In vitro oral and inhalation bioaccessibility of hydrophobic organic contaminants (HOCs) in airborne particles and influence of relevant parameters

Article *in* Environmental Research · December 2018

DOI: 10.1016/j.envres.2018.12.025

6 authors, including:

Yuan Zeng
Chinese Academy of Sciences
3 PUBLICATIONS **1** CITATION

SEE PROFILE

Environmental Research 170 (2019) 134–140

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

In vitro oral and inhalation bioaccessibility of hydrophobic organic contaminants (HOCs) in airborne particles and influence of relevant parameters

Yuan Zeng[a,b], Yun Fan[a,b], Xiao Yan[c], Jing Zheng[c], She-Jun Chen[b,*], Bi-Xian Mai[a]

[a] State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Environmental Protection Key Laboratory of Environmental Pollution Health Risk Assessment, South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou 510655, China

ARTICLE INFO

Keywords:
Halogenated flame retardants (HFRs)
Organophosphorus flame retardants (OPFRs)
Polycyclic aromatic hydrocarbons (PAHs)
$PM_{2.5}$
Lung fluids
Hydrolysis

ABSTRACT

The bioaccessibility of environmental contaminants has been assessed widely using in vitro simulation; however, the physiological parameters used vary greatly. In this study, we assessed the influence of various physiological parameters and food material on the oral or inhalation bioaccessibility of $PM_{2.5}$-bound hydrophobic organic contaminants (HOCs), including halogenated flame retardants (HFRs), organophosphorus flame retardants (OPFRs), and polycyclic aromatic hydrocarbons (PAHs). The results showed that physiologically based pepsin and pancreatin have a small influence on the HOC liberation from particles. The bioaccessibility increased dramatically when the bile salt concentrations exceeding the critical micelle concentration, and application of porcine bile salts probably lead to underestimated bioaccessibility. Protein and carbohydrates significantly increased the bioaccessibility of most HOCs, while a significant bioaccessibility reduction was caused by green tea. The bioaccessibility of most HOCs was not promoted by liquor under normal physiological condition, but was significantly promoted under fast condition. Long residence time of $PM_{2.5}$ in the lung (15 days) would result in higher mobilization of PAHs into the lung fluid than short time (one day). However, the inverse time-dependence for OPFRs suggests degradation in the lung fluid. A mechanism of hydrolysis of organophosphorus ester is hypothesized, and the half lives ranged from 17 to 90 days.

1. Introduction

In risk assessments, assumption of 100% bioaccessibility of the amount of contaminants in matrices tends to overestimate human exposure, because many pollutants are not completely available. Contaminant bioavailability is therefore essential for risk assessment and attracts growing body of scientific research (Rostami and Juhasz, 2011; Collins et al., 2015). Physiologically based in vitro methods, which utilize artificial gastrointestinal and lung fluids to simulate the digestive and respiratory conditions, are widely used for bioaccessibility assessment because they are rapid, inexpensive and have no ethical issues (Cui et al., 2016; Kastury et al., 2017). The methods have also been applied for hydrophobic organic contaminants (HOCs) in matrices such as soil, indoor dust, combustion particles, and food (Xing et al., 2008; Yu et al., 2013; Fang and Stapleton, 2014; Li et al., 2015; Juhasz et al., 2016; Zhang et al., 2016).

A series of digestive enzymes (e.g., pepsin, trypsin, pancreatin, and amylase), bile salts, and bicarbonate are basic or central constituents of artificial gastrointestinal fluids used for both static and dynamic in vitro bioaccessibility assays (Shani-Levi et al., 2017). These components perform their functions in digesting food and consequently also in mobilization of contaminants from the matrices in the gastrointestinal tract (Rostami and Juhasz, 2011). However, there is enormous variation in their compositions between the in vitro models described in the literature (see references cited in the Supplementary material). For instance, a wide range of contents of pepsin (1.5–3125 U/mL) and pancreatin consisting of lipase (0.12–144 U/mL), α-amylase (0.24–288 U/mL), and trypsin (0.023–27 U/mL) have been used in previous studies. The commercial bile salts from different sources such as porcine, bovine, and chicken origins have also been used, differing in their activity/concentration and physicochemical properties. Although they vary with individuals and fast/fed conditions in the body, the

* Corresponding author.
E-mail address: chenshejun@gig.ac.cn (S.-J. Chen).

https://doi.org/10.1016/j.envres.2018.12.025
Received 22 October 2018; Received in revised form 25 November 2018; Accepted 13 December 2018
Available online 14 December 2018
0013-9351/

inconsistency in these and other parameters in in vitro models may exert a substantial influence on the bioaccessibility evaluation, hindering the possibility to compare results across studies. The effects of some simulated parameters on oral bioaccessibility of HOCs have been assessed, such as bile salt (Tang et al., 2006; Zhang et al., 2015), incubation time (Yu et al., 2011; Starr et al., 2016), simulated fluid pH (Zhang et al., 2015), food materials (Hack and Selenka, 1996; Cave et al., 2010; Zhang et al., 2015), and microbiota (Molly et al., 1993; Van de Wiele et al., 2005). However, most studies assessed only a few parameters, and the influence of multiple factors on HOC bioaccessibility is not adequately understood.

Most in vitro studies have focused on oral bioaccessibility, whereas the bioaccessibility of particle-bound HOCs inhaled into the respiratory system is poorly understood, which may lead to significant adverse human health effects (Anderson et al., 2012). Recently, bioaccessibility of phthalate esters via indoor dust inhalation were evaluated using artificial lung fluids (Kademoglou et al., 2018). Parameters used in lung models are also variable and have rarely been elaborated (Julien et al., 2011).

In the present study, oral and inhalation bioaccessibility of a range of HOCs, including halogenated flame retardants (HFRs), organophosphorus flame retardants (OPFRs), and polycyclic aromatic hydrocarbons (PAHs), in airborne fine particles ($PM_{2.5}$, aerodynamic diameters less than 2.5 μm) collected in a megacity in China was assessed. These chemicals have been proved ubiquitous in various environmental media and have a wide range of physicochemical properties (Lafontaine et al., 2015; Wang et al., 2018). The main objective is to assess the influence of some basic parameters used in in vitro models on the bioaccessibility of HOCs, which are rarely or not adequately elucidated. Such a study will improve the in vitro HOC bioaccessibility assessment methodology. We also aim to understand the overall bioaccessibility of these HOCs associated with urban fine PM which may be inhaled into the deep parts of the lung or trapped in the upper respiratory tract and swallowed into the digestive tract (Maynard and Kuempel, 2005), to aid in the health risk assessment for HOCs.

2. Material and methods

2.1. In vitro assay design

Urban $PM_{2.5}$ samples were collected on Whatman quartz fiber filters for 48 h, using active large-volume air samplers (TE-6001, Tisch Environment Inc., US) at a flow rate of 1.13 m^3/min. The loaded filter was wrapped in aluminum foil, sealed in a polyethylene zip bag, and stored at − 20 °C prior to the assays. The reagents used for the assays are described in Table S1 in the Supplementary material.

The basic compositions, conditions, and assessed physiological parameters of the simulated fluids (saliva, gastric fluid, and intestinal fluid) in the digestion models are summarized in Table 1. For this simulation, parameters of pepsin, pancreatin, bile salts, and food materials were assessed. Specifically, pepsin with activity levels of 0, 2.67, 26.7, and 267 U/mL, two types of pancreatin (designated as pancreatin #1 and pancreatin #2), and bile salts with levels of 0, 0.3, 1.5, 3, and 4.5 g/L were involved. Pancreatin is a mixture of lipase, α-amylase, and trypsin. Pancreatin #1 prepared in our laboratory has a composition close to the human physiology (64.26:1.81:1 for lipase, α-amylase, and trypsin), and pancreatin #2 is commercial pancreatin P7545 (5.33:10.7:1) that has been used in many studies (Table S2). Also, two commonly used bile salts (bovine and porcine) were compared. Based on the developed gastrointestinal fluids, the effects of food on the bioaccessibility were further evaluated separately via addition of carbohydrates and protein, tea, and liquor which are ingested by humans every day or have been scarcely investigated (Table S3).

For pulmonary simulation, two typically used artificial lung fluids (Gamble's solution and artificial lysosomal fluid) and extraction time (1 and 15 days, representative of different residence times of particles in the lung) were assessed. Gamble's solution (with pH of 7.4) simulates the interstitial fluid deep in the human lung; while the acidic artificial lysosomal fluid (pH = 4.5) represents the ingestion venue of $PM_{2.5}$ by macrophages (Pelfrene et al., 2017).

2.2. Bioaccessible extraction

Several $PM_{2.5}$ loaded filters used in an assay were combined and divided into subsample replications, each corresponding to a 48-h sampling period. For each extraction, triple subsamples were applied. The procedures used for gastrointestinal and lung simulations here have been given in recent studies (Dean and Ma, 2007; Yu et al., 2011; Zereini et al., 2012; Li et al., 2016). The subsample filters were cut into small pieces and immerged into artificial saliva (about 11 mL) in a 500 mL flask based on a solid/liquid (S/L) ratio of 1:160. The flasks were hand-shaken for 5 s and artificial gastric fluid (about 140 mL) was added based on a S/L ratio of 1:2000 (Ellickson et al., 2001). The flasks were filled with nitrogen, sealed, and shaken in an incubator at 37 °C for 2 h under conditions. Same volume of intestinal fluid was then added and incubated for 5 h. The filters for lung fluid assays were prepared in the same way described above. The cut filters were mixed with lung fluid (about 70 mL) in a 250 mL flask based on a S/L ratio of 1:1000 as suggested previously (Julien et al., 2011). The solutions were incubated at 37 °C and shaken at a rate of 50 rpm for 10 min every 4 h, continuing for 1 and 15 days, respectively.

2.3. Chemical analysis and quality control

The analytical method was given in detail in the Supplementary material and was described briefly here. After the bioaccessible extraction, the solutions were filtered successively through a filter paper and a Whatman quartz fiber filter (pore size, 0.70 μm). The aqueous solution was treated with liquid-liquid extraction method, after being spiked with surrogate standards. The extracts were then purified and

Table 1
Compositions, conditions, and preparation of the stimulated fluids.

Type	*Composition, condition, and preparation*
Saliva	Mucin (4.0 g), urea (1.0 g), $CaCl_2{\cdot}4H_2O$ (0.99 g), Na_2HPO_4 (0.6 g), KCl (0.4 g), and NaCl (0.4 g) was dissolved in 1 L deionized water. Solution's pH was adjusted to 5.5 with HCl (aq) (Dean et al., 2007; Yu et al., 2011).
Gastric fluid	*NaCl (2 g) was dissolved in 7 mL HCl (aq) and diluted with 250 mL deionized water. Pepsin (0, 0.0107 g, 0.1068 g, and 1.0680 g) was added and diluted with deionized water to 1 L.* Solution's *pH = 2* (Dean et al., 2007; Yu et al., 2011).
Small intestine fluid	*$NaHCO_3$ (16.8 g) was dissolved in deionized water, and pancreatin (5.968 g) and bile salts (0, 0.624 g, 3.12 g, 6.24 g, and 9.36 g) were added. The solution was diluted with deionized water to 1 L.* Solution's *pH = 7.5* (Dean et al., 2007; Yu et al., 2011).
Gamble's solution	$MgCl_2{\cdot}6H_2O$ (0.203 g), NaCl (6.019 g), KCl (0.298 g), Na_2HPO_4 (0.126 g), Na_2SO_4 (0.063 g), $CaCl_2{\cdot}2H_2O$ (0.368 g), CH_3COONa (0.574 g), $NaHCO_3$ (2.604 g), sodium citrate (0.097 g), NH_4Cl (0.118 g), dipalmitoylphosphatidylcholine (0.202 g) was *dissolved in 1* L *deionized water.* Solution's *pH = 7.4* (Li et al., 2016; Zereini et al., 2012).
Artificial lysosomal fluid	$MgCl_2{\cdot}6H_2O$ (0.107 g), NaCl (3.210 g), Na_2HPO_4 (0.071 g), Na_2SO_4 (0.039 g), $CaCl_2{\cdot}2H_2O$ (0.128 g), sodium citrate (0.077 g), NH_4Cl (0.118 g), NaOH (6 g), citric acid (20.8 g), glycine (0.059 g), disodium tartrate dihydrate (0.090 g), sodium lactate (0.085 g), sodium pyruvate (0.086 g) was *dissolved in 1* L *deionized water.* Solution's *pH = 4.5* (Li et al., 2016; Zereini et al., 2012).

fractionated with a solid-phase extraction cartridge (Supelclean ENVI-Florisil, 3 mL, 500 mg). The solid phase was freeze-dried and Soxhlet extracted with a mixture of acetone/hexane (1:1). Subsequent treatment for the extracts was identical to that for the aqueous solution. HOCs consisting of 12 OPFRs, 20 HFRs, and 18 PAHs (Table S4) were analyzed using an Agilent 7890 gas chromatograph coupled with an Agilent 5975 mass spectrometer.

Procedural blanks (simulating fluid for aqueous solutions and clean quartz fiber filter for solid phases, $n = 14$) were run with samples. Only trace amount (< 3% of their amounts in most sample extracts) of HOCs were found in the blanks, and the results were blank-corrected accordingly. The recoveries of surrogate standards (mean ± standard deviation) ranged from 32.7 ± 33.0% to 101 ± 17.8% for PAHs, and from 76.1 ± 24.2% to 102 ± 13.1% for OPFRs, and from 92.5 ± 23.3% to 106 ± 26.4% for HFRs. Reported concentrations were not surrogate-recovery corrected.

3. Results and discussion

3.1. HOC concentrations in $PM_{2.5}$

HOC concentrations in $PM_{2.5}$ used in the assays are given in Table S5. The mass-normalized total concentrations of OPFRs, PAHs, and HFRs ranged from 50.4 to 158 μg/g, from 17.5 to 456 μg/g, and from 0.9 to 28.9 μg/g, with means of 86.9, 132, and 6.8 μg/g, respectively. Of the HFRs, polybrominated diphenyl ethers (PBDEs) were dominant, with mean concentration of 6826 ng/g (890–28,940 ng/g), but lower brominated congeners (tri- through hexa-BDEs) were not found in the samples. The mean concentrations were 3811 ng/g (400–11,280 ng/g) for decabromodiphenyl ethane (DBDPE) and 113 ng/g (< 308 ng/g) for Dechlorane Plus (DPs).

3.2. Effect of enzymes and bile salts

The bioaccessibility under different pepsin enzymatic activities was shown in Fig. 1. The bioaccessibility values for tri(2-chloroethyl) phosphate (TECP), tris(1-chloro-2-propyl) phosphate (TCPP), tris(1,3-dichloropropan-2-yl) phosphate (TDCPP), and triphenyl phosphate (TPhP) (72–99%) were apparently higher than those for other OPFRs such as 2-Ethylhexyl diphenyl phosphate (EHDPP), tricresyl phosphate (TCrP), and tris(2-ethylhexyl) phosphate (TEHP) (< 23%). The bioaccessibility results for PAHs ranged from 4.2% to 25%, higher values were observed for phenanthrene (PHE), fluoranthene (FLT), and pyrene (PYR) (19–26%). HFRs had the lowest bioaccessibility, which was nearly not found in the simulated gastric fluid. In previous bioaccessibility models, pepsin activities from 1.5 to 3125 U/mL have been applied (Table S6). The pepsin activities of the gastric juices measured from the 18 volunteers by Ulleberg et al. (2011) ranged from only 7–70 U/mL (with an average of 26.7 U/mL). Our result showed a slight increase in the bioaccessibility for most of these HOCs under normal physiological pepsin level compared to default condition (with no pepsin addition), except for a few compounds with an increase of over 30% (Fig S1 for individual HOCs). Application of 10-fold higher level of pepsin (267 U/mL) in in vitro assay also resulted in small increases in the bioaccessibility (12 ± 11% for PAHs and 3.1% for OPFRs) relative to normal condition. This indicated that pepsin activity has a small influence on the liberation of HOCs from airborne particles in the stomach. This probably because pepsin enzyme in the stomach breaks down proteins (into smaller peptides) but not carbohydrates and fats. Thus, it is possible that pepsin may have a significant influence on HOC liberation from protein-rich food (e.g., fish meat).

In the presence of pancreatin #1, there was no noticeable increase in the bioaccessibility for most HOCs either (1.6%–166% for PAHs, −32% to 68% for HFRs, and −6.55% to 44% for OPFRs) compared to the default intestinal fluid (Fig. 1 and Fig. S2). In contrast, pancreatin #2 significantly elevated the bioaccessibility of most HOCs (1.8–2.2 times on average, excluding four OPFR with high bioaccessibility) ($p = 0.013$, Mann-Whitney Rank Sum test). The enhancing effect of pancreatin #2 in the assay was likely related to the α-amylase activity in the synthetic fluids (26.8 U/mL for pancreatin #1 versus 91.8 U/mL for pancreatin #2). α-Amylase has a hydrophobic cavum for binding in particular planar molecules and is more hydrophobic than the other two enzymes (lipase and trypsin) (Xiao et al., 2011). The finding has an implication that employment of different pancreatins in in vitro models may lead to considerable uncertainties in the bioaccessibility of HOCs.

Although bile salts have been proved to be a key factor influencing HOC bioaccessibility and have been identified and explained by formation of bile salt micelles with HOCs in numerous studies (Oomen et al., 2000; Tang et al., 2006; Zhang et al., 2015), the concentration-dependent effects is scarely elucidated. Furthermore, bile salts of various origins (e.g., porcine, bovine, chicken) have been used.

The effects of bile salts (Fig. 1 and Fig. S3) showed that the bioaccessibility values of HOCs increased with the increase of bile salt concentrations, except for four OPFRs (TCEP, TCPP,TDCPP, and TPhP) which had consistently high bioaccessibility. There was an obvious increase in the bioaccessibility for most HOCs even with the addition of small amount of bile salts (with a concentration of 0.03% in the digestive fluid). The increases were relatively modest (approximately 2-fold on average for the HOCs) when bile salt concentrations increased from 0.03% to 0.15% compared to the dramatical increase (5.0, 5.6, and 7.1 times for OPFRs, PAHs, and HFRs, respectively) for bile salt concentration increase from 0.15% to 0.30%. It was followed by a more modest increase (20%–23% on average) for bile salt concentrations increasing from 0.30% to 0.45%. The results revealed that bile salts are a critical parameter for bioaccessibility of HOCs in the gastrointestinal tract compared to pepsin and pancreatic enzymes in vitro models.

Although the considerably enhancing mobilization effect of bile salts for HOCs has been identified in numerous studies (Oomen et al., 2004; Zhang et al., 2015), the dependence of HOC bioaccessibility on bile salt concentrations is not well known. We found that the strong effect occurred when bile salt concentrations exceed the critical micelle concentration (CMC), which was determined at 1.73 g/L in this study (Fig. S4). This result confirmed a mobilization mechanism of formation of bile salt micelles with HOCs. Oomen et al. also observed bile concentration-dependent mobilization of polychlorinated biphenyls from soil, but the CMC of chicken bile used in that study was unknown (Oomen et al., 2000). A recent study found the bioaccessibility of soot PAHs increased with increasing amounts of bile salts (Zhang et al., 2015). However, the concentrations of porcine bile salts (with hyodeoxycholic acid salts being the primary component) were all below the CMC (around 20 g/L) (Alava et al., 2013), substantially differing from that of the human bile salts (around 1.8 g/L, with main components of cholic acid sodium and deoxycholic acid sodium) measured here and a previous studies (Luo, 2014). The typical bile salt concentrations in the intestinal tract vary from 0.1% to 0.3% under fast condition and from 0.5% to 1% under fed condition (Rautureau et al., 1981). Therefore, physiologically normal bile salt levels would play a very important role in mobilization of HOCs in the digestive system. A large number of studies have used porcine bile salts for in vitro bioaccessibility assessment as shown in Table S7. We therefore compared the HOC bioaccessibility between porcine bile salts and bovine bile salts that have constituents similar to those in humans. The values for bovine bile salts were substantially higher than those for porcine bile salts (10, 7.7, and 1.3 times for PAHs, OPFRs, and HFRs, respectively), both at a concentration of 0.3% in the fluid (Fig. S5). The porcine bile salts couldn't mobilize the HOCs at a concentration of 0.3% due to it is under the CMC and couldn't form micelle. Bile salts (concentration as well as type) therefore are key variables that should be paid particular attention in in vitro models. Our results indicated that application of porcine bile salts probably lead to underestimation of HOCs bioaccessibility.

Overall, although the human physiological conditions vary with food ingestion or other factors, we recommend that parameters

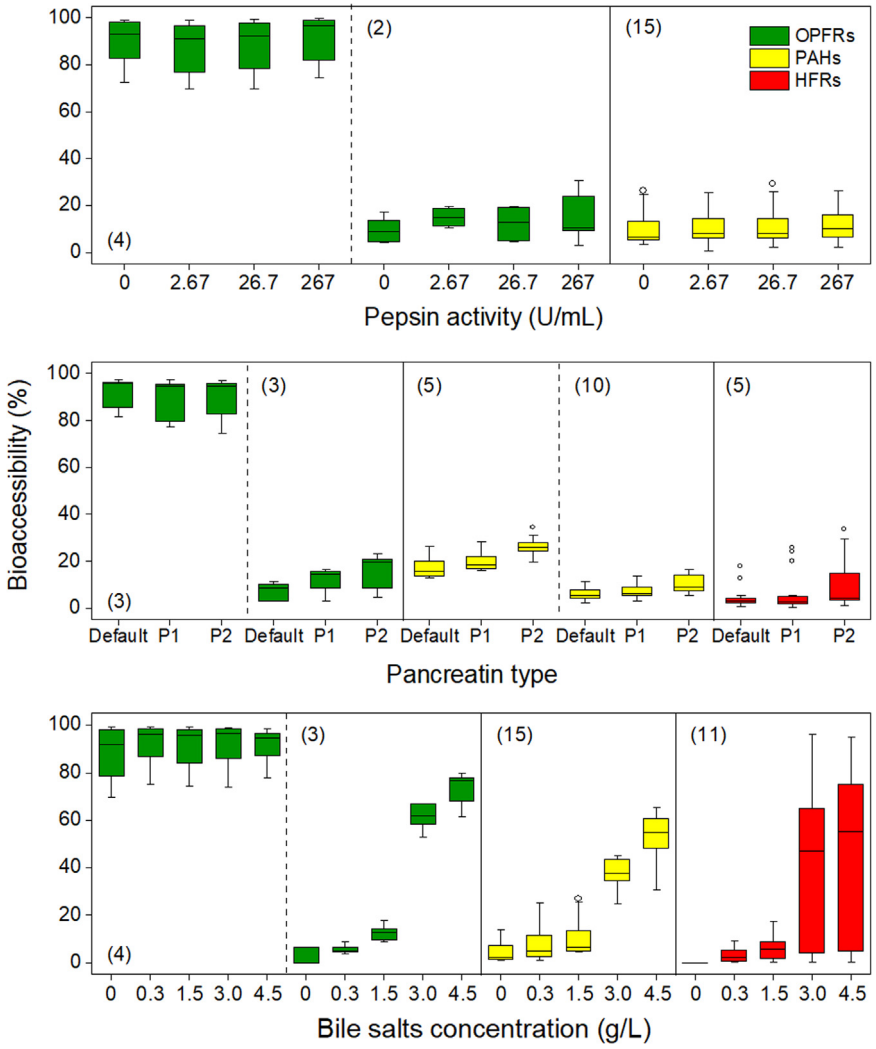

Fig. 1. Effect of pepsin, pancreatin, and bile salts on the oral bioaccessibility of $PM_{2.5}$-bound HOCs. P1 and P2 represent the pancreatin #1 and #2, respectively. HOCs with similar bioaccessibility were grouped, and numbers in the brackets are the numbers of compounds included in the groups.

representative of the physiology be used in digestive model for assessment of bioaccessibility. A standardized method should be established for general population for comparison between studies. Using the physiologically normal parameters (26.7 U/mL pepsin, pancreatin #1, and 0.3% bovine bile salts) the oral bioaccessibility values of $PM_{2.5}$-bound HOCs were 16%–44% for PAHs, 1.4%–64% for HFRs, and 19%–97% for OPFRs.

3.3. Effect of food material

It has been shown that inclusion of food additives containing lipid (e.g., milk powder, vegetable oil, and cream) can facilitate mobilization of HOCs from solid matrices into digestive fluid (Hack and Selenka, 1996; Cave et al., 2010; Zhang et al., 2015). Our results indicated that addition of these food nutrients (carbohydrates and protein) also significantly increased the bioaccessibility (2-fold on average for the HOCs, $p < 0.001$) (Fig. 2 and S6). This was consistent with previous results that HOCs can sorb to proteins and carbohydrates after digestive degradation (Oomen et al., 2000; Tilston et al., 2011), but in contrast to the weak effect for soot PAHs (Zhang et al., 2015).

In the presence of green tea, there was a significant reduction (33 ± 14% for PAHs, 15 ± 9.5% for OPFRs, and 23 ± 16% for HFRs) in the bioaccessibility for the HOCs ($p < 0.014$), with exception of several high molecule weigh FRs such as BDE209, 1,2-bis-(2,4,6-tribromophenoxy)ethane (BTBPE) andDechlorane Plus (DPs) (Fig. S7). Tea catechins (derivatives of flavans) are a class of polyhydroxy compounds with high polarity. Thank to this property, catechins can enter into the internal of bile salt micelles and bond with amphipathic molecules directly in form of hydrogen bond, which is stronger than the van der Waals force between the bile salt molecules and HOCs. Thus, the competition between catechins and HOCs for bile salt molecules reduced the release of HOCs from particles in the small intestine.

Liquor did not obviously promote the bioaccessibility of these HOCs under both the conditions (0.67% and 6.7% of alcohol in the simulated fluid), except for PAHs (32 ± 13%) under the 6.7% alcohol condition (Fig. 2 and S8). This was surprising considering the high solubility of these HOCs in alcohol. We also assessed their mobilization under simulated fast condition (bile salt concentration = 0.15%). Interestingly, the bioaccessibility values were promoted by liquor for most of the HOCs (1.2–3.2 times under 0.67% alcohol condition and 2.6–7.9 times under 6.7% alcohol condition) (Fig. S9). Our simulation suggests that bile salts are the predominant factor mobilizing of HOCs from the matrix in the intestinal tract compared to alcohol. However, it is worth note that alcohol administration can increase the permeability of the intestinal mucosa, enhancing the transport of toxins across the intestinal walls as shown in previous research (Bode and Bode, 1997).

3.4. Difference in HOC bioaccessibility

Fig. S1–9 indicate varying bioaccessibility values for the individual

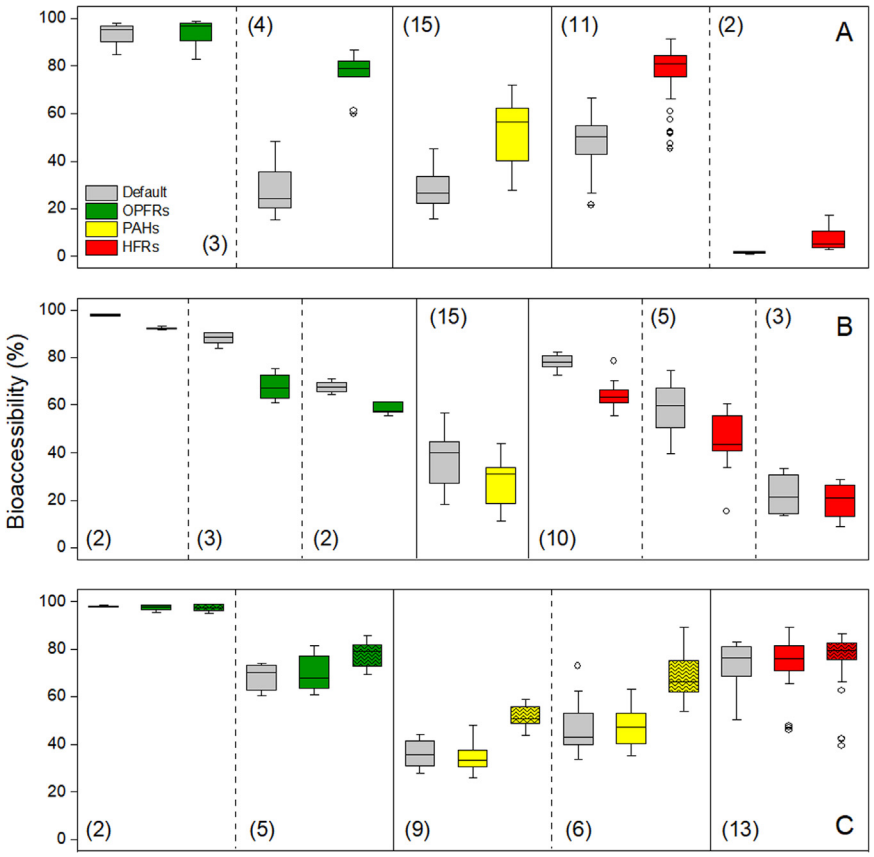

Fig. 2. Effect of nutrients (A), green tea (B), and liquor under 0.67% and 6.70% (with zigzag pattern in the boxes) vol of alcohol conditions (C) on the oral bioaccessibility of $PM_{2.5}$-bound HOCs. HOCs with similar bioaccessibility were grouped, and numbers in the brackets are the numbers of compounds included in the groups.

HOCs. Generally, compounds with lower octanol/water partition coefficients (K_{OW}) (Table S8) had higher bioaccessibility. Four OPFRs (TCEP, TCPP, TDCPP, and TPhP) showed apparently the highest bioaccessibility and the values were little influenced by the investigated parameters. The lowest bioaccessibility was observed for HFRs with high K_{OW}. However, under condition of high-concentration bile salts (0.30%–0.45%), the bioaccessibility values showed small differences among each class of HOCs due to the enhancing effect of bile salts for high K_{OW} compounds. It should be noted that the bioaccessibility was affected by various factors such the concentrations in particles, affinity to particles, and particle physicochemical properties (Yu et al., 2013).

The influence of the measurement parameters on the bioaccessibility was assessed for the individual HOCs by calculating the percentage differences between the control and parameterized conditions. As displayed in Fig. S10, there was roughly exponential increase in the percentage differences with the log K_{OW} values for lower log K_{OW} HOCs (< 8, including OPFRs, PAHs, and several PBDEs), indicating a more robust effect of these parameters for higher K_{OW} HOCs. The low concentrations in the particles may be a possible reason for the deviation of HFRs from this trend.

3.5. Lung fluid simulation

The bioaccessibility values of PAHs in the 1-day simulation ranged from 0.03% to 24% (with a mean of 2.5%), which were significantly lower than those of the 15-day simulation ranging from 0.7% to 24.5% (with a mean of 6.5%) ($p = 0.006$) (Fig. 3 and S11). Similar to the results of digestive tract simulation, TCEP, TCPP, and TDCPP showed the highest bioaccessibility (55%–97%) and the values were little affected by the simulation time (one- versus 15-day) due to the high water solubility of these compounds. Relatively, the results for TPhP (26%), EHDPP (1.2%), and tricresyl phosphate (TCrP) (2.3%) were obviously lower. Surprisingly, however, the apparent bioaccessibility for some OPFRs such as TPhP, EHDPP, and TCrP in the 15-day simulation was even lower than the one-day simulation result. HFRs were nearly bioaccessible even in the 15-day simulation, partly because of their low concentrations in the $PM_{2.5}$. Thus, inhalation risk of these contaminants without considering their bioaccessibility in the lung fluid would be substantially over-evaluated. There were no significant differences in the extraction efficiency between the Gamble's solution and artificial lysosomal fluid for most HOCs (Fig. S11).

Our explanation for the inverse dependence of mobilization of some OPFRs on simulation time is degradation of these compounds in the lung fluid. The reduced amount (liquid + solid phases) in the 15-day simulated fluid (Fig. S12) also supported this explanation. TPhP showed the most efficient hydrolysis (97% within 15 days), followed by TCrP (86%) and EHDPP (78%) in Gamble's solution. The degradation in the weakly basic Gamble's solution was much more significant than that in the acidic artificial lysosomal fluid. We therefore hypothesize a mechanism of hydrolysis of ester in the simulated fluid that organophosphorus esters are catalyzed by hydroxyl ions (Su et al., 2016).

An experiment was conducted to examine the kinetics of the hydrolytic degradation of OPFRs in lung fluid during a period of 31 days (Fig. 4). The most pronounced degradation was observed for TPhP, which begun between 1 and 3 day, with a half life of 17 days. TCrP, TEHP, and EHDPP showed similar degradation rates (with half lives of 85–90 days) starting between the 3–5 day. No degradation was found for other OPFRs in the fluid except for TDCPP with a slow rate. Several degradation products (phosphate diesters) were also found in the solution (Fig. 4). To the best of our knowledge, this is the first observation of degradation of OPFRs in the lung fluid. A recent study investigated the hydrolysis of house dust OPFRs in digestive fluids over a physiological residence time (27 h), but no significant degradation was observed (Fang and Stapleton, 2014). The results also indicated that the degradation of OPFRs (commercial standards) in the kinetics experiment (16%–70%) were substantially slower than those ($PM_{2.5}$-bound)

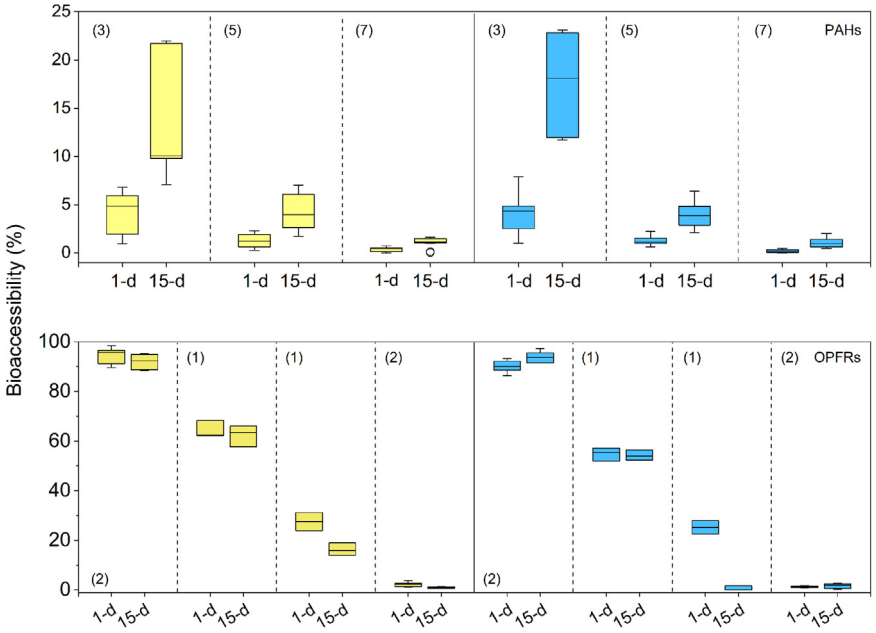

Fig. 3. Comparison of the inhalation bioaccessibility of HOCs in PM2.5 between 1- and 15-day simulations and in Gamble's solution (yellow boxes) and artificial lysosomal fluid (blue boxes). Numbers in the brackets are the numbers of compounds included in the groups.

in the bioaccessibility assay (10%–97%). Our explanation for the rapid hydrolysis of $PM_{2.5}$-bound OPFRs is the catalysis by metals ions or metallic oxides present in the particles as suggested in previous studies (Huang and Zhang, 2012).

4. Conclusion

We assessed the influence of various physiological parameters and food materials on the in vitro bioaccessibility of $PM_{2.5}$-bound HOCs. Bile salt is a critical factor influencing on the bioaccessibility of HOCs, and addition of food material in the simulated fluids also has a significant impact on the results; while the influence of pepsin and pancreatin is small. For the first time, we revealed that green tea would reduce significantly human oral bioaccessibility of HOCs, and liquor under normal physiological condition does not motivated the HOC liberation from particles. A standardized method should be established for comparison between studies. We recommend that parameters close to human physiology be used. The simulated time, but not the lung fluid types, influences the HOC inhalation bioaccessibility. The results suggest degradation of OPFRs in the deep lung fluid and mechanisms of hydrolysis was hypothesized. However, potential biotransformation mechanisms of oxidative metabolism in the lung fluid should be further examined.

Acknowledgements

This work was supported by the Program of Guangzhou Science and Technology (No. 20170702003); the National Natural Science Foundation of China (Nos. 41573088); the Key Research Program of Frontier Sciences of Chinese Academy of Sciences (No. QYZDJ-SSW-DQC018); the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (No. 2017BT01Z134); and the Science and Technology Project of Guangdong Province, China (No. 2014B030301060).

Competing interests

The authors declare no competing financial interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.12.025.

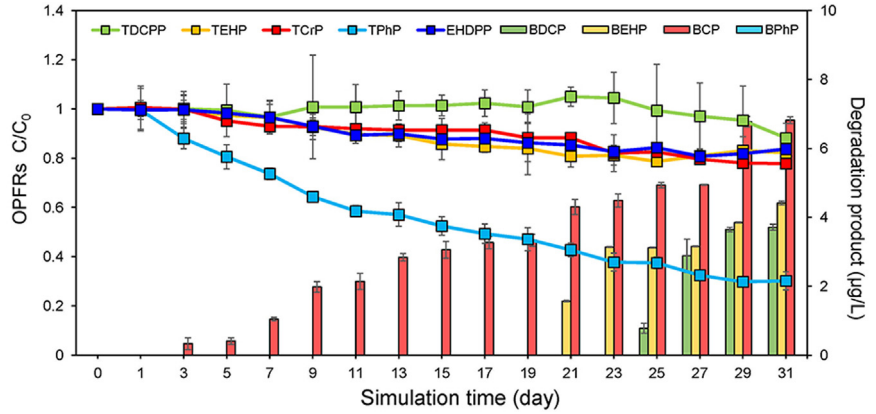

Fig. 4. Kinetics of hydrolytic degradation and hydrolyzates of OPFRs in Gamble's solution within 31 days.

References

Alava, P., Du Laing, G., Odhiambo, M., Verliefde, A., Tack, F., Van de Wiele, T.R., 2013. Arsenic bioaccessibility upon gastrointestinal digestion is highly determined by its speciation and lipid-bile salt interactions. J. Environ. Sci. Heal A 48, 656–665.

Anderson, J.O., Thundiyil, J.G., Stolbach, A., 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. J. Med. Toxicol. 8, 166–175.

Bode, C., Bode, J.C., 1997. Alcohol's role in gastrointestinal tract disorders. Alcohol Health Res. World 21, 76–83.

Cave, M.R., Wragg, J., Harrison, I., Vane, C.H., Van de Wiele, T., De Groeve, E., Nathanail, C.P., Ashmore, M., Thomas, R., Robinson, J., et al., 2010. Comparison of batch mode and dynamic physiologically based bioaccessibility tests for PAHs in soil samples. Environ. Sci. Technol. 44, 2654–2660.

Collins, C.D., Craggs, M., Garcia-Alcega, S., Kademoglou, K., Lowe, S., 2015. 'Towards a unified approach for the determination of the bioaccessibility of organic pollutants'. Environ. Int. 78, 24–31.

Cui, X.Y., Xiang, P., He, R.W., Juhasz, A., Ma, L.Q., 2016. Advances in in vitro methods to evaluate oral bioaccessibility of PAHs and PBDEs in environmental matrices. Chemosphere 150, 378–389.

Dean, J.R., Ma, R.L., 2007. Approaches to assess the oral bioaccessibility of persistent organic pollutants: a critical review. Chemosphere 68, 1399–1407.

Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B.T., Lioy, P.J., 2001. Oral bioavailability of lead and arsenic from a NIST standard reference soil material. Arch. Environ. Contam. Toxicol. 40, 128–135.

Fang, M.L., Stapleton, H.M., 2014. Evaluating the bioaccessibility of flame retardants in house dust using an in vitro Tenax bead-assisted sorptive physiologically based method. Environ. Sci. Technol. 48, 13323–13330.

Hack, A., Selenka, F., 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicol. Lett. 88, 199–210.

Huang, X.L., Zhang, J.Z., 2012. Hydrolysis of glucose-6-phosphate in aged, acid-forced hydrolysed nanomolar inorganic iron solutions-an inorganic biocatalyst? RSC Adv. 2, 199–208.

Juhasz, A.L., Tang, W., Smith, E., 2016. Using in vitro bioaccessibility to refine estimates of human exposure to PAHs via incidental soil ingestion. Environ. Res. 145, 145–153.

Julien, C., Esperanza, P., Bruno, M., Alleman, L.Y., 2011. Development of an in vitro method to estimate lung bioaccessibility of metals from atmospheric particles. J. Environ. J. Environ. Monitor. 13, 621–630.

Kademoglou, K., Giovanoulis, G., Palm-Cousins, A., Padilla-Sanchez, J.A., Magner, J., de Wit, C.A., Collin, C.D., 2018. In vitro inhalation bioaccessibility of phthalate esters and alternative plasticizers present in indoor dust using artificial lung fluids. Environ. Sci. Tech. Let. 5, 329–334.

Kastury, F., Smith, E., Juhasz, A.L., 2017. A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust. Sci. Total Environ. 574, 1054–1074.

Lafontaine, S., Schrlau, J., Butler, J., Jia, Y.L., Harper, B., Harris, S., Bramer, L.M., Waters, K.M., Harding, A., Simonich, S.L.M., 2015. Relative influence of trans-pacific and regional atmospheric transport of PAHs in the Pacific Northwest, U.S. Environ. Sci. Technol. 49, 13807–13816.

Li, K., Li, C., Yu, N.Y., Juhasz, A.L., Cui, X.Y., Ma, L.N.Q., 2015. In vivo bioavailability and In vitro bioaccessibility of perfluorooctanoic acid (PFOA) in food matrices: correlation analysis and method development. Environ. Sci. Technol. 49, 150–158.

Li, S.W., Li, H.B., Luo, J., Li, H.M., Qian, X., Liu, M.M., Bi, J., Cui, X.Y., Ma, L.Q., 2016. Influence of pollution control on lead inhalation bioaccessibility in PM2.5: a case study of 2014 Youth Olympic Games in Nanjing. Environ. Int. 94, 69–75.

Luo, P.X., 2014. The Interactions between Amphiphilic Molecules-bile Salts and Cationic Surfactants (Master's thesis). He'nan Normal University, Henan, China.

Maynard, A.D., Kuempel, E.D., 2005. Airborne nanostructured particles and occupational health. J. Nanopart. Res. 7, 587–614.

Molly, K., Woestyne, M.V., Verstraete, W., 1993. Development of a 5-step multichamber reactor as a simulation of the human intestinal microbial ecosystem. Appl. Microbiol. Biotechnol. 39, 254–258.

Oomen, A.G., Rompelberg, C.J.M., Van de Kamp, E., Pereboom, D.P.K.H., De Zwart, L.L., Sips, A.J.A.M., 2004. Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. Arch. Environ. Contam. Toxicol. 46, 183–188.

Oomen, A.G., Sips, A.J.A.M., Groten, J.P., Sijm, D.T.H.M., Tolls, J., 2000. Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. Environ. Sci. Technol. 34, 297–303.

Pelfrene, A., Cave, M.R., Wragg, J., Douay, F., 2017. In vitro investigations of human bioaccessibility from reference materials using simulated lung fluids. Int. J. Environ. Res. Pub Health 14.

Rautureau, M., Bisalli, A., Rambaud, J.C., 1981. Bile-salts and lipids in aqueous intraluminal phase during the digestion of a standard meal in normal man. Gastroen Clin. Biol. 5, 417–425.

Rostami, I., Juhasz, A.L., 2011. Assessment of persistent organic pollutant (POP) bioavailability and bioaccessibility for human health exposure assessment: a critical review. Crit. Rev. Environ. Sci. Technol. 41, 623–656.

Shani-Levi, C., Alvito, P., Andres, A., Assuncao, R., Barbera, R., Blanquet-Diot, S., Bourlieu, C., Brodkorb, A., Cilla, A., Deglaire, A., et al., 2017. Extending in vitro digestion models to specific human populations: perspectives, practical tools and bio-relevant information. Trends Food Sci. Technol. 60, 52–63.

Starr, J.M., Li, W.W., Graham, S.E., Bradham, K.D., Stout, D.M., Williams, A., Sylva, J., 2016. Using paired soil and house dust samples in an in vitro assay to assess the post ingestion bioaccessibility of sorbed fipronil. J. Hazard. Mater. 312, 141–149.

Su, G.Y., Letcher, R.J., Yu, H.X., 2016. Organophosphate flame retardants and plasticizers in aqueous solution: PH-dependent hydrolysis, kinetics, and pathways. Environ. Sci. Technol. 50, 8103–8111.

Tang, X.Y., Tang, L., Zhu, Y.G., Xing, B.S., Duan, J., Zheng, M.H., 2006. Assessment of the bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an in vitro test. Environ. Pollut. 140, 279–285.

Tilston, E.L., Gibson, G.R., Collins, C.D., 2011. Colon extended physiologically based extraction test (CE-PBET) increases bioaccessibility of soil-bound PAH. Environ. Sci. Technol. 45, 5301–5308.

Ulleberg, E.K., Comi, I., Holm, H., Herud, E.B., Jacobsen, M., Vegarud, G.E., 2011. Human gastrointestinal juices intended for use in in vitro digestion models. Food Dig. 2, 52–61.

Van de Wiele, T., Vanhaecke, L., Boeckaert, C., Peru, K., Headley, J., Verstraete, W., Siciliano, S., 2005. Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites. Environ. Health Perspect. 113, 6–10.

Wang, T., Tian, M., Ding, N., Yan, X., Chen, S.J., Mo, Y.Z., Yang, W.Q., Bi, X.H., Wang, X.M., Mai, B.X., 2018. Semivolatile organic compounds (SOCs) in fine particulate matter (PM2.5) during clear, fog, and haze episodes in winter in Beijing, China. Environ. Sci. Technol. 52, 5199–5207.

Xiao, J.B., Kai, G.Y., Ni, X.L., Yang, F., Chen, X.Q., 2011. Interaction of natural polyphenols with alpha-amylase in vitro: molecular property-affinity relationship aspect. Mol. Biosyst. 7, 1883–1890.

Xing, G.H., Yang, Y., Chan, J.K.Y., Tao, S., Wong, M.H., 2008. Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. Environ. Pollut. 156, 1218–1226.

Yu, Y.X., Pang, Y.P., Zhang, X.Y., Li, C., Yu, Z.Q., Fu, J.M., 2011. Optimization of an in vitro method to measure the bioaccessibility of polybrominated diphenyl ethers in dust using response surface methodology. J. Environ. Sci. 23, 1738–1746.

Yu, Y.X., Yang, D., Wang, X.X., Huang, N.B., Zhang, X.Y., Zhang, D.P., Fu, J.M., 2013. Factors influencing on the bioaccessibility of polybrominated diphenyl ethers in size-specific dust from air conditioner filters. Chemosphere 93, 2603–2611.

Zereini, F., Wiseman, C.L.S., Puttmann, W., 2012. In vitro investigations of platinum, palladium, and rhodium mobility in urban airborne particulate matter (PM10, PM2.5, and PM1) using simulated lung fluids. Environ. Sci. Technol. 46, 10326–10333.

Zhang, Y.Y., Pignatello, J.J., Tao, S., 2016. Bioaccessibility of nitro- and oxy-PAHs in fuel soot assessed by an in vitro digestive model with absorptive sink. Environ. Pollut. 218, 901–908.

Zhang, Y.Y., Pignatello, J.J., Tao, S., Xing, B.S., 2015. Bioaccessibility of PAHs in fuel soot assessed by an in vitro digestive model with absorptive sink: effect of food ingestion. Environ. Sci. Technol. 49, 14641–14648.