn, in the modified Gamble's solution was higher than in the original Gamble's solution (Berlinger et al., 2008). Julien et al. (2011) found that amino acids might promote metal(loid) release by forming amino acid structures. These studies all implied that certain amino acids

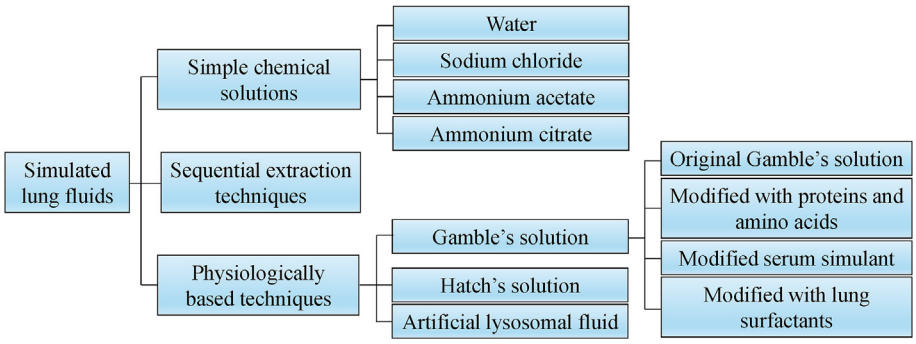

Fig. 4. Classification of simulated lung fluids.

Table 3
Compositions of simulated human lung fluids (g/L).

Type	Original Gamble solution [a]	Modified Gamble solution with proteins and amino acids [b]	Modified serum simulant [c]	Modification with lung surfactants [d]	Hatch's solution [e]	ALF [f]
NaCl	6.019	0.2	6.8	8.47	7	3.21
$NaHCO_3$	2.604	2.7	2.27	2.016	2.27	
KCl	0.298	0.298		0.298	0.37	
Na_2HPO_4	0.142	0.15			0.1196	0.071
NaH_2PO_4			0.144			
$Mg(C_2H_3O_2)_2{\cdot}4H_2O$					0.0342	
Na_2SO_4	0.071	0.072		0.071		0.039
$CaCl_2{\cdot}2H_2O$	0.4	0.256				0.128
CaCl2			0.022	0.277	0.225	
$MgCl_2{\cdot}6H_2O$	0.203				0.21	
$MgCl_2$				0.095		0.05
$C_6H_5O_7Na_3.2H_2O$	0.097					
$C_6H_5O_7Na_3$			0.052	0.774		
$C_2H_3O_2Na{\cdot}3H_2O$	0.9526					
$C_2H_3O_2Na$				0.574		
KH_2PO_4					0.03	
Citrate						
Sodium lactate						0.085
Disodium tartrate						0.09
NH_4Cl		6.02	0.535			
Sodium pyruvate						0.172
H_2SO_4			0.049			
$C_6H_8O_6$					0.05	
$C_5H_4N_4O_3$					0.025	
$C_{10}H_{17}N_3O_6S$					0.05	
D-Glucose					1	
Glycine		0.376	0.45			0.059
Dipalmitoyl				0.2		
Lysozyme					2.5	
Citric acid						20.8
a-Tocopherol					0.001	
Albumin					10	
Apo-transferrin					0.2	
choline					10	
NaOH						6
L-Cysteine		0.122				
DPPC		0.1				
Ascorbic acid		0.018				
Uric acid		0.016				
Glutathione		0.03				
Albumin		0.26				
Mucin		0.5				

[a] Moss (1979).
[b] Boisa et al. (2014).
[c] Kanapilly et al. (1973).
[d] Dennis et al. (1982).
[e] Berlinger et al. (2008).
[f] Colombo et al. (2008). ALF: artificial lysosomal fluid.

and proteins are efficient in promoting the release of certain metal(loid)s.

Modified serum simulant was derived from Gamble's solution with a series of modifications by Kanapilly et al. (1973). This formulation contained citrate as a substitute for acetate to represent organic acids, omitting magnesium and potassium ions, but

adding ammonium chloride to stabilize the pH at 7.3. According to a study by Ansoborlo et al. (1998), there was a good correlation between *in-vitro* experiments involving UF_4, UO_2, and U_3O_8 in uranium fuels using the modified serum simulant and *in-vivo* results by intratracheal instillation using rats with the same uranium compounds. A modified serum simulant called serum ultra-filtrate was also used, in which diethylene triamine penta-acetic acid and L-cysteine were added to the serum simulant because of their good chelation of L-cysteine and the ability of diethylene triamine penta-acetic acid to prevent precipitation of plutonium ions during the experiments (Eidson and Mewhinney, 1983). Subsequently, serum ultra-filtrate was used by Huang et al. (2014) and Voutsa and Samara (2002).

Beyond the modified solutions just described, there is a kind of Gamble's solution modified with lung surfactants, which is a mixture consisting of lipids and some proteins secreted by epithelial type II cells with DPPC as the main ingredient. The lung surfactants secreted in the alveolar region and lower bronchioles can play an important role in reducing surface tension at the gas-liquid surface (Bernhard, 2016; Veldhuizen and Haagsman, 2000). Therefore, adding DPPC as a surrogate for surfactant would mimic natural human lung fluids more closely. Recently, Pelfrêne et al. (2017) suggested that the DPPC concentration in simulated lung fluids should be approximately 100 mg/L, assuming a median concentration of 7.4 mg/L for phosphatidylcholine in bronchoalveolar fluid and a DPPC content 13 times that of phosphatidylcholine.

In addition, DPPC, acting as a weak chelating agent, plays a significant role in releasing contaminants from particulates because it can increase the wettability of hydrophobic particulates, reconnect the leaching solution with the contaminants, and prevent particulates from aggregating (Martin et al., 2018). For example, when DPPC was removed from this modified Gamble's solution, Pb solubility in $PM_{2.5}$ decreased from 23%–43% to 5.6%–18% (Li et al., 2016b). Davies and Feddah (2003) demonstrated that a concentration-dependent increase of three glucocorticoids in desorption occurred after DPPC was added to simulated lung fluids. Moreover, DPPC was also used to test the release of inhaled products (such as poorly soluble drug substances) in the pharmaceutical sector (Davies and Feddah, 2003; Riley et al., 2012). Considering the important effects of DPPC on both metal and organic chemicals, the use of DPPC in the release bioaccessibility measurement of contaminants in simulated human lung fluids merits further study.

3.2.3. Hatch's solution

Hatch's solution is an additional type of simulated lung fluid that has been used in some studies (Kastury et al., 2018a; Sysalová et al., 2014). The human respiratory system consists of two layers of extracellular secretions lining the tracheal bronchus. The upper layer is a thicker "gel" layer of mucus, in which inhaled particulates first deposit. The composition of the mucous layer of the respiratory tract was considered in formulating Hatch's solution, as described by Hatch (1992). This simulated lung fluid contains elevated concentrations of protein, enzymes, lung surfactant, and complex organic molecules. The release of As in mining and smelting incubated in Hatch's solution was approximately four times greater than in four other simulated lung fluids, including the original Gamble's solution, serum simulant, simulated epithelial lung fluid, and another formulation, after 24 h incubation (Kastury et al., 2018a). Similar results were also observed for Pb (Berlinger et al., 2008). The higher release efficiency in Hatch's solution than in Gamble's solution may be partly attributed to the elevated concentrations in Hatch's solution of albumin and DPPC, which are known as chelators for their capability to bind metal(loid)s.

3.2.4. Artificial lysosomal fluid

It is well known that a fraction of particulates will be swallowed by macrophages within hours after deposition and that the acidic condition inside macrophages is quite different from the extracellular neutral environment. Consequently, some studies have proposed an acidic solution with a similar composition to Gamble's solution, but adding hydrochloric acid or buffers to alter the pH (Guldberg et al., 1998; Thélohan and de Meringo, 1994). Stopford et al. (2003) substituted glycine for glycerine and called the acidic solution ALF. As a result, metals (such as platinum, palladium, and rhodium) in particulate matter could be more thoroughly dissolved in ALF than in Gamble's solution because of the low pH of ALF (Colombo et al., 2008; Fathi et al., 2012; Marques et al., 2011). Currently, ALF has been widely used to mimic the acidic intracellular lung environment of macrophages in many release bioaccessibility measurements (Kastury et al., 2018b; Xie et al., 2018).

4. Factors influencing release bioaccessibility

Many factors can affect the release bioaccessibilities of pollutants as measured using simulated human lung fluids (Fig. 4). Generally, there are two kinds of factors: external and internal. External factors mainly include the compositions of the simulated lung fluid and the conditions for *in-vitro* methods, including extraction time, sorption sinks, solid to liquid (S/L) ratio, and agitation. The internal factors mainly include the characteristic properties of chemicals and the matrices themselves. Significant differences existing among the external and internal causes for release bioaccessibility measurements by *in-vitro* methods have made it difficult to construct a standardized method. The present review highlights the influences of external and internal factors on release bioaccessibility. A systematic discussion of the factors can be helpful for constructing a standard *in-vitro* method simulating human lung fluid for release bioaccessibility measurement of contaminants in inhaled particulates.

4.1. Physiological parameters

To date, there has been no standardized *in-vitro* protocol for release measurement of particulate-bound contaminants using simulated lung fluids. The diverse parameters (pH, extraction time, S/L ratio, and others) adopted in release bioaccessibility measurements are highly variable, resulting in a variety of results.

4.1.1. pH

As mentioned earlier, the compositions of simulated human lung fluids have an important influence on the release of pollutants, including metal(loid)s and organic contaminants. The different compositions result in various pH values, which may play an important role in measurement of the release of bioaccessible contaminants, especially for metals, although no consistent results could be found in the literature (Liu et al., 2019b; Zeledón-Toruño et al., 2007). For example, cobalt (Co) release from cobalt powder is higher in ALF (pH = 4.5) than in Gamble's solution (pH = 7.4) (Stopford et al., 2003). However, according to Guo et al. (2017), pH is not an affecting factor on the release of phenanthrene and pyrene from BC700 in aqueous solution. On the one hand, phenanthrene and pyrene, as non-ionic organic compounds, are not ionized in water solution. On the other hand, this phenomenon may have occurred because phenanthrene and pyrene are adsorbed in BC700 mostly through hydrophobic partitioning interaction instead of electrostatic interaction at pH below 10, which is the point of zero electric charge of BC700.

Similarly, Liu et al. (2019b) reported that pH showed no obvious

effect on the release of phenanthrene bound in biochar. It had been suggested that PAHs in water undergo a hydrophobic interaction with leonardite and that C═C bond linkages of PAHs adsorbed in carbonaceous materials are chemically inert and less affected by pH (Zeledón-Toruño et al., 2007). However, Gao et al. (2019) suggested that the surface positive charge on $PM_{2.5}$ decreases with increasing pH as the OH ions compete to adsorb on active sites with PAH molecules, reducing adsorption efficiency and promoting PAH release in Gamble's solution. Considering the varied influences of pH on contaminant release, further investigating the mechanisms by which pH influences the release of particulate-bound metals or HOCs in simulated lung fluids is needed.

4.1.2. Compositions of simulated lung fluids

As mentioned earlier, several kinds of simulated lung fluids exist. The compositions of simulated human lung fluids can have a significant effect on contaminant release from particulates. For example, Julien et al. (2011) found that Gamble's solution can dissolve more Pb and Zn in four SRMs than water with a similar pH because the chemical composition of the extraction solutions has a significant impact on metal desorption. This is highlighted in a report by Liu et al. (2019b), who found that low-molecular-weight organic acids (such as citric acid) in ALF can increase the specific surface area and micropore volume of the matrix, promoting absorption of HOCs such as phenanthrene. However, this result is not consistent with the assay conducted by Sun et al. (2016), who found that low-molecular-weight organic acids (citric and malic acids) in aqueous solution can disrupt the linkages between a black carbon surface and HOCs (such as PAHs) bound on it, thus increasing HOC release from black carbon.

Moreover, inorganic and organic salts were found to reduce desorption of phenanthrene from biochar because of pore blockage caused by ions (Liu et al., 2019b). These results are consistent with the study by Gao et al. (2019), who observed that black carbon-bound PAHs have higher release bioaccessibility using Gamble's solution than using ALF because of the much lower ionic strength of Gamble's solution. Furthermore, both Gamble's solution and ALF should be used to evaluate release bioaccessibility in future studies because the two solutions represent different areas in the lung (Xie et al., 2018).

4.1.3. Extraction time

The extraction time in release bioaccessibility studies has been found to be intimately related with the clearance time of matrices from the respiratory tract after particulates are initially deposited in the alveolar region (Julien et al., 2011; Kastury et al., 2017). Most of the factors influencing matrix clearance, such as characteristics of target materials and immune response within an organism, are not easily quantified. It has been confirmed that the half-life of particulate clearance in the alveolar region is 110 days, as suggested by Lay et al. (1998). In recent years, human lung clearance has been addressed in experimental studies, which found that the half-life of 30% insoluble particulate deposits in the alveolar region is up to 30 days and that more than 50% of insoluble particulates are retained at 300 days (Bailey et al., 2008; Kastury et al., 2017). This information was also used to determine the parameter values of the human respiratory tract models developed by the International Commission on Radiological Protection (Bailey et al., 2008). Therefore, the extraction times used in *in-vitro* methods using simulated lung fluids for release bioaccessibility measurement were generally much longer than those in oral bioaccessibility determinations using a simulated human gastrointestinal tract.

By investigating desorption of pollutants on particulates and according to the time when they reach the ultimate equilibrium state, extraction times in release bioaccessibility ranging from a few minutes to 360 days were investigated, and the released contaminants were found to be highly time-dependent for all kinds of matrices (Lima et al., 2013). The desorption kinetics of both metals and organic compounds in matrices in simulated lung fluids followed a similar pseudo-second-order model consisting of a rapid desorption phase followed by an asymptotically slow desorption phase, although they arrived at balance at different times (Julien et al., 2011; Kastury et al., 2018b; Liu et al., 2019b). Surveys such as that conducted by Zeledón-Toruño et al. (2007) have suggested that PAHs can adsorb onto adsorbents rapidly in the early phase through hydrophobic interactions, after which they can slowly migrate into inaccessible areas in the second desorption phase. Some researchers have reported that the most suitable Pb, Zn, and As release time is 24 h (Julien et al., 2011; Kastury et al., 2018a), but all experiments reached apparent desorption equilibrium for phenanthrene and pyrene release within approximately 30 min and remained roughly flat afterwards (Liu et al., 2019b).

In addition, Hofmann and Asgharian (2003) calculated the mucociliary clearance velocities in human bronchial airways by asymmetric, multiple-path models and suggested that 85%–90% of initial particulates deposited in the human terminal bronchioles would be removed within 24 h after deposition. Wragg and Klinck (2007) suggested an extraction time of 100 h when studying Pb release using Gamble's solution. In a recent study, a 10-day extraction time was chosen by Xie et al. (2018) after evaluating PAH solubility in modified Gamble's solution and ALF. The longer extraction time may be attributed to the S/L ratio or the characteristics of PAHs in these studies. More accurate investigations of the correlation between extraction time and contaminant release should be conducted.

4.1.4. S/L ratio

As expected, the S/L ratio is an important factor when other conditions are the same because the ratio can influence the mass of dissolved contaminants in simulated human lung fluids. Considering the size and concentration of inhaled particulates in the air, the number of particulates loading in the alveolar region is also influenced by individual human physiology (Pelfrêne et al., 2017). Many studies have shown that the average total surface of the lung fluid volume in the alveolar regions is relatively small. For example, the total volume of epithelial lining fluid was identified as 20 mL by Macklin (1955), assuming an alveolar region average surface area of 100 m^2 covered by an average of 0.2 μm depth of alveolar fluid. A smaller fluid volume was suggested by Weibel (1973), who used an average thickness of 0.068 μm covering the same surface area of the alveolar region to obtain a total alveolar fluid volume of 7 mL. Hence, individual human physiology makes a precise S/L ratio difficult to specify.

In the literature, S/L ratios varying from 1:50000 to 1:30 have been used for release bioaccessibility measurement of inhaled standard reference material-bound metals, including Mn, Ni, Pb, Cd, Ce, Co, Cu, Zn, etc., in water and Gamble's solution (Julien et al., 2011). The study considered inhalation exposure scenarios over a 24-h period over a large concentration range of 20–500 $\mu g/m^3$. As a conservative estimate, it was assumed that all the inhaled particulates reached the pulmonary alveoli with an average daily air uptake of 10–20 m^3 and a total alveolar fluid volume ranging from 5 to 20 mL. Finally, Julien et al. (2011) found that the release of metallic elements was independent of S/L from 1:500 to 1:5000. Studies have been carried out using very wide ranges of S/L ratios for release measurement of metals such as Ni, Cd, Zn, and Mn using *in-vitro* simulated human lung fluid (Lima et al., 2013; Wiseman and Zereini, 2014).

Comparatively, for HOCs, there have been fewer studies on the influence of S/L ratio. Wide ranges of these ratios have been examined, although the organic chemicals in particulates exhibited different properties from metal(loid)s. In experiments conducted by Li et al. (2019), 4.3–34.3 mg $PM_{2.5}$ samples were added to 20 mL simulated epithelial lung fluid to investigate PAH release with S/L ratios ranging from 1:600 to 1:4000, a range that was similar to that used by Julien et al. (2011). A larger ratio was used by Liu et al. (2019b), who investigated desorption of biochar-bound phenanthrene and pyrene using 4 mg samples added to 20 mL desorption media (S/L = 1:5000), i.e., Gamble's solution and ALF. In comparison, Kademoglou et al. (2018) used a much lower ratio of 1:100 in experiments in which 0.2 g indoor dust samples were added to 20 mL ALF. Unfortunately, that study did not further discuss the effect of S/L ratio on the release of particulate-bound HOCs. Currently, information on the influence of S/L ratio on contaminant release and the associated mechanisms, especially for HOCs, is too limited.

4.1.5. Agitation

Although agitation as a mechanical step has some immediate effect on the solubility of a substance, it is traditionally thought of as a uniform experimental procedure applied during *in-vitro* methods. In ordinary air, the concentrations of particulates such as $PM_{2.5}$ are low. Particulate matter can be completely dispersed in lung fluids in the human body without agglomeration. However, the available surface area of particulates influencing the contaminant-chelator interaction decreases when particulates agglomerate together spontaneously without agitation after they are added to simulated lung fluids (Ansoborlo et al., 1990). To investigate the effect of agitation on the release of metal(loid)s, the optimal type and frequency of agitation methods should be understood.

Several available reports have been released on the influence of agitation methods on substance release in simulated human lung fluids. Julien et al. (2011) observed that orbital shaking tended to concentrate the particulates, which limited full contact between the particle surface and the lung simulant fluid, resulting in lower Pb and Zn release in SRMs. Kastury et al. (2018a) studied the effect of orbital rotation, end-over-end rotation, magnetic stirring, and occasional stirring to assess their influences on the release of PM_{10}-bound As and Pb. In addition to magnetic stirring, which is not congruent with lung mixing processes, the authors found no significant differences among the other three agitation methods after extraction for 24 h. They finally chose end-over-end rotation as their mixing approach because the particulates were the most dispersed in the simulated human lung fluid by visual inspection when using this rotation method. Similar end-over-end rotation has been generally used in oral contaminant bioaccessibility measurement using a simulated human gastrointestinal tract (Oomen et al., 2002; Yu et al., 2012a, 2019). However, to the authors' best knowledge, there is no available information on the influence of agitation on HOC release in simulated human lung fluids, and hence further investigation is required.

4.1.6. Sorption sinks

Beyond the factors described above, there is another very important factor that can influence chemical release, especially for HOCs. In the human gastrointestinal tract, because HOCs with high hydrophobicity in general have high *n*-octanol-water partition coefficients ($\log K_{OW}$), the chemicals tend to partition into the lipid membrane continuously and to maintain a concentration gradient for further mobilization in intestinal enterocytes (Cui et al., 2016; Zhang et al., 2016). Similarly, absorption of a substance in the lung epithelial fluid is also a dynamic and complex process (Collins et al., 2013; Gouliarmou et al., 2013). For example, the solubility of Ce, Zr, and Nb in radioactive aerosol particulates in simulated lung fluids under an equilibrium condition could not reflect actual release in natural human lung fluids because of the static simulation method (Kanapilly et al., 1973). Many traditional static studies evaluating HOC release through *in-vitro* methods with simulated human lung fluid with little liposome may underestimate the results, especially for extremely hydrophobic compounds (Xie et al., 2018).

Recently, with the development of oral bioaccessibility measurements using *in-vitro* methods in a simulated human gastrointestinal tract, many absorption sinks, such as Tenax, C18 membrane, silicon rod, and silicon combined with activated carbon, have been added to the digestion solution to improve HOC release (Fang and Stapleton, 2014; Li et al., 2016a; Yu et al., 2013). The absorption sinks, which simulate the dynamic HOC uptake process, can improve the correlation between oral bioaccessibility determined by *in-vitro* methods and oral bioavailability using animals. Investigation of release bioaccessibility measurement using a similar dynamic process has been very limited.

Tenax, a porous resin with high affinity, reusability, sensitivity, accuracy, desirable adsorption, and conductive back-extraction for HOCs, has been widely regarded as an ideal absorption sink for oral bioaccessibility measurement (Harwood et al., 2013; Yu et al., 2013). A similar absorption sink was also used by Xie et al. (2018), who found that PAH releases with the assistance of Tenax in Gamble's solution and ALF were higher than those without Tenax. At present, many questions remain regarding the use of these absorption sinks. First, studies using Tenax extraction to determine release HOC bioaccessibility are too scarce, and further verification by *in-vivo* methods should be conducted. Second, the combined effect of the absorption sink and the components of simulated lung fluids on release bioaccessibility remains to be investigated.

4.1.7. Selection of the physiological parameters

If the objective of a study involves only the release mechanism or the adsorption and desorption mechanism of pollutants deposited in lung particles, the selection of these parameters is comparatively easy. However, to protect human health, the simulated human fluids should, on the one hand, be as close as possible to the composition of natural human lung fluid, and on the other hand, the other incubation conditions should represent the worst-case scenario. In other words, this scenario will lead to the largest release bioaccessibilities of contaminants under the conditions. Therefore, lower pH is suggested for metals, and Tenax as a sorption sink is suggested as an addition. End-over-end rotation agitation is recommended. Considering the effectiveness, an extraction time of 24 h is suitable. As for the S/L ratio, very different conditions were appropriate for various matrices and target substances. The authors suggested an S/L ratio at the inflection point of the correlation curve between the release bioaccessibility and the S/L ratio.

4.2. Physiochemical factors of samples

4.2.1. Particulate types

Contaminant release from particulates can be affected by various matrices of atmospheric particulate matter or dust. Studies evaluating the influence of different particulate types using the same methodological parameters are lacking (Table 1 and 2). Julien et al. (2011) added four certified reference materials to Gamble's solution to investigate the release of metals and found that the released Cu in the SRMs was similar, but that Cd, Pb, and Zn were highly variable. At the same time, the solubility of metals in BCR 038, a fly ash powder composed of an aluminosilicate, glass, and an iron oxide matrix, was weak compared with those in the other

three materials, including NIST 1648a, NIES 8, and NIST 2584 in Gamble's solution (Julien et al., 2011). Similar releases of metals from various materials such as Saharan aerosol particulates, Arizona dust, fly ashes, and SRM 1648 provided by the National Institute of Standards were also observed by Desboeufs et al. (2005), who studied the release of trace metals using aluminosilicated and carbonaceous samples under atmospheric water conditions. They found that the metals in the aluminosilicated matrix were more easily dissolved than those in the carbonaceous matrix because of the type of bonds between the metals and the matrices.

In the literature, different matrices have been generally considered, and a variable set of parameters has been used in experiments, which has made it difficult to evaluate the effect of particulate types on the release of particulate-bound HOCs (Table 2). Fortunately, the influence of particulate types on phenanthrene and pyrene release using Gamble's solution and ALF was evaluated by Liu et al. (2019b), who studied the release of these chemicals from seven biochar samples produced by various plant materials with different heat-treatment temperatures. Higher heat-treatment temperatures resulted in a greater volume of micropores in the particulates and led to lower phenanthrene desorption in ALF and Gamble's solution. The release of phenanthrene from four matrices, including corn straw, wheat straw, peanut shells, and shaddock peels after heat-treatment at the same temperature (500 °C), showed no obvious differences in Gamble's solution, but there were slight differences in ALF. Currently, the number of studies investigating particulate influences is severely limited.

4.2.2. Particle size

In the atmosphere, particle size varies from several nanometers to hundreds of microns. Although many studies have been carried out on the influence of particle size on the oral bioaccessibilities of metals or organic chemicals using a simulated human gastrointestinal tract (Girouard and Zagury, 2009; Yu et al., 2013), the number of studies investigating the influence of particle size on the release bioaccessibilities of contaminants using simulated human lung fluids is still very limited. According to (Xie et al., 2018), the larger the particle size, the higher is the release rate of e-waste burning particulate-bound PAHs using ALF and modified Gamble's solution with lung surfactants. They suggested that lower reactive surface area per unit mass with similar carbon content was the main reason for the higher PAH releases. Similar trends have been proposed in other studies for HOCs (Mehler et al., 2011; Sun et al., 2008).

In contrast to organic chemicals, some particulate-bound metals exhibited different trends in simple chemical solutions (e.g., nitric acid, deionized water, or acetate buffer) (Birmili et al., 2006; Canepari et al., 2010). For example, the solubilities of Pb, Co, and Cd in fine particulates tested using water as a simulated human lung fluid were higher than those in coarse particulates (Birmili et al., 2006). However, Zn and Ba exhibited the opposite trend (Birmili et al., 2006). In addition, higher Cu release was found for larger particle sizes in nitric acid, but no relationship between solubility and particle size in acetate buffer was observed (Canepari et al., 2010). Although the results of earlier studies may have been different for various chemicals, the influence of particle size on contaminant release should be carefully considered.

4.2.3. Organic carbon content

The release of contaminants in simulated lung fluids may be affected by the physiochemical properties of the particulate compositions. Li et al. (2019) observed a strong negative correlation between desorption and the EC/(EC+OC) (ratio of elemental carbon to the sum of elemental and organic carbon) rather than with organic carbon content in $PM_{2.5}$, especially for high-molecular-weight PAHs tested using simulated epithelial lung fluid. However, to the authors' best knowledge, few studies have investigated the release effect of carbon content on particulate-bound metals. Actually, many studies on release or desorption have observed this influence. For example, a larger particle size of dichlorodiphenyltrichloroethane (DDT)-containing estuarine sediment resulted in lower desorption using salt water as the desorption medium because of the higher total organic carbon (TOC) in larger particulates (Wu et al., 2016). Hence, desorption of DDTs may be driven by TOC rather than particle size. Similar results were also observed in oral bioaccessibility measurement using a simulated gastrointestinal tract by Ruby et al. (2016), who found that soil TOC content is inversely related to oral PAH bioaccessibilities. Therefore, the influence of particulate composition as well as carbon content on the release bioaccessibilities of contaminants using simulated human lung fluids should be further considered.

4.2.4. Hydrophobicity

For HOCs, $\log K_{OW}$ has an important influence on the solubility of chemicals in various solutions. The release of particulate-bound HOCs in simulated lung fluids has been studied by many investigators. For example, Li et al. (2019) found a decreasing release of $PM_{2.5}$-associated PAHs using simulated epithelial lung fluid with increasing $\log K_{OW}$. Xie et al. (2018) also suggested that the release of low-ring PAHs in e-waste burning particulates was higher than that of high-ring PAHs measured using modified Gamble's solution and ALF. Similarly, Kademoglou et al. (2018) found that desorption of phthalate esters and alternative plasticizers in indoor dust or SRM 2585 in Gamble's solution and ALF decreased with increasing hydrophobicity of the chemicals. These studies all demonstrated that released HOCs in simulated lung fluids had a negative relationship with their $\log K_{OW}$, which can be mainly attributed to the compositions of the simulated lung fluids belonging to the aqueous system and therefore to the lower solubility capacity for HOCs with higher $\log K_{OW}$.

5. Application of inhalation bioaccessibility in health risk assessment

Risk assessment is proposed as an approach to avoid, reduce, or manage hazardous substances that harm human health. To effectively characterize these substances and then to reduce human health risks, it is necessary to know what the risks are and how high they are. Atmospheric particulate matter such as $PM_{2.5}$ emitted from human activities or formed during photochemical processes can be inhaled and penetrate deeply into the lungs, presenting potential health risks, including chronic and acute respiratory disease, cardiovascular disease, lung cancer, and others (Polezer et al., 2019; Wu et al., 2015). Beyond the risks of atmospheric particulate matter itself, the harmful effects of organic contaminants and heavy metals in the particulates cannot be overlooked. It is currently recommended to factor inhalation bioaccessibility (including deposition and release bioaccessibility) into the evaluation of human health risk from atmospheric particulate matter by inhalation.

Nowadays, although many studies on the solubility or release of contaminants from matrices have been performed, few available reports have added release bioaccessibilities of pollutants measured by simulated human lung fluids into health risk assessments by inhalation (Li et al., 2019). As is well known, although the technologies of simulated human lung fluids and the simulated gastrointestinal tract were developed simultaneously, they are developing at different speeds. Compared to the simulated gastrointestinal tract, the technology of inhalation bioaccessibility

measurement is more complex and difficult. For oral bioaccessibility measurement, only the release fraction of contaminants should be considered in the human gastrointestinal tract. However, for contaminants in particulates, both the fraction of contaminants released from particulates and the fraction of particulates deposited in the human respiratory tract should be considered (Fig. 1). In addition, some particulates deposited in the human respiratory tract, especially the upper respiratory tract, can be cleared by mucociliary transport from the lung, which means that the inhaled particulates will reach the gastrointestinal tract. Consequently, Kastury et al. (2018a) developed an assay for inhalation-ingestion bioaccessibility to estimate human exposure to Pb and As in PM_{10}. They found that the highest Pb inhalation-ingestion bioaccessibility was measured using a simulated lung-gastric solution. However, the bioaccessibility of As was measured using a solution that simulated the lung-gastric-small intestinal tract.

Compared with release bioaccessibility, deposition bioaccessibility has been occasionally factored into contaminant risk assessments available in the literature (Yang et al., 2017), although this assessment factor should be considered as recommended by the United States Environmental Protection Agency (Liu et al., 2019a). This factor should also be added to the assessment according to the technical guidelines for risk assessment of contaminated sites of the National Environmental Protection Standard of the People's Republic of China (HJ25.3–2014) (Ministry of Ecology and Environment of the People's Republic of China, 2014). In these recommendations, a deposition fraction of 0.75 is suggested for air particulates. For example, to better assess the potential inhalation exposure risks of polychlorinated biphenyls, polychlorinated naphthalenes, polychlorinated dibenzo-p-dioxins, and dibenzofurans in metallurgical plants, Yang et al. (2017) estimated inhalation exposure to the chemicals on the basis of an alveolar fraction of particulates retained in the lung of 0.75. Then the inhalation exposure to the contaminants was estimated according to the following equation:

$$\mathrm{Inh} = (V_r \times C_{air} \times f_r \times t_f) \Big/ \mathrm{BW} \quad (1)$$

where Inh (fg TEQ/kg/day) is inhalation exposure of a contaminant; V_r (20 m^3/day) is ventilation rate; C_{air} (fg TEQ/m^3) is the ambient air concentration of the contaminant; t_f (dimensionless) is the exposed time fraction; BW (kg) is the body weight; f_r (dimensionless) is the alveolar fraction retained in lung, i.e., deposition bioaccessibility named in the present review. In addition, to estimate the health risks, hazard quotient (HQ) for non-carcinogenic pollutants and lifetime cancer risk (LCR) for carcinogenic pollutants, respectively, which are calculated based on the following equations:

$$\mathrm{HQ} = \mathrm{Inh}/\mathrm{RfD} \quad (2)$$

$$\mathrm{LCR} = \mathrm{Inh} \times \mathrm{CSF} \quad (3)$$

where RfD (mg/kg-bw/day) is reference dose; CSF is the cancer slope factor (mg/kg-bw/day)$^{-1}$. The risk level of 1×10^{-6} is often used as the lower end of the range of acceptable risk for carcinogenic pollutants.

However, particle size in the atmosphere varies from several nanometers to hundreds of microns, with different deposition mechanisms in the respiratory tract according to size. Depending on particle size, several deposition mechanisms, including impaction, gravitational sedimentation, and Brownian diffusion, occur in the respiratory tract (Morman and Plumlee, 2013). It is generally accepted that only particulate matter with an aerodynamic diameter less than 10 μm (PM_{10}) is easy to suspend in the atmosphere for a sufficient time and to deposit in the tracheal-bronchial system together with the alveolar region to cause health risks by inhalation (Schaider et al., 2007). Impaction takes place when particulates with aerodynamic diameters larger than 1.5 μm maintain their trajectory and hit the airway walls in the upper respiratory tract, although the direction of the air stream changes along the respiratory tract. For particulates with aerodynamic diameters larger than 0.5 μm, gravitational sedimentation occurs mainly in mid-size and smaller bronchioles and alveoli. Brownian diffusion involves sufficiently small particulates with aerodynamic diameters less than 0.5 μm and molecular bombardment of these particulates leading to random motion as gas molecules (Carvalho et al., 2011).

Different particulates lead to different deposition fractions in the respiratory tract. According to the International Commission on Radiological Protection (Carvalho et al., 2011), the total deposition fraction of inhaled particulates decreased in the respiratory tract with decreasing particle size to submicron dimensions. Then this value gradually increased back to 100% as particle size decreased further to nanometric scale. Only 20% of 0.1–1 μm particulates can deposit in the respiratory tract, and 80% are subsequently exhaled. It has been argued that the minimum may be determined by the balance of diffusion of impaction and gravitational sedimentation mechanisms (Finlay, 2001). Half of inhaled particulates with 0.02–0.1 μm deposit in the alveolar region, and 20% deposit both in the tracheobronchial and extrathoracic region. Unfortunately, particulates with aerodynamic diameters less than 0.02 μm mostly deposit in the extrathoracic region. In addition, Rissler et al. (2017) investigated the deposition fraction of inhaled particulates over a wide size range (15–5000 nm) for 67 healthy subjects aged 7–70 years at relaxed breathing using an aerodynamic particle sizer. They found that the minimum deposition fraction in the respiratory tract occurred in the 300–500 nm diameter range for both adults and children. The average deposition fraction for adults was 11% lower than that for children, although the difference was not statistically significant ($p = 0.21$). The gender difference observed in the deposition fraction was minor and not significant (6% lower deposition fraction for males, $p = 0.13$).

Therefore, to estimate more accurately the human exposure to contaminants in particulates, it is necessary to consider not only deposition bioaccessibility, but also the influence of particle size on deposition bioaccessibility. Recently, to estimate more accurately the human exposure to phthalates in $PM_{2.5}$ from Shenzhen, China, Lu et al. (2018) used different deposition bioaccessibility rates for children and adults, males and females. For different populations, particulates of different sizes can deposit in varied fractions in various regions of the respiratory tract, resulting in different bioaccessible fractions of contaminants in the human lung. These differences should be taken into account when assessing exposure to air particulates by inhalation. Unfortunately, there is too little information on the effect of deposition bioaccessibility on human exposure to particulates in air through inhalation, although this factor is recommended to be added to assessments in China and the United States.

6. Summary and perspective

The harmful effects of contaminants in particulate matter should not be overlooked because of the possibility that they are toxic to humans. To estimate human exposure to air contaminants more accurately, inhalation bioaccessibility should be added to the calculation. Currently, estimation methods for oral ingestion of contaminants are shifting away from total contaminant concentrations towards methods that factor oral bioaccessibility into the

estimate. The release bioaccessibilities of contaminants in particulates has been studied, although these studies were mainly focused on the factors influencing contaminant release. Although assessment of release bioaccessibilities of particulate-bound contaminants is progressively being used as a low-cost and rapid approach to assess inhalation exposure risk, significant methodological differences are tremendous impediments to standardization of *in-vitro* methods, especially for external factors including pH, extraction time, sorption sinks, and the S/L ratio. Existing methods for release bioaccessibility measurement of particulate-bound pollutants by simulated human lung fluids lack uniform standards. The composition of simulated lung fluids and the relative influencing factors of *in-vitro* methods can exert a significant effect on the calculated release of particulate-bound contaminants. In this present review, the measurement of release bioaccessibilities of pollutants was reviewed to investigate human exposure and to refine the criteria of bioaccessibility assays.

Considering the complexity of fluids in the lung with their biological pH levels and ionic strengths, many studies have demonstrated that simple chemical solutions and sequential extraction techniques are not suitable. With the development of physiologically based simulated human lung fluid, Gamble's solution has undergone several modifications while keeping similar basic chemical constituents. Some modifications have been made by adding specific chemicals and/or changing the amount of chemicals added. It is well known that a fraction of particulates will be swallowed by macrophages within hours after deposition and that the acidic condition inside macrophages is quite different from the extracellular neutral environment. Therefore, Gamble's solution and ALF are commonly used in current studies to represent two areas of the lung. However, it is difficult to ascertain which kinds of solution represent the most appropriate simulation of the interstitial region of the lung because of the lack of validation by *in-vivo* methods.

Until now, no research has reported on the harmonisation of in vitro methods for bioaccessibility and calibration with in vivo data on bioavailability in terms of particle inhalation, although studies on the relationship of bioaccessibility and bioavailability via oral ingestion have been carried out (Juhasz et al., 2009; Yu et al., 2019). Currently, novel in vitro method containing simulated human lung fluid should urgently be developed to measure the deposition bioaccessiblity of particulate matter. In addition, the experimental parameters should be optimized based on the deposition data obtained by subjects. Subsequently, a lung simulator containing simulated human lung fluid simulating the process of human breathing was developed, and the particulate matter deposits in the lung simulator (Yu et al., 2020). Then contaminants dissolved in simulated human lung fluids will be collected and particle-associated insoluble fractions will be leached by organic solvents. Thus, insoluble bioaccessible contaminants can be incorporated into predictive models. Of course, this is just the beginning. Much more studies should be conducted in the future.

Currently, there are two major challenges for the measurement and application of inhalation bioaccessibility in human health risk assessment. First, a unified, accurate, stable, simple, and systematic *in-vitro* release bioaccessibility method is needed that is biologically relevant. As the authors suggested in the section on selection of physiological parameters, the release bioaccessibility can be measured under a worst-case scenario. However, in terms of our knowledge, the authors believe that the investigation on release bioaccessibility using simulated human lung fluid is more suitable to explore the release mechanism of pollutants in particulate matters, rather than using the release bioaccessibility obtained in the assessment of human health risk through inhalation, which is similar to oral bioaccessibility measured in a simulated human gastrointestinal tract. This is the case because the matrices entering human bodies have very different behavior between the human lung and the gastrointestinal tract, as mentioned earlier. Second, to perform an accurate assessment of the human health risk of airborne particulates through inhalation and to add inhalation bioaccessibility to the assessment, the factor used should be deposition bioaccessibility, rather than release bioaccessibility. Actually, although some data are available on the deposition bioaccessibility of inhaled particulates through inhalation by means of modeling approaches and animal subjects, the results of which have been well reviewed by Wei et al. (2018), the data on deposition bioaccessibility of air particulates in the human lung are too limited. In addition, the lack of *in-vivo* and *in-vitro* correlations for particle-bound metal(loid)s or organic contaminants in various matrices is a knowledge gap when this deposition bioaccessibility is used to assess exposure risk. With the further development of online particulate measurement techniques, more deposition data in the human lung can be obtained, similar to the study by Rissler et al. (2017). Overall, there is a long way to go before an *in-vitro* inhalation bioaccessibility measurement becomes available and before its application to the human health risk assessment of contaminants in particulates by inhalation becomes commonplace.

CRediT authorship contribution statement

Helong Ren: Methodology, Writing - original draft. **Yingxin Yu:** Methodology, Writing - review & editing. **Taicheng An:** Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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