In Vivo Bioavailability and In Vitro Bioaccessibility of Perfluorooctanoic Acid (PFOA) in Food Matrices: Correlation Analysis and Method Development

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ABSTRACT: Food is a major source of human exposure to perfluorooctanoic acid (PFOA), however, PFOA bioavailability in food has not been studied. An in vivo mouse model and three in vitro methods (unified BARGE method, UBM; physiologically based extraction test, PBET; and in vitro digestion method, IVD) were used to determine the relative bioavailability and bioaccessibility of PFOA in the presence of 17 foods. PFOA was mixed with foods of different nutritional compositions and fed to mice over a 7-d period. PFOA relative bioavailability was determined by comparing PFOA accumulation in the liver following PFOA exposure via food to that in water. PFOA bioavailability relative to water ranged from 4.30 ± 0.80 to 69.0 ± 11.9% and was negatively correlated with lipid content (r = 0.76). This was possibly due to competitive sorption of free fatty acids with PFOA onto transporters on intestine epithelial cells. Besides, cations in the gastrointestinal tract, such as Ca²⁺ and Mg²⁺, are capable of complexing PFOA and partitioning to the lipid phase. On the other hand, when assessed using in vitro assays, PFOA bioaccessibility varied with methods, being 8.7–73% (UBM), 9.8–99% (PBET), and 21–114% (IVD). PFOA bioaccessibility was negatively correlated with lipid content when assessed using UBM (r = 0.82); however, a poor correlation with food composition was observed for PBET and IVD (r = 0.01–0.50). When in vivo and in vitro data were compared, a strong correlation was observed for UBM (r = 0.79), but poor relationships were observed for PBET and IVD (r = 0.11–0.22). This was probably because the higher lipolysis ability and presence of Ca²⁺ and Mg²⁺ in the gastrointestinal fluid of UBM resulted in a lower potential to form stable micelles compared to PBET and IVD. These results indicated that PFOA relative bioavailability was mainly affected by lipid content in foods, and UBM has the potential to determine PFOA bioaccessibility in food samples.

INTRODUCTION

Perfluoralkyl substances (PFAS), a class of persistent organic pollutants (POPs), have been widely used in industrial and consumer products such as Teflon and Gore-Tex since the 1950s.¹ PFAS are amphiphilic compounds, consisting of a fully fluorinated hydrophobic alkylchain of varying length (typically C4 to C16) and a hydrophilic end group, such as sulfonates or carboxylates. Due to their strong carbon–fluorine bonds, PFAS are biochemically and thermally stable. As a consequence, they tend to bioaccumulate in the environment, with perfluorooctanoic acid (PFOA) being one of the most detected PFAS.² Toxicity tests have shown that PFOA induces multiple toxicity, including hepatotoxicity,³ developmental effects,⁴ and immunotoxicity⁵ in mammals.

A major human exposure pathway for PFOA is via ingestion of contaminated food.⁶ The occurrence of PFOA in food has been reported worldwide, especially in industrial regions such as Europe, North America, and East Asia.⁷–⁹ In a 10-year survey conducted in Europe, PFOA was detected in different foods, ranging from 0.01 to 161 μg/kg.¹⁰ Because of its high aqueous solubility, aquatic organisms such as fish accumulate higher concentrations of PFOA than terrestrial animals and plants, reaching several hundreds to thousands of μg/kg.¹¹,¹²

Because of its adverse effects on human health,²,⁶ it is important to evaluate human exposure to PFOA via food ingestion pathway. Traditionally, exposure assessment has been conducted by assessing the total concentration of POPs in foods. However, human exposure and its associated risk may be overestimated if contaminant bioavailability is not taken into account. For POPs to be bioavailable, food must first be digested, releasing the contaminant from the food matrix, which
may then be absorbed in the gastrointestinal (GI) tract where it reaches the systemic circulation. However, little information is available regarding the bioavailability of POPs let alone PFOA in food matrices.

Bioavailability studies of POPs in foods have mainly focused on single food composition, such as oil, glucose, and milk solution. For example, the impact of lipid content on the bioavailability of polycyclic aromatic hydrocarbon (PAH) and dichlorodiphenyl trichloroethane (DDT) has been studied. Because of its surfactant properties, high aqueous solubility, and propensity to form colloids in aqueous solutions, PFOA may behave differently from traditional hydrophobic POPs. To date, there are only limited studies assessing the bioavailability of amphiphilic and/or ionizable POPs. For example, using an in vivo rat model, Pu et al. demonstrated the influence of soil organic carbon on the bioavailability of pentachlorophenol (PCP) in contaminated soils. In addition, the bioavailability of drugs (e.g., lipic acid and cilazapril) with molecular structures similar to PFOA is reduced when taken with foods. PFOA orally administered to rats has been shown to be readily absorbed (93%). Mice also absorbed a substantial amount of PFOA after oral administration (>50–74%). However, the influence of food matrices on PFOA bioavailability is yet to be investigated.

Although animal-based in vivo assays are suitable to determine the bioavailability of POPs in food matrices, they are expensive and time-consuming. To overcome these limitations, in vitro methods have been developed to mimic the solubilization of POPs in the human GI tract (i.e., bioaccessibility). In vitro bioaccessibility refers to the soluble fraction of a chemical in the GI tract, which is potentially available for absorption into the systemic circulation. In recent years, several in vitro methods have been used to measure the bioaccessibility of POPs in soils, including in vitro digestion method (IVD), unified BARGE method (UBM), and the physiologically based extraction test (PBET), which have been applied to assess the bioaccessibility of POPs in foods. For example, Yu et al. determined the bioaccessibility of polybrominated diphenyl ethers (PBDEs) in different foods using a modified Simulator of the Human Intestinal Microbial Ecosystem (SHIME). PBDE bioaccessibility ranges from 2.6 to 41% and is positively correlated with lipid content. Similarly, Han et al. found that the bioaccessibility of polychlorinated biphenyls (PCBs) in meat and fish is also positively correlated to lipid content (2.3–59%) using the SHIME assay.

In vitro methods mimic processes in GI fluid including steps before lipids reach enterocytes. Dietary lipids, such as triglycerides, may be lipolyzed by lipase into free fatty acids and monoglycerides in the intestine at pH of 6–7. In the presence of bile salts in intestinal solution, the released free fatty acids and monoglycerides may aggregate to form mixed micelles. The mixed micelles enable the transport of hydrophobic compounds through an unstirred water layer (≈200–500 μm thick) before reaching the microvillus membrane of enterocytes. The presence of an aqueous layer is an important absorption barrier by the intestines for hydrophobic substances such as PAHs and PBDEs. This may explain why nonpolar POPs absorption is enhanced by lipid absorption in some reports. However, there is limited information regarding the effect of food compositions on PFOA bioaccessibility. In addition, the differences between the properties of PFOA and nonpolar POPs make it necessary to measure PFOA bioaccessibility in the presence of food matrix.

In this study, an in vivo mouse model and three in vitro methods (PBET, IVD, and UBM) were employed to determine PFOA relative bioavailability and bioaccessibility in the presence of 17 different food matrices. The objectives of the study were to (1) determine the impact of different food composition on PFOA relative bioavailability using an in vivo mouse model, (2) evaluate the impact of different food compositions on PFOA bioaccessibility using three in vitro methods, and (3) test the ability of in vitro methods to predict PFOA relative bioavailability in food matrices.

### MATERIALS AND METHODS

**Chemicals and Materials.** PFOA (98%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). PFOA stock solutions at 500 mg/kg were prepared in both Milli-Q water and food-grade corn oil. The PFOA stock solution was stored in polypropylene tubes and kept at 4 °C. Labware handling PFOA were made of polypropylene, which has little ability in PFOA absorption. The recovery of PFOA concentration in stock solution was 100 ± 3.8% after being stored in polypropylene bottle for 1 d (data not shown). All solvents and chemicals were of HPLC or analytical grade.

**Food Sample Preparation.** All food samples were bought from local supermarkets in Nanjing, China. Two major food categories were covered: i.e., animal-based food including mutton, beef, shelled shrimp, and two brands of milk, and plant-based food including haricot bean, pea, corn oil, orange juice, and carrot juice. In addition, pure protein/starch powder was mixed with corn oil to generate food mixtures with different protein/carbohydrate portions at 20, 40, or 60% (w:w). A detailed description of all 17 food samples is provided in Supporting Information (SI) Table S1.

Solid food samples were initially homogenized using a food processor for 5 min, then vacuum sealed, and stored at −20 °C prior to use. An aliquot of solid food (45 g) was placed in a PP centrifuge tube, PFOA in corn oil (5 mL; 10 mg/kg PFOA) was added to achieve a concentration of 1 mg/kg PFOA (wet weight), then the food was mixed thoroughly with a PP rod. For liquid foods (50 mL), PFOA in water (0.1 mL; 500 mg/kg PFOA) was added to achieve a concentration of 1 mg/L PFOA, then the solution was mixed by shaking. All samples were prepared a day before the assessment of bioavailability and bioaccessibility with storage at 4 °C prior to use. Before commencement of the assays, the liquid and solid samples were homogenized again and a subsample was collected to confirm PFOA concentration. The concentration of PFOA used (1 mg/kg) is at the higher end of PFOA concentrations reported in foods and was used to minimize potential analytical uncertainty.

**PFOA Bioavailability Using a Mouse Model.** Female Balb/c mice weighing 22–25 g were purchased from Aiermaite Company in Suzhou, China. All mice were raised under standard animal house conditions (12 h light/dark cycle, 22 ± 2 °C, and 50 ± 5% humidity) and were acclimated for 1 week before bioavailability assays. Mice were housed in cages with water supplied ad libitum. After which mice were fasted for 5.5 h prior to exposure to PFOA-containing food. All PFOA-spiked food was fed at noon and consumed within an hour, after which mice were fasted for another 5.5 h prior to the supply of standard mouse chow (Qinglong Mountain Company, Nanjing, China). This process was used to minimize the effect of mouse chow on the bioavailability of PFOA-spiked foods.

Relative bioavailability (RBA) refers to comparative bioavailability of different forms of a chemical or from different exposure media containing the chemical, e.g., bioavailability of a chemical in soil relative to its bioavailability in water.36 When determining PFOA-RBA (n = 3), 0.3 mL of PFOA in Milli-Q water (1 mg/L) was used as the reference dose with Milli-Q water as the control. PFOA-containing food was fed to individually caged mice in small polypropylene dishes. Food samples containing 1 mg/kg PFOA were administered to mice at 0.3 g/d or 0.3 mL/d for 7 d. After 7 d, mice were sacrificed 12 h after final food intake with their livers being collected for PFOA quantification. Previous research has shown that PFOA accumulates primarily in the liver and serum of both male and female mice,37 with a small proportion being excreted in the urine. In the study of Hundley et al.,23 liver accounted for >70% of PFOA accumulated in mice tissues. Our preliminary studies also showed that the liver accumulated the highest concentration of PFOA (compared to other organs) and was correlated with PFOA dose administered to mice (SI Figure S1). Hence, liver was chosen as the target organ for PFOA accumulation and RBA determination. PFOA-RBA in food matrices was calculated according to the following equation:

\[
\text{PFOA relative bioavailability (\%)} = \left( \frac{\text{Liver PFOA}_{\text{[food]}}}{\text{Liver PFOA}_{\text{[water]}}} \times \frac{D_{\text{[water]}}}{D_{\text{[food]}}} \right) \times 100
\]

(1)

where \( \text{Liver PFOA}_{\text{[food]}} \) = PFOA (\( \mu \text{g} \)) accumulated in the liver following dosage of foods containing PFOA; \( \text{Liver PFOA}_{\text{[water]}} \) = PFOA (\( \mu \text{g} \)) accumulated in the liver following dosage of water containing PFOA; \( D_{\text{[water]}} \) = dose of PFOA administered in water containing PFOA (\( \mu \text{g} \)); and \( D_{\text{[food]}} \) = dose of PFOA administered in foods containing PFOA (\( \mu \text{g} \)).

PFOA Bioaccessibility Determined Using Three In Vitro Methods. Three methods (UBM, PBET, and IVD) were used to assess PFOA bioaccessibility in the presence of different food matrices. In vitro assays were conducted following standard protocols,25,27,38 except that 0.3 g of food was used (instead of soil) at sample/solution ratios of 1:97.5 for UBM, 1:100 for PBET, and 1:150 for IVD. The IVD assay is an inexpensive method for organic contaminants with simple components adapted from an inorganic method,39 while PBET is an unfiltered assay developed by Ruby et al.,39 which has been used to assess PAH bioaccessibility in contaminated soils.\(^{26}\)

The UBM is a standard European assay to assess bioaccessibility of inorganic contaminants in soils,38 which has also been used to assess DDT bioaccessibility in contaminated soils.\(^{25}\)

After intestinal extraction, samples were centrifuged (4000 rpm, 10 min), and 10 mL of the supernatant was used to determine PFOA concentration. Detailed composition of gastrointestinal fluids for the three methods is listed in Table S2. PFOA bioaccessibility in food matrices was calculated following the completion of intestinal phase extraction according to the following equation:

\[
\text{In vitro PFOA bioaccessibility (\%)} = \left( \frac{\text{In vitro PFOA}}{\text{Total PFOA}} \right) \times 100
\]

(2)

where in vitro PFOA = PFOA (\( \mu \text{g} \)) extracted from foods containing 1 mg/kg PFOA following gastrointestinal phase extraction, and total PFOA = 1 mg/kg PFOA (\( \mu \text{g} \)) in foods prior to in vitro treatment.

PFOA Extraction from Mice Liver and GI Fluids. PFOA in liver samples and intestinal fluids was extracted according to Hansen et al.\(^{40}\) and Powley et al.\(^{41}\) Briefly, whole livers were placed in 15-mL polypropylene (PP) tubes containing 10 mL of milli-Q water and homogenized by blending at 10 000 rpm (PRO200, PRO Scientific, USA). An aliquot of the sample (1 mL of homogenized sample) was transferred to another PP tube, to which 1 mL of 0.5 M tetrabutyl ammonium hydrogen sulfate (TBHAS) solution and 2 mL of sodium carbonate buffer (0.25 M, pH 10) were added.\(^{13}\)C\(_4\)-PFOA (>99%, Wellington Laboratory, Canada) was added as surrogate. After mixing, the slurries were extracted with 5 mL of methyl tert-butyl ether (MTBE) by shaking for 20 min. After centrifugation at 4000 rpm for 10 min, the MTBE layer was transferred into a clean PP tube, and the extraction procedure was repeated twice. The collected extract was reduced in volume to ~0.5 mL under nitrogen and reconstituted to 1 mL through the addition of methanol. The methanol extract was then purified by transferring to a 2-mL PP tube containing 25 mg of Envi-Carb graphitized carbon adsorbent. The extract was sonicated for 30 s, shaken for 20 min at 250 rpm, and then centrifuged at 3000 rpm for 15 min. The purification procedure was repeated twice, and then all supernatants were combined in a 5-mL PP tube. The collected supernatants were then evaporated to near dryness under nitrogen and reconstituted in 0.5 mL of methanol for PFOA analysis. For GI fluids, 1 mL was transferred to the PP tube containing TBHAS, and then processed according to the procedure for liver samples as detailed above.

Analysis of PFOA. PFOA extracts in methanol were filtered through a PP mesh (0.2 \( \mu \text{m} \)) into HPLC vials for PFOA analysis on a Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system (Waters, Milford, MA, USA) equipped with a Waters ACQUITY TQD triple quadrupole mass spectrometer (TQMS). An isolator column for perfluorinated organic compound (ACQUITY, Waters) was inserted in-line between the solvent mixer and the injector to reduce the instrument background. Detailed information regarding the method can be found in Yu et al.\(^{42}\)

Briefly, PFOA separation was performed on an ACQUITY BEH C18 column (2.1 mm \( \times \) 50 mm, 1.7 \( \mu \text{m} \), Waters) at 50 \( ^\circ \)C with 2 mM ammonium acetate in water and methanol as the mobile phase. The TQMS was operated in negative electrospray ionization multiple reaction monitoring mode. For each sample, 10 \( \mu \text{L} \) was injected into the UPLC–TQMS with a flow rate of 400 \( \mu \text{L} / \text{min} \). Initially, the mobile phase containing 10% methanol was held for 0.50 min), after which it was increased to 25% for 1 min, 85% for 6 min, 100% for 7 min, and then back to 10% for 9 min for equilibration.

The instrument detection was conducted for \( m/z \) parent ion 413, and product ion 369, with a quantifiable limit of 0.23 ppb. The recovery efficiency for liver samples spiked at 10 \( \mu \text{g} / \text{kg} \) PFOA was 79 ± 4.1%, and for UBM GI fluid, it was 94 ± 5.2%.

Analysis of Free Fatty Acids and Zeta Potential in Gastrointestinal Fluids. To determine their impact on PFOA bioaccessibility, the concentration of free fatty acids in GI fluids was determined. Following bioaccessibility assessment using in vitro assays, samples were centrifuged (4000 rpm, 10 min) and supernatants (2.5 mL) were transferred to PP tubes for free fatty acid titration following the Chinese national standard GB/
Table 1. PFOA Relative Bioavailability in the Presence of Food Determined Using an In Vivo Mouse Model and PFOA Bioaccessibility Determined Using Three In Vitro Methods

<table>
<thead>
<tr>
<th>Food</th>
<th>PFOA relative bioavailability (%)</th>
<th>PFOA bioaccessibility (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBET&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>animal-based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutton</td>
<td>36.9 ± 2.0</td>
<td>22.8 ± 5.8</td>
</tr>
<tr>
<td>beef</td>
<td>18.3 ± 2.4</td>
<td>27.3 ± 7.4</td>
</tr>
<tr>
<td>shrimp</td>
<td>15.1 ± 1.1</td>
<td>42.1 ± 5.9</td>
</tr>
<tr>
<td>milk1</td>
<td>58.7 ± 5.9</td>
<td>94.8 ± 13</td>
</tr>
<tr>
<td>milk2</td>
<td>45.1 ± 3.2</td>
<td>99.5 ± 8.4</td>
</tr>
<tr>
<td>plant-based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chips</td>
<td>29.0 ± 3.4</td>
<td>50.3 ± 4.4</td>
</tr>
<tr>
<td>pea</td>
<td>69.2 ± 12</td>
<td>19.3 ± 1.7</td>
</tr>
<tr>
<td>haricot bean</td>
<td></td>
<td>38.6 ± 10</td>
</tr>
<tr>
<td>corn oil</td>
<td>4.30 ± 0.8</td>
<td>15.8 ± 2.9</td>
</tr>
<tr>
<td>carrot juice</td>
<td></td>
<td>81.4 ± 4.7</td>
</tr>
<tr>
<td>orange juice</td>
<td></td>
<td>73.6 ± 12</td>
</tr>
<tr>
<td>mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60% starch</td>
<td></td>
<td>10.7 ± 5.0</td>
</tr>
<tr>
<td>40% starch</td>
<td></td>
<td>17.2 ± 4.3</td>
</tr>
<tr>
<td>20% starch</td>
<td></td>
<td>26.3 ± 7.5</td>
</tr>
<tr>
<td>60% protein</td>
<td></td>
<td>55.3 ± 7.2</td>
</tr>
<tr>
<td>40% protein</td>
<td></td>
<td>28.7 ± 7.3</td>
</tr>
<tr>
<td>20% protein</td>
<td></td>
<td>9.83 ± 5.6</td>
</tr>
</tbody>
</table>

Data represent average PFOA relative bioavailability (n = 4) and bioaccessibility (n = 3) in the presence of different foods (% ± SD) and PFOA bioaccessibility was determined following gastrointestinal extraction of PFOA added to different foods. PBET = Physiologically based extraction test; UBM = Unified Bioaccessibility Research Group of Europe Method; and IVD = in vitro digestion method.

RESULTS AND DISCUSSION

To better understand the effects of food composition on human exposure to PFOA, an in vivo mouse model and three in vitro assays (UMB, PBET, and IVD) were used to determine the relative bioavailability (RBA) and bioaccessibility of PFOA. Two different food types were assessed, including packed food (n = 11) and food mixtures (n = 6) containing different proportions of starch and protein. The presence of PFOA in different foods has been widely reported with ng/g concentrations in market foods and up to several hundreds of ng/g in samples (e.g., fish) from potentially polluted areas. In dietary corn oil is increased from 5% to 30%. Weber and Lanno also observed that BaP bioavailability in catfish decreases by ~30% after adding monoglycerides, free fatty acids, or triglycerides to intestinal fluid. It was hypothesized that hydrophobic BaP was absorbed by undigested lipids, which were unable to pass through the brush border membrane of enterocytes and was subsequently excreted via the feces. In this study, PFOA may be complexed with cations such as Ca²⁺ and Mg²⁺ in the intestinal tract (pH > 6.5), which are preferably absorbed compared to aqueous phase. A similar phenomenon has been observed for ionizable drugs, such as alendronic acid, ciprofloxacin, and etidronic acid. As a consequence, PFOA salts were probably mixed with undigested lipids and excreted in the feces, explaining the decrease of PFOA-RBA in foods with higher lipid content. A second possible reason for the decrease in PFOA-RBA was that during lipid digestion, lipase broke down lipids into free fatty acids and monoglycerides. With a structure similar to octanoic acid, PFOA absorption onto monocarboxylate transporters (the plasma membrane transporters that carry molecules having one
Three in vitro methods were used to determine PFOA bioaccessibility in the presence of foods. PFOA bioaccessibility determined using UBM, PBET, and IVD was 8.7–73%, 16–100%, and 29–114% (Table 1). Generally, PFOA bioaccessibility was the highest in liquid food including milk, carrot juice, and orange juice, with bioaccessibility being >60% irrespective of the method. In contrast, PFOA bioaccessibility was the lowest in corn oil, ranging from 8.70 ± 2.50% (UBM) to 28.8 ± 1.10% (IVD) (Table 1).

When food composition was correlated to PFOA bioaccessibility, different relationships were observed for the three assays. Similar to PFOA-RBA, a significant negative correlation was observed between PFOA bioaccessibility and lipid content \((r = 0.82, p < 0.01)\) for UBM (Figure 2). The strong correlation with lipid content was confirmed when food mixtures (containing starch and protein) were analyzed separately \((r = 0.90, p < 0.05)\). However, a poor correlation was observed between PFOA bioaccessibility determined using the UBM and protein and carbohydrate content \((r = 0.09–0.11)\). For PBET and IVD, no significant correlation was observed between food composition and PFOA bioaccessibility \((r = 0.01–0.50)\) (Figure 2).

**Relationship between PFOA Relative Bioavailability and Bioaccessibility in Food.** To determine the ability of in vitro assays to predict PFOA-RBA in food matrices, the in vivo and in vitro correlation (IVIVC) was determined. A strong correlation was observed between PFOA-RBA and bioaccessibility determined using UBM \((r = 0.79, p < 0.01)\) (Figure 3), with slope and y-intercept being 0.77 and 11.7.

The fact that both UBM and PFOA-RBA showed a strong correlation with lipid content in foods \((r = 0.76–0.82)\) indicated the similarity between the two methods. Unlike UBM, no significant IVIVC \((p > 0.05)\) was established for PBET \((r = 0.11)\) and IVD \((r = 0.22)\). Among the 11 individual foods, pea had the largest influence on the IVIVC for PBET. Excluding pea data, PBET showed a strong linear correlation with PFOA-RBA \((r = 0.82, p < 0.001)\) although it did not improve the relationship for IVD (Figure 3).

**Impacts of in Vitro Methods on PFOA Bioaccessibility in Foods.** The difference in PFOA bioaccessibility determined using the three assays may stem from compositional differences in their GI fluids. As detailed above, lipid content was negatively correlated with PFOA-RBA (Figure 1) and PFOA bioaccessibility determined using UBM (Figure 3). Among the three assays, IVD contained the lowest concentration of pancreatin \((0.3 \text{ g/L})\), a mixture of amylase, lipase, and protease, to digest lipid.\(^{45}\) In addition, its intestinal extraction period \((2 \text{ h})\) was the shortest of the three assays.\(^{25}\) In contrast, UBM contained the highest pancreatin \((1.38 \text{ g/L})\) and lipase \((0.23 \text{ g/L})\) concentrations with the longest intestinal extraction period \((4 \text{ h}; \text{Table S2})\).\(^{38}\) Higher lipase concentration and longer incubation time may enhance lipid digestion during intestinal extraction,\(^{46}\) and as a result, lipids may be digested to a greater extent during UBM extraction compared to PBET and IVD (SI Table S1).

To test this hypothesis, corn oil (i.e., pure lipid) was added to the three assays, and the production of free fatty acids was determined. Following intestinal extraction, \(180 ± 11.0, 167 ± 21.0, \text{ and } 105 ± 17.0 \text{ mM/ml of free fatty acids was produced by UBM, PBET, and IVD, respectively (Figure 4A), indicating that UBM had the strongest lipolysis ability. The released free fatty acids have the potential to reduce the dissolution of PFOA by saturating the intestinal solution. Considering the concentration of these acids was far below the solubility of PFOA (9.5 g/L at 25 °C), other factors may also influence PFOA bioaccessibility in foods. As detailed above, under near
neutral conditions in intestinal solutions, PFOA may form complexes with cations such as Ca\(^{2+}\). Among the three methods, UBM was the only assay that contained CaCl\(_2\) in GI fluids (799 mg/L Ca in gastric phase, plus 200 mg/L Ca in intestinal phase). As a consequence, this may decrease PFOA bioaccessibility compared to results from other assays.

In addition, as a surfactant, the solubility of PFOA is largely determined by colloidal stability in the GI fluids. Therefore, micelle formation was also determined based on zeta potential (\(\zeta\)). The stability of colloidal suspensions and formation of lipid micelles are related to its \(\zeta\). A higher zeta potential confers greater stability, which indicates micelles may be resistant to aggregation and therefore minimize aggregation within the chyme.\(^{47,48}\) Among the three methods, the bile content varied from 0.92 g/L (UBM), to 1.78 g/L (PBET), to 2.0 g/L (IVD). The difference in bile concentrations suggests that UBM should have the least potential to form stable micelles, therefore with the lowest \(\zeta\).

To test this hypothesis, the zeta potential of micelles in GI fluids before and after intestinal digestion was determined after the addition of corn oil (Figure 4B). The higher the absolute value of \(\zeta\), the more stable the colloid suspension.\(^{47,48}\) The \(\zeta\) in the intestine solutions of UBM, PBET, and IVD before incubation was variable, ranging from \(-13 \pm 4.4\) to \(-32 \pm 0.5\) to \(-43 \pm 6.8\) mV. The IVD produced the lowest \(\zeta\), indicating that micelles were the most stable, while they were least stable in UBM. The measured \(\zeta\) values were consistent with their bile content, i.e., higher bile content resulted in higher micelle stability. However, after intestinal extraction, the \(\zeta\) decreased to \(-5.2 \pm 0.7\), \(-6.7 \pm 3.5\), and \(-15 \pm 1.6\) mV, indicating that colloid stability decreased after intestinal digestion (Figure 4B). Both UBM and PBET showed lower \(\zeta\), suggesting potential coagulation of micelles in intestinal solutions. The results supported the hypothesis that assays with higher bile content resulted in higher micelle stability in intestinal solution, leading to higher PFOA bioaccessibility.

Compared to hydrophobic POPs, due to its high solubility in water (9.5 g/L at 25 °C) and surfactant-like properties, PFOA has a greater propensity to partition from a lipid phase to an aqueous phase in intestinal solution. However, when coexposed with foods, PFOA may complex with cations and partition to the lipid phase, thereby lowering its bioaccessibility. In addition, colloid stability also affects PFOA concentration in intestinal solutions. Because colloidal suspensions were unstable in UBM intestinal solution, this resulted in lower PFOA bioaccessibility in corn oil compared to the other two methods (Figure 3). Compared to gastric solution, all three methods showed lower \(\zeta\) and therefore lower colloid stability in intestinal solution, indicating that the hydrolysis of lipids from corn oil reduced colloid stability after intestinal extraction. This was consistent with Charman and Stella\(^{49}\) who reported that DDT-RBA was reduced when administered in peanut oil compared to oleic

Figure 2. Impact of food composition on PFOA bioaccessibility using three different in vitro methods. “—” represents regression line of all foods (\(n = 17\)). “- - -” represents regression line of individual foods (\(n = 11\)). ■ represents individual foods, and □ represents mixture foods. Error bar represents ± SD, \(n = 3\).
fatty acid, suggesting that oil digestion reduces the absorption of POPs. Data were consistent with the fact that lipid content in food was negatively correlated with PFOA-RBA and PFOA bioaccessibility (UBM), suggesting that micelle stability was an important controlling factor.

Implications for PFOA Exposure Assessment. In this study, an in vivo mouse model and three in vitro assays (UMB, PBET, and IVD) were used to determine the relative bioavailability and bioaccessibility of PFOA, an emerging organic contaminant, in the presence of 17 different foods. On the basis of PFOA concentration in mice liver after 7-d exposure, PFOA-RBA ranged from 4.3 to 69%, with lipid content being a major factor negatively affecting PFOA bioavailability. This indicates that absorption of this emerging organic contaminant will vary depending on diet, with high-fat diets reducing PFOA absorption thereby bioavailability.

Potential exists for predicting PFOA-RBA for the refinement of human health exposure using a simple, inexpensive in vitro assay. Although PFOA bioaccessibility varied among methods due to differences in lipolysis ability and the potential to form stable micelles, a strong correlation \( r = 0.79 \) was observed between PFOA-RBA and PFOA bioaccessibility determined using UBM. Bioaccessibility data indicated that colloidal stability in intestinal solutions was a major factor influencing PFOA bioaccessibility, which is different from typical hydrophobic POPs. The results suggest that UBM has the potential to predict PFOA-RBA in food samples although additional sample assessment is required to confirm the in vivo/in vitro correlation.

ASSOCIATED CONTENT

Supporting Information

Protein, lipid, and carbohydrate composition of 17 different foods used to assess PFOA relative bioavailability (Table S1), composition and operation parameters for three in vitro methods used to assess PFOA bioaccessibility in foods (Table S2), PFOA concentrations in different tissues in mice after different dosing for 7 d (Figure S1), and concentrations of PFOA in mouse chow and spiked to different foods (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.
spiked milk with 14C-phenanthrene, 14C-benzo [a] pyrene or 14C-TCDD in growing pigs. Chemosphere 2002, 48 (8), 843−848.


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