

Factors Affecting the Bioaccessibility of Polybrominated Diphenylethers in an in Vitro Digestion Model

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The factors affecting the bioaccessibility of polybrominated diphenyl ethers (PBDEs) in foodstuffs were investigated using a static in vitro model. The results showed that the gastrointestinal digestion increased the bioaccessibility of PBDEs in the intestinal solution. The incubation time significantly affected the bioaccessibility, which increased to about 25% in 4–6 h and reached equilibrium. The adsorption and release processes followed a Langmuir isotherm equation ($R^2 > 0.99$). The concentrations of PBDEs in foodstuffs did not affect the bioaccessibility, while the ratios of liquid to the mass of foodstuffs had a significant effect when the ratios were less than 90. The bioaccessibility of PBDEs increased with increasing pH, reached a maximum at a pH of 7.3 ± 0.1 , and then decreased in the intestine. To our knowledge, this is the first report demonstrating the digestive parameters playing such an important role for the bioaccessibility of PBDEs in foodstuffs. Even though in vitro tests are often conducted to study bioaccessibility, the extrapolation of such results to explain what is happening inside the highly dynamic environment of the human gastrointestinal tract is still sometimes uncertain and sometimes underestimated.

KEYWORDS: PBDEs; bioaccessibility; gastrointestinal tract; in vitro test; digestion

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) were first introduced on the market in the 1960s and have since then been used as flame retardants to improve fire safety (1). Because of the growing popularity of personal computers and other electronic equipment and stricter fire regulations, a substantial increase in production has been seen since the end of the 1970s (2). Structurally similar to polychlorinated biphenyls (PCBs) and dioxins, PBDEs are considerably persistent, bioaccumulative in the fatty tissues of organisms, and biomagnifiable throughout the food chain (3). The presence of PBDEs in humans is of particular concern due to their potential ability to cause thyroid hormone disruption, neurodevelopmental deficits, and cancer

(4, 5). As a consequence, the European Union banned the use of penta- and octa-BDE in 2004 and deca-BDE in 2008.

In the past years, many studies have highlighted the bioaccumulation behavior of PBDEs in different trophic levels of ecosystems, whereas little literature is available on human (2, 6, 7). The concentrations of PBDEs in human samples have increased by a factor of about 100 over the last 30 years (3). Similar to other persistent organic pollutants (POPs), the diet is the main route for human exposure to PBDEs, although some researchers have recently suggested that house dust might play an important role as well (8). Clearly, many researchers have found a positive relationship between PBDE concentrations in the human body and dietary intake of fish and shellfish, which are the most predominant sources of PBDEs intake by humans (9).

To accurately assess the health risk of the food exposure route, we need to know the fraction of PBDEs that is absorbed in the gastrointestinal (GI) tract. At present, such factors are not taken into account to assess risks from human exposure to PBDEs (3). The fraction of compounds that do not dissolve in the

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digestive tract are not available for intestinal absorption, whereas those compounds that are mobilized during digestion represent the fraction that is available for absorption across the small intestine epithelium (10). Although a compound is generally absorbed in its freely dissolved form, it seems likely that compounds sorbed to micelles and proteins are available for absorption provided that there is digestive degradation and disintegration of the contaminant–micelle complex. That fraction that is mobilized from the matrix during digestion, and that is considered to be available for absorption, is defined as the bioaccessible fraction (10).

In recent years, several *in vitro* digestion models have been developed as tools to estimate the bioaccessibility of contaminants or nutritional materials ingested in food or soil matrices (11, 12). To improve our insight of human exposure assessment of PBDEs in foodstuffs, the objectives of the present study are to study the factors affecting the bioaccessibility of PBDEs from foodstuffs using a static *in vitro* digestion model of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) (13, 14). The model is based on a gastric and intestinal phase mimicking the enzymatic and physicochemical conditions prevailing in the human GI transit for fed conditions. In the present study, spiked Grass carp has been used as the test sample. As far as we know, this is the first report about measuring the human bioaccessibility of PBDEs in foodstuffs and other environmental media and the first time demonstrating that digestive parameters play a very important role for PBDE bioaccessibility.

MATERIALS AND METHODS

Materials. The standards mixture of PBDE congeners (BDE17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190, and 209) was purchased from AccuStandard (BDE-COC, United States). ^{13}C -PCB208 (EC-1419-1.2, United States) and ^{13}C -PCB141 (EC-1426-1.2, United States) were purchased from Cambridge Isotope Laboratories (Andover, MA) and were used as an internal standard and surrogate, respectively. Mucin from porcine stomach, type II (EC 282-010-7, United States), starch from potato (EC 232-686-4, India), bile bovine (EC 232-369-0, United States), and D-(+)-glucose SigmaUltra, 99.5% GC (EC 200-075-1, United States), were purchased from Sigma (St. Louis, MO). Pectin from apples (EC 2325530, Switzerland), xylan from birch wood (EC 2327606, Germany), and (+)-arabinogalactan from larch wood (EC 2329100, United States) were purchased from Fluka (Buchs, Switzerland). Peptone from poultry (VL377645 449), pancreatin from porcine pancreas (F1339630 536), pepsin from porcine gastric mucosa (F1424885 608), and yeast extract (VM469326 527) were purchased from Merck (Darmstadt, Germany). Silica gel (80–100 mesh) was purchased from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China). Water was prepared by a Milli-Q system (Millipore Co., Billerica, MA). Acetone, dichloromethane (DCM), and *n*-hexane were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and distilled in a glass system. Other chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. Silica and alumina (100–200 mesh) were extracted with *n*-hexane:DCM (1:1, v/v) for 72 h before use.

Preparation of Samples. The Grass carp samples used for *in vitro* digestion were prepared as follows. Grass carp was purchased from Yanping market in Shanghai, China. The edible muscle was taken, homogenized, freeze-dried, and ground into a fine powder. The dry powder was added to DCM solutions containing different concentrations of PBDE, homogenized and vaporized, and then lyophilized. The dry powders were used as test samples for *in vitro* digestion and stored at $-20\text{ }^{\circ}\text{C}$ less than 3 days before use.

Preparation of Nutritional Medium and GI Solution. The medium and GI solutions were synthesized according to previous studies (13, 14). Typically, the nutrition contained 1.0 g L^{-1} (+)-arabinogalactan, 2.0 g L^{-1} pectin, 1.0 g L^{-1} xylan, 3.0 g L^{-1} potato starch, 0.4 g L^{-1} D-(+)-glucose, 3.0 g L^{-1} yeast extract, 1.0 g L^{-1} peptone, 4.0 g L^{-1} mucin, and 0.5 g L^{-1} L-cysteine. The mixture was autoclaved at $121\text{ }^{\circ}\text{C}$ for 15 min. The gastric acid containing 0.0890 g L^{-1} pepsine in 0.1 mol L^{-1}

HCl was sterilized using a $0.22\text{ }\mu\text{m}$ filter. The gastric solution was prepared by adding 400 mL of nutrition and 50 mL of gastric acid. The pH of obtained gastric solution was 3.0 ± 0.1 . The small intestinal solution contained 12.5 g L^{-1} NaHCO_3 , 6.0 g L^{-1} bile bovine, and 0.9 g L^{-1} pancreatin. Prior to the GI digestion, all solutions were brought to $37\text{ }^{\circ}\text{C}$.

Physiologically Based GI Digestion. A slightly modified protocol from previously described static *in vitro* digestion tests was used in this study (13, 14). Typically, the sample of test Grass carp powder was accurately weighed into labeled glass screw vessels. The gastric solution was added, and the vessel was crimp sealed with an open screw cap including a Teflon-faced silicon septum. The mixtures were then manually shaken to thoroughly mix the powder and the gastric solution. The oxygen in the headspace was removed by flushing the headspace with ultrapure nitrogen to simulate the anaerobic environment. The mixture was then incubated for 2 h at $37\text{ }^{\circ}\text{C}$ using head-over-heel rotation. After that, small intestinal solution containing NaHCO_3 , bile, and pancreatic enzymes was added at a ratio of 2:1 (stomach solution to small intestinal solution, v/v), and the oxygen in the headspace was removed. The mixture (pH = 7.0 ± 0.1) was further incubated for 6 h at $37\text{ }^{\circ}\text{C}$. All of the samples were protected from light during digestion and storage.

Sample Preparation. After incubation, the suspensions were centrifuged at 7000g for 10 min, yielding supernatant and precipitate. The supernatant was filtered with a glass fiber membrane ($0.45\text{ }\mu\text{m}$). From the filtrate, 10 mL was removed, and surrogate was added and extracted using liquid–liquid shaking with 40 mL of acetone and 80 mL of *n*-hexane:DCM (1:3, v/v) for three times. The extracts were concentrated to ca. 1 mL using a rotary evaporator, and then, 30 mL of *n*-hexane was added. After they were treated with 10 mL of concentrated sulfuric acid, the extracts were rinsed with water and dried over anhydrous sodium sulfate and then concentrated to 1 mL. Finally, the extract was cleaned up using a 10 mm i.d. silica–alumina column packed, from the bottom to the top, with neutral alumina (6 cm, 3% deactivated), neutral silica gel (2 cm, 3% deactivated), 25% sodium hydroxide silica (5 cm), neutral silica gel (2 cm, 3% deactivated), and 50% sulfuric acid silica (6 cm). PBDEs congeners were eluted with 70 mL of *n*-hexane:DCM (1:1, v/v) and collected. The collected eluate was concentrated, and internal standard was added. The samples were stored at $4\text{ }^{\circ}\text{C}$ until analysis using gas chromatography (GC)/NCIMS.

Chemical Analysis. Instrumental analysis of PBDEs was done with a Hewlett-Packard (HP) 6890N GC coupled to a 5975 mass spectrometer (MS). An HP-5MS capillary column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$, J&W Scientific, United States) coated with 5% phenyl methyl siloxane capillary column was used. The GC operating conditions were as follows: the oven temperature was programmed from $110\text{ }^{\circ}\text{C}$ (held for 1 min) to $180\text{ }^{\circ}\text{C}$ (held for 1 min) at a rate of $8\text{ }^{\circ}\text{C min}^{-1}$, to $240\text{ }^{\circ}\text{C}$ (held for 5 min) at $2\text{ }^{\circ}\text{C min}^{-1}$, to $280\text{ }^{\circ}\text{C}$ (held for 5 min) at $2\text{ }^{\circ}\text{C min}^{-1}$, to $300\text{ }^{\circ}\text{C}$ (held for 15 min) at $20\text{ }^{\circ}\text{C min}^{-1}$, and finally postrun for 10 min. Splitless injection of a $1\text{ }\mu\text{L}$ sample was performed. The injector and ion source temperatures were set at 280 and $250\text{ }^{\circ}\text{C}$, respectively. Ultrapure Helium was used as a carrier gas under constant flow mode at a flow rate of 1 mL min^{-1} , and it was used as a makeup gas at 60 mL min^{-1} . The selective ion monitoring (SIM) mode was operated, and ions $m/z = 79$ and 81 were monitored for tri- to hepta-BDE congeners, while $m/z = 476/478$ and $372/374$ were monitored for ^{13}C -PCB208 and ^{13}C -PCB141, respectively.

Quality Assurance and Quality Control (QA/QC). The analytical procedures were monitored using strict QA/QC. Procedural blanks were run every seven samples for *in vitro* digestion process. Instrumental QC was done by regular injection of solvent blanks and standard solutions. Identification and quantification of PBDEs were undertaken according to the retention times and peak areas of the corresponding calibration standards. Seven standard stock solutions (from 0.5 to 100 ng mL^{-1}) covering the expected concentrations for the samples were used. Calibration plots had excellent linear regression coefficients for all of the compounds ($R^2 > 0.998$). Recovery and standard deviation (SD) of the surrogate were $104.6 \pm 13.1\%$. Therefore, PBDEs were efficiently collected during the

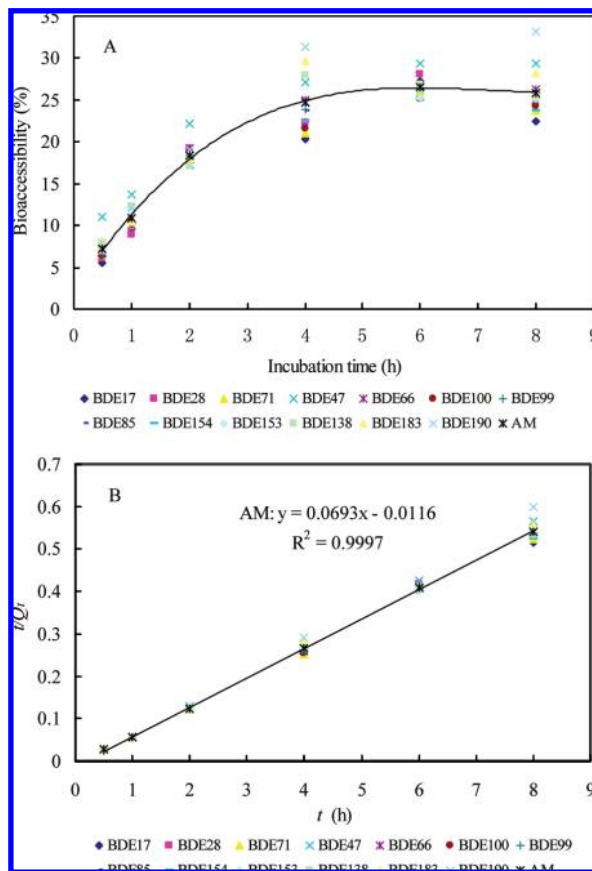


Figure 1. (A) Bioaccessibility (%) of PBDEs in intestinal solution as a function of incubation time. (B) Plot of the amount of unreleased PBDEs Q vs time for the release kinetics of PBDEs from Grass carp matrix into digestive juice [experimental parameters: 0.2 g of dry Grass carp powder digested in 18 mL of intestinal solution (pH of 7.0) after 2 h of incubation in gastric solution (pH of 3.0) with each PBDEs concentration of 20 ng g^{-1} dry weight].

extraction procedures. Because of high laboratory background, decabromodiphenyl ether (BDE209) was not discussed in this paper. The data used in the paper were measured in triplicate.

Calculation of Bioaccessibility. In this study, bioaccessibility is defined as the fraction of PBDEs present in the filtrate of the centrifuged GI digests. This was calculated as the percentage of the amount of each PBDE in the filtrate to the total amount of each compound as follows (14, 15).

$$\begin{aligned} \text{bioaccessibility (\%)} &= \frac{m_{\text{released-PBDEs}}}{m_{\text{total-PBDEs}}} \times 100 \\ &= \frac{m_{\text{released-PBDEs}}}{m_{\text{fish}} \times C_{\text{PBDEs}}} \times 100 \end{aligned} \quad (1)$$

RESULTS AND DISCUSSION

Influence of GI Phase Toward PBDEs Bioaccessibility.

Bioaccessibility values of all PBDE congeners were ranged from lower than the detection limit to 3.58% with average of 0.76% in the gastric solution whether the incubation time was prolonged or not. In correspondence with studies for other organic compounds (12, 16), the bioaccessibility of PBDEs in intestinal solution was much higher (5–34%, **Figure 1A**) than in gastric solution and was dependent on the incubation time discussed in detail below. The higher bioaccessibility in the intestine vs the gastric phase can be explained by the presence of bile salts and pancreatin. In the human body, bile is a complex fluid containing water, electrolytes, and a battery of organic molecules

including bile acids, cholesterol, phospholipids, and bilirubin that flows through the biliary tract into the small intestine. Pancreatin is a mixture of several digestive enzymes containing trypsin, amylase, and lipase produced by the exocrine glands of the pancreas and is necessary for the digestion of starch, fat, and protein. Previous studies showed that the surface tension of the intestinal solution is important for mobilizing contaminants from solid matrix, thereby affecting their solubilities (15). The research by Tang et al. (16) showed that pepsin and pancreatin could only slightly lower the surface tension of simulated GI solutions, which indicated their limited abilities to increase the solubilities of polycyclic aromatic hydrocarbons (PAHs) in the digestive juice.

In contrast, bile salts act as a surfactant-like component during digestion, thereby decreasing the surface tension of the intestinal solution. Moreover, bile may create an apolar environment in the interior of bile salt micelles for hydrophobic compounds and thereby increase their solubility (15). The concept of critical micelle concentration (CMC) has previously been used to explain the influence of bile concentration on PAHs transfer, showing that the bile extract could decrease the surface tension of digestive juices substantially when the bile concentration is higher than the CMC of 0.15 $g L^{-1}$ (16). Similar results were obtained by Wright et al. (17) when investigating the efficiency of β -carotene micellarisation. These researchers showed that increasing bile concentrations brought about increased carotenoid transfer. In the present study, the concentration of bile extract was 2 $g L^{-1}$ in the small intestinal solution, about 13.3 times the CMC, which means that it could significantly lower the surface tension of the digestion solution, thereby enabling PBDEs solubilization.

Kinetics of the Release Process. The influence of incubation time on the release of PBDEs from Grass carp (initial concentration, 20 $ng g^{-1}$) in the simulated GI tract is presented in **Figure 1A**. A time-dependent release of PBDEs before 6 h of incubation time was observed in the intestinal solution. There are two successive release phases, a rather fast phase occurring within the first 2 h, where the bioaccessibility rapidly increased up to about 20%, and then followed by a slower phase as the released amount of PBDEs reached its equilibrium value about 25% in the next 4 h. After that, no apparent change in bioaccessibility was found during the prolonged incubation period. An equilibrium of PBDEs between intestinal solution and food matrix was reached.

To study the kinetics of the sorption process of a compound onto solid, several kinetic models, such as pseudofirst-order, pseudosecond-order, Elovich equation, and intraparticle diffusion, are available to explain the mechanisms of the process and to fit experimental data (18). Among them, the pseudosecond-order equation is often successfully used to describe the sorption kinetics for compounds on an adsorbent. To study PBDE release kinetics from the simulating GI tract in this study, the same equation was used. According to the pseudosecond-order equation, the amount of PBDEs unreleased from the food matrix is a function of time presented by the following equation:

$$\frac{dQ_t}{dt} = k(Q_e - Q_t)^2 \quad (2)$$

where k ($g ng^{-1} h^{-1}$) is the second-order rate constant, Q_e ($ng g^{-1}$ dry weight) is the amount of PBDEs unreleased from the Grass carp at equilibrium, and Q_t ($ng g^{-1}$ dry weight) is the amount of PBDEs unreleased at any time t (h). A linear relationship is obtained when eq 2 is integrated for the boundary conditions $t = 0$ ($Q_t = 0$) to t_e ($Q_t = Q_e$):

$$\frac{t}{Q_t} = \frac{1}{Q_e} t + \frac{1}{kQ_e^2} \quad (3)$$

The plot of t/Q_t against t provides a linear relationship, from which the amount of unreleased PBDEs at equilibrium Q_e (same as adsorption capacity) and the pseudo-second-order rate constant k can be calculated from the slope and intercept of the plot, respectively. In this study, Q_t was calculated according to the bioaccessibility of PBDEs at sampling time, and eq 3 was used to fit the experiment data. A linear relationship with excellent correlation coefficients (each of the PBDE congeners showing $R^2 > 0.9955$) was observed between t/Q_t and t , which indicates the applicability of the pseudo-second-order model to describe the release process of PBDEs in the simulating GI tract (**Figure 1B**). The rate constant of the pseudo-second-order k ranged from -0.30 to $-0.53 \text{ g ng}^{-1} \text{ h}^{-1}$ with an average of $-0.42 \text{ g ng}^{-1} \text{ h}^{-1}$. The calculated amount of unreleased PBDEs at equilibrium Q_e varied from 13.32 to 14.90 ng g^{-1} with the average of 14.44 ng g^{-1} dry weight, which meant that the calculated bioaccessibility of PBDEs according to the data of Q_e ranged between 25.48 and 33.42% with an average of 27.80% . According to the study, the average experimental bioaccessibility of PBDEs after 6 h of incubation (at equilibrium) ranged between 24.36 and 29.70% with an average of 26.23% . The difference between the calculated and the experimental average bioaccessibility of PBDEs varied between 0.59 and 3.73% with an average of 1.57% . The values of calculated bioaccessibility at equilibrium showed a good agreement with the experimental values. For subsequent batch experiments, 6 h of incubation time was used, which was sufficient to establish equilibrium during the digestion.

Isotherms of Adsorption and Release. Adsorption is a surface phenomenon that is generally characterized by the concentration of a compound from a solution onto or near the surfaces of pores of the solid at constant temperature (19). This surface excess occurs in general when the attractive energy of the compound with the solid surface (i.e., the adhesive work) is greater than the cohesive energy of the substance itself. It is often described by a graphic representation of the distribution ratio of adsorbate adsorbed per unit mass of the adsorbent and the concentration of the nonadsorbed adsorbate, which is known as the adsorption isotherm. Many types of adsorption isotherms, such as Langmuir isotherm, Freundlich equation, and linear equation, are available in the literature to study desorption or adsorption processes. Yet, the most widely used are the Langmuir and the Freundlich isotherms (20). The former is a theoretical expression obtained by assuming a uniform monolayer of the adsorbate molecules, while the latter is purely empirical.

To quantitatively describe release (similar to desorption) or adsorption processes of PBDEs in the simulating GI tract, these equations were used to fit the experimental data. Among them, linear relationships with excellent correlation coefficients (all the PBDE congeners $R^2 > 0.97$) were observed when using the following equation:

$$\frac{C_e}{Q_e} = \frac{1}{\alpha Q_s} + \frac{1}{Q_s} C_e \quad (4)$$

which derived from the commonly used Langmuir equation:

$$Q_e = \frac{\alpha Q_s C_e}{1 + \alpha C_e} \quad (5)$$

where C_e (ng mL^{-1}) is the concentration of each PBDEs in the digestive solution at equilibrium. Q_e (ng g^{-1}) is the amount adsorbed or unreleased PBDEs per unit mass of the food matrix

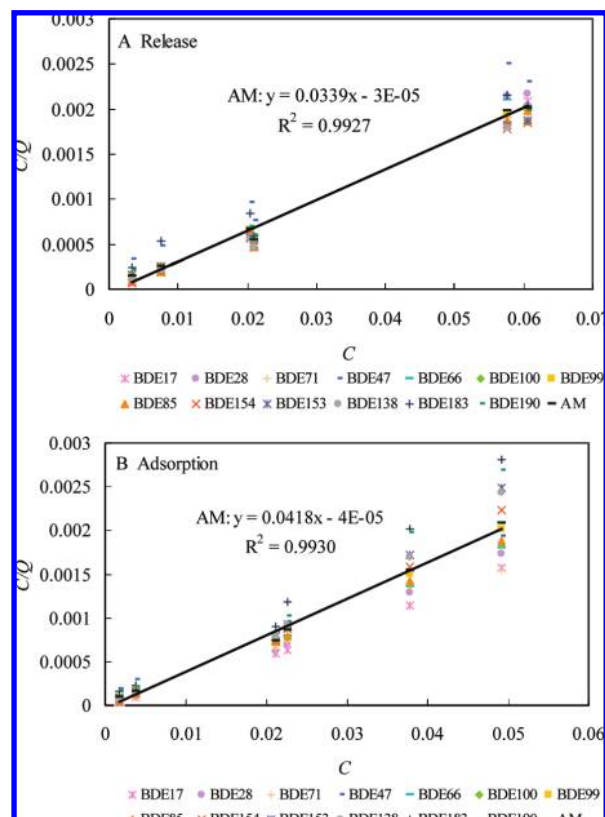


Figure 2. Fitting of the Langmuir equation for release and adsorption of PBDEs on the Grass carp matrix during different digestion times in simulating the human GI tract [experimental parameters: 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 g of dry Grass carp powder digested in 18 mL of intestinal solution (pH of 7.0) for 6 h after 2 h of incubation in gastric solution (pH of 3.0) with the mass of each PBDE congeners of 4 ng].

at equilibrium as described above. α (mL ng^{-1}) is Langmuir adsorption constant. Q_s (ng g^{-1}) is saturated adsorption capacity.

Given by the curves in **Figure 2A** (for release) and **Figure 2B** (for adsorption), the applicability of the linear form of Langmuir isotherm eq 4 can be examined by considering the plots of C_e/Q_e (Y-axis) vs C_e (X-axis) for PBDE release and adsorption during digestion. Langmuir adsorption constant (α) values could be calculated from the intercepts. A wide range of α values were obtained for each PBDE congener during release (between -1665.0 and 326.0 mL ng^{-1}) and adsorption processes (between -9050.0 and 368.0 mL ng^{-1}). Averages of -1130.0 and $-1045.0 \text{ mL ng}^{-1}$ were obtained for release and adsorption processes, respectively. Q_s is a rough measure of adsorption capacity of the surface area of the Grass carp matrix. The Q_s values obtained from the slopes in the Langmuir isotherm equations ranged from 26.2 to 32.1 ng g^{-1} with an average of 29.5 ng g^{-1} and from 18.0 to 31.3 ng g^{-1} with an average of 23.9 ng g^{-1} , respectively. These data show that the calculated Q_s for the release process was slightly higher than those of the adsorption process. This might be due to aging effects for the storage of prepared samples. The organic compounds on the surfaces of solid would initially form outer-sphere complexes followed by the formation of inner-surface complexes. This might lead to higher calculated Q_s during the release process for the PBDEs.

Influence of the PBDEs Levels. The small intestine is the main place where substances are absorbed into the human body. Compounds that enter the small intestine from the stomach can be absorbed across the intestinal epithelium either along the

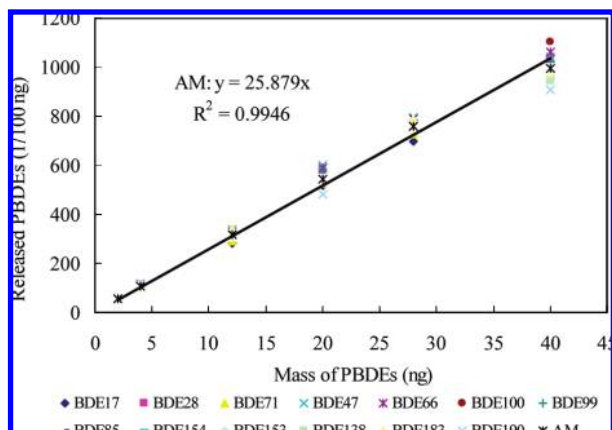


Figure 3. Released (ng) PBDEs at different concentrations with the same amount of Grass carp powder [experimental parameters: 0.2 g of dry Grass carp powder digested in 18 mL of intestinal solution (pH of 7.0) for 6 h after 2 h of incubation in gastric solution (pH of 3.0) with the concentrations of each PBDE congeners of 10, 20, 60, 100, 140, and 200 ng g⁻¹ dry weight].

cells (the paracellular route) or through the cells (the transcellular route) as soon as the substance is released into the chyme from its matrix (10). The concentration of the substance remains low in the chyme if the rate of absorption is faster than release (21). However, in the present static digestion model, the PBDEs released from Grass carp were not removed from the solution during digestion; therefore, bioaccessibility may be underestimated when saturation of PBDEs occurs in the chyme. Thus, the levels of the compounds in matrix may be an important factor potentially affecting bioaccessibility of PBDEs.

To study how much the level of PBDEs in Grass carp affects the bioaccessibility, 0.2 g of dry Grass carp powder with PBDEs concentrations from 10 to 200 ng g⁻¹ for each compound were incubated in 12 mL of gastric solution and 18 mL of intestinal solution for 2 and 6 h, and the pH values of gastric and intestinal solution were 3.0 ± 0.1 and 7.0 ± 0.1, respectively. Given by the graph in **Figure 3**, a dose proportional relationship was found between the contamination level and the released PBDEs. There is an excellent linear correlation between the released (ng) PBDE congeners and the total masses of them ($R^2 > 0.98$). The values of coefficients of regression (k) denote the bioaccessibility of PBDEs according to eq 1. The bioaccessibility of PBDE congeners ranged between 24.3 and 27.4%, with an average of 25.9%. This shows that no saturation of the chyme with PBDEs occurred with neither of the tested contaminant levels in Grass carp, and the bioaccessibility of PBDEs had no relation to their concentrations under a settled liquid to solid (L/S) ratio. The results are consistent with the study by Oomen et al. (10) that linear relationships were found between the concentrations of PCBs in the chyme and the digested matrix with the intercepts closed to zero.

Ratios of Liquid to Solid. Because the amount of fluid in the GI tract will vary depending on the amount of food ingested, the L/S ratio range was chosen to encompass a variety of conditions that could be plausible in the human GI tract under fed conditions (22). In this study, 0.08–1.0 g of dry Grass carp powders with each PBDEs concentration of 20 ng g⁻¹ and 18 mL of intestinal solution were used. The results of the influence of the L/S ratio on the bioaccessibility of PBDEs in Grass carp are shown in **Figure 4**. An L/S ratio-dependent release of PBDEs was observed

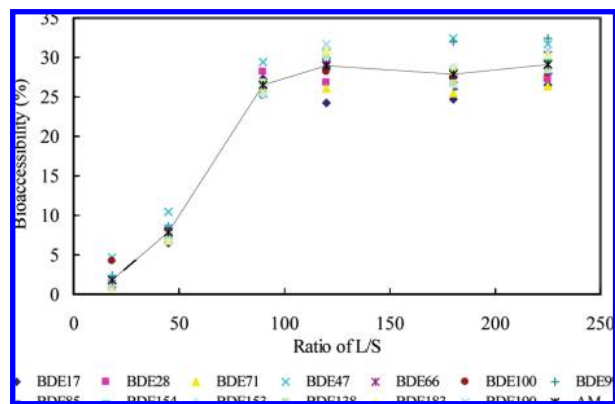


Figure 4. Bioaccessibility of PBDEs under different L/S ratios with the same PBDEs concentrations [experimental parameters: 0.2 g of dry Grass carp powder digested in 18 mL of intestinal solution (pH of 7.0) for 6 h after 2 h of incubation in gastric solution (pH of 3.0) with the concentrations of each PBDE congeners of 20 ng g⁻¹ dry weight].

at L/S ratios lower than 100. This suggests that complete release of the PBDEs was not achieved at the lower ratios. If the released PBDEs would have reached saturated levels in solution, a decrease of bioaccessible PBDEs would be expected when higher L/S ratios are employed. Similar to the graph of **Figure 1**, there are two effect phases; a rather great effect of the bioaccessibility of PBDEs occurs within the ratio lower than 90. The bioaccessibility increased rapidly up to about 26.5% from lower than 5% and then followed by a slower effect stage increased to about 28.9% at L/S ratios higher than 100. After that, there was a consistent percentage of each PBDE congener across all of the L/S ratios used in the experiments. According to a previous study (23), there was a logarithmic correlation between PAH release and L/S ratios when low L/S ratios (<40) were used to study the PAH release from a soil matrix using the SHIME in vitro model. A study by Hamel et al. (22) showed that metal bioaccessibility within a given soil was only slightly affected by changes in L/S ratios for the range of 100:1–5000:1 in synthetic gastric fluid. The present results are in agreement with these data when we consider their study results for the bioaccessibility at both low and high L/S ratios. Our data showing the biphasic relationship between PBDEs bioaccessibility from Grass carp and the wide range of L/S ratios is important information to elucidate the PBDEs release processes during GI transit.

Influence of pH Values. The presence of food can markedly alter the physicochemical conditions in the GI tract. An important difference is the gastric pH values. It can be as low as 1 and as high as 6 for the fasted state and the fed state condition, respectively. Moreover, the secretions of the gastric juice, duodenal juice, and bile increase for the fed state, while the presence of food also delays the gastric emptying. In contrast, the pH values in the human small intestine range from 5.5 to 7.5 and are hardly affected by the presence of food (24). The gastric pH for fasting condition is between 1.5 and 2, while eating a meal results in a temporary rise of gastric pH from 3 to 7 because of the diluting and buffering effects of the ingested food components (25). In this study, different pH values were applied to study this influence. A gastric pH of 1.4, 1.9, 2.2, 3.0, 3.5, and 4.1 was used, which resulted in a pH of 5.9, 6.5, 6.8, 7.0, 7.3, and 7.46 in the intestinal solution after bile and pancreatin were added. The results given in **Figure 5** indicate that the bioaccessibility of PBDEs increases with increasing pH,

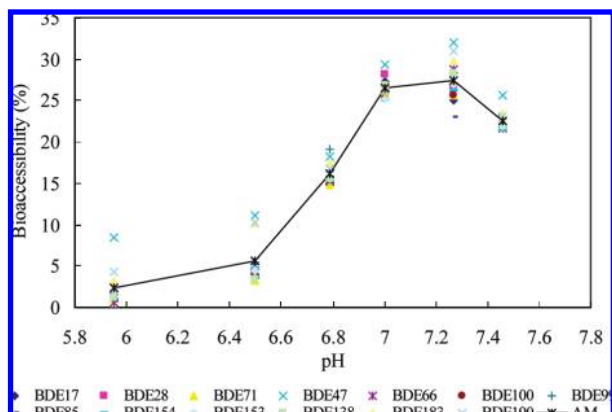


Figure 5. Bioaccessibility of PBDEs in the intestinal solution with different pH values [experimental parameters: 0.2 g of dry Grass carp powder digested in 18 mL of intestinal solution for 6 h after 2 h of incubation in gastric solution with the concentrations of each PBDE congeners of 20 ng g⁻¹ dry weight].

reaching a maximum at pH of 7.3 ± 0.1 and then decreasing. These observations are likely related to the precipitation of bile salts under the acidic conditions used. At low pH, bile salts, which would otherwise form micelles and solubilize PBDEs, precipitate. Below pH 4, up to 60% of bile salts are precipitated (17). Porcine pancreatic lipase has an isoelectric point of about 5.7 and shifts to roughly 6.0 in the presence of bile salts (17). Oomen et al. (11) compared five different *in vitro* digestion models, currently in use in different countries, to study the bioaccessibility of soil contaminants and concluded that gastric pH values could be responsible for the different bioaccessibility of metals in the results. Many studies showed the pH could affect the bioaccessibility in the simulating GI tract (17, 26), while another previous study described no increase in PAH release when the gastric pH of 2 was increased to an intestinal pH of 7 (23).

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