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Determination of perfluorooctane sulfonate and perfluorooctanoic acid in food packaging using liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

This research aimed to monitor the amounts of PFOS and PFOA in food packaging and study the migration of PFOS and PFOA from food packaging, using a saliva simulant and pressurized liquid extraction (PLE) technique. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) was employed to determine residues of PFOS and PFOA by using a gradient reversed-phase method with ammonium acetate/acetonitrile buffer. A good linearity was established for PFOS and PFOA in a range of 0.05–10 $\mu g\,L^{-1}$, with $R^2 \geq 0.9998$. Of the samples extracted by methanol, the highest concentration of PFOS was found in fast-food container samples, at a level of 92.48 $ng\,dm^{-2}$. For PFOA, the highest concentration in samples extracted by methanol was found in ice cream cup samples, at a level of 16.91 $ng\,dm^{-2}$. The amounts of PFOS and PFOA that migrated from food packaging samples through contact with saliva simulant were 4.80 and 4.55 $ng\,dm^{-2}$, respectively. Saliva simulant could leach PFOS and PFOA from the group of the thickest paper samples ($\leq 1\,dm^2\,g^{-1}$) at levels of 7.01 and 6.41 $ng\,dm^{-2}$, respectively, indicating that paper with greater thickness and less area might release larger quantities of coated/added PFOS or PFOA.

1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used in numerous industrial and commercial applications – including paper and textile treatments, production of fluoropolymers and cosmetics, and in insecticide formulations and firefighting foams – because of their unique properties as synthetic organic chemicals consisting of a fully fluorinated carbon chain and a sulfonate group or carboxylic group, respectively [1]. The presence of strong C—F bonds makes them chemically and thermally very stable, and resistant to hydrolysis, photolysis, microbial degradation or metabolism. However, PFOS and PFOA have been observed to persist in the environment, bioaccumulate in human and animal tissue, and biomagnify in food chains, and thus may have potentially significant adverse impacts on human health and the environment [1,2].

PFOS and PFOA belong to the wide group of perfluorinated compounds (PFCs). In the year 2000, growing concern about this class of chemicals resulted in the announcement by the largest producer, the 3M Company, to phase out the production of PFOS. Since then, a number of papers reporting environmental concentrations of PFOS and PFOA have been published. PFOS was recently included as a persistent organic pollutant (POP) in Annex B of the Stockholm Convention [3]. However, PFOA and the homologous chemicals of PFOS, which potentially may degrade to PFOS, are not regulated yet.

The concentrations of various PFCs have been determined in the human blood of individuals from a number of regions and countries around the world [4–6]. The half-lives of human serum elimination of PFOS and PFOA have been estimated at 5.4 and 3.8 years, respectively [7]. To mitigate any future risks associated with PFOS and PFOA, there is thus an urgent need for improved understanding of the pathways of human exposure.

Paper is the most widely used as packaging material. The surface of paper is treated to improve its properties, including physical strength, oil/grease resistance, and wettability. Food packaging products made of paper material usually contain coatings/additives with PFOS and PFOA for oil and water resistance [8,9]. Therefore, the analysis of PFOS and PFOA leached from the package into its contents is important for quality assurance and food safety. This research aims to monitor the amount of PFOS and PFOA in food packaging products made of paper material, and study the migration of PFOS and PFOA from food packaging using a saliva simulant and pressurized liquid extraction (PLE) technique.

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2. Materials and methods

2.1. Chemicals

Perfluorooctane sulfonate (PFOS standard of 98% purity) and perfluorooctanoic acid (PFOA standard of >95% purity) were purchased from Wako (Japan). HPLC-grade methanol (>99.99% purity) and acetonitrile (>99.8% purity) were from Merck (Germany). Ammonium acetate (99.9999% purity) was from Sigma–Aldrich (USA). In addition, the following reagents were used to prepare the saliva simulant: potassium chloride (>99.0% purity, Sigma–Aldrich), potassium carbonate (>99% purity, Merck), dipotassium hydrogen phosphate (99% purity, Merck), sodium chloride (>99.5% purity, Sigma–Aldrich), calcium chloride dehydrate (>99.5% purity, Merck), magnesium chloride hexahydrate (>99% purity, Merck), and 1 N hydrochloric acid (Merck).

The saliva simulant was prepared with 0.82 mM magnesium chloride (0.0781 g L^{-1}), 1.0 mM calcium chloride (0.1110 g L^{-1}), 3.3 mM dipotassium hydrogen phosphate (0.5748 g L^{-1}), 3.8 mM potassium carbonate (0.5252 g L^{-1}), 5.6 mM sodium chloride (0.3273 g L^{-1}), and 10 mM potassium chloride (0.7455 g L^{-1}). The potassium and sodium salts were dissolved in distilled water before adding the magnesium and calcium salts, making up to 1 L. The pH of the solution was adjusted to 6.8 by dropwise addition of 1 N hydrochloric acid [10].

2.2. Food packaging samples

2.2.1. Sample collection

The package samples were fresh packages that had never been used to contain food products. Thirty-four samples of food packaging made of paper (10 instant food cups, 3 microwave-popcorn bags, 3 beverage cups, 2 ice cream cups, 8 fast-food containers, 7 dessert containers and 1 baking paper) were purchased from domestic and international restaurants/cafes located in Bangkok, Thailand. They represented various paper packaging methods for containing food.

2.2.2. Sample preparation

Before the analysis of paper samples, the printing and outside layer of the containers were deliberately removed with the aid of a cutter. The remaining paper was cut with scissors into smaller size pieces (approximately 5 mm × 5 mm). The pieces were kept in a desiccator, and then divided into 2 g (dry weight) samples for use in the analysis.

2.3. Pressurized liquid extraction (PLE) with methanol and saliva simulant

The paper samples were extracted using two extractants: methanol and saliva simulant. Each of the paper samples was prepared in duplicate for each type of extraction solvent, using PLE technique. Firstly, methanol was used as a PLE solvent to determine the overall concentrations of PFOS and PFOA. Secondly, saliva simulant was used as a PLE solvent to initiate the migration of PFOS and PFOA from food packaging samples. This was conducted to simulate the effect of "mouthing" in order to understand its potential impact on human health.

Pressurized liquid extraction (PLE) was performed using a Dionex ASE 200 accelerated solvent extractor (ASE) equipped with a solvent controller. Two grams of each paper sample were inserted into a 33 mL stainless steel ASE cell, which was filled with stainless steel balls. Cellulose filters (Dionex, P/N 049458) were placed at the bottom of the extraction cell. In this work, a precise and accurate PLE method is proposed for the extraction of PFOS and PFOA from food packaging made of paper. PLE was performed using a 100% flush volume with a 60 s purge. The extraction conditions were 80 °C, 100 psi, preheating for 0 min, heating for 5 min, static extraction time of 30 min, and one extraction cycle.

After extraction, the liquid phase sample was decanted into 50 mL polypropylene bottle for subsequent solvent extract preparation. As PFOS and PFOA are ubiquitous environmental contaminants and can be adsorbed by glassware, considerable care was taken to avoid sample contamination. All labware and equipment used for PFOS and PFOA preparation or experiments were made from plastic (polypropylene grade), and washed twice with methanol and then twice with ultrapure water prior to use.

2.4. Preparation of extracted sample for analysis

2.4.1. Extracted sample in methanol

After the PLE technique, 1 mL of each of the extracted samples in methanol was placed into a 2 mL centrifuge tube together with 1 mL of ultrapure water, and the tube was capped. The addition of ultrapure water helps to precipitate and remove soluble sample components [11]. The tubes were shaken to mix the contents homogenously, and then centrifuged for 20 min at 12,000 rpm and 25 °C to remove any suspended particles in the extracted sample. After centrifugation, the liquid phase sample was transferred into a vial for analysis by LC–MS/MS.

The calibration standards of PFOS and PFOA were prepared at seven concentration levels: 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg L^{-1}, in a composition of 50:50 (v/v) methanol and ultrapure water.

2.4.2. Extracted sample in saliva simulant

The extracted sample in saliva simulant (2 mL) was placed into a 2 mL centrifuge tube, and capped. Tubes were centrifuged for 20 min at 12,000 rpm and 25 °C to precipitate any suspended particles. Then, 1 mL of the extracted sample was transferred into an 8 mL polypropylene tube. The sample tube was placed on a nitrogen purge (model MG-2200, Eyela, Japan) to evaporate under high purified nitrogen gas at 50 °C for 30 min or until the sample in the tube was completely dry. The sample was reconstituted in 1 mL acetonitrile, then shaken and transferred into a vial for analysis by LC–MS/MS.

The calibration standards were prepared in 40:60 (v/v) acetonitrile and ultrapure water by adding PFOS and PFOA mixed standard at seven concentration levels: 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg L^{-1}.

2.5. Instrumental analysis

Analysis was performed using an Agilent 1200 SL high-performance liquid chromatograph (HPLC) interfaced with an Agilent 6400 Triple Quadrupole mass spectrometer (MS/MS) (Agilent Technologies, USA). The HPLC column were an Agilent Eclipse XDB-C_{18} 4.6 mm × 50 mm, 1.8 μm particle size, and an Agilent Eclipse Plus C_{18}, 2.1 mm × 100 mm, 1.8 μm particle size, maintained at a temperature of 40 °C. The sample injection volume was 10 μL. The mobile phase was comprised of: (A) 10 mM ammonium acetate (CH_3COONH_4) in ultrapure water; and (B) HPLC-grade acetonitrile (CH_3CN). The column was flushed with the mixture using a gradient that increased the acetonitrile to 90% by a process that started with an initial condition of 45% (B), increased to 50% (B) at 5.0 min, then to 60% (B) at 5.5 min, was held at 60% (B) for 4.5 min, and then went up to 90% (B) at 15 min, at a flow rate of 0.25 mL min^{-1}. One hour of washing with the mobile phase mixture at a flow rate of 0.25 mL min^{-1} was used to remove experimental PFOS and PFOA background for every ten analyzed samples.

The mass spectrometer parameters were optimized to transmit the parent ions, fragment them, and monitor the daughter ions.

Table 1
Mass spectrometer characteristics for PFOS and PFOA.

Target compound	Retention time (min)	Parent ion (m/z)	Productivity ion (m/z)	Dwell time (ms)	Collision energy (V)
PFOS	10.9	499	80	50	55
PFOA	4.8	413	369	50	5

The MS/MS was operated to detect liquid samples in electrospray ionization (ESI) negative mode with capillary voltage 3500 V. Analyte ions were monitored by using multiple reactions monitoring (MRM) mode. Ions selected were 499 (parent) for PFOS and 413 (parent) for PFOA. Ions monitored were 80 (daughter) for PFOS and 369 (daughter) for PFOA. Nitrogen was the collision gas, and the collision energy was 55 V for PFOS and 5 V for PFOA. The PFOS and PFOA retention time (RT) under these conditions were 10.9 and 4.8 min, respectively. MS/MS characteristics for the target compounds are shown in Table 1.

The instrument was calibrated for PFOS and PFOA by seven concentration levels of PFOS and PFOA mixed solution in a range of 0.05–10.00 $\mu g\,L^{-1}$. Most samples were analyzed shortly after preparation. Otherwise, they were stored in a refrigerator in polypropylene vials at 4 °C and analyzed within 3 days. A blank sample was analyzed with every set of samples prepared. If the concentration result was not less than the upper calibration standard, the sample was diluted and reanalyzed.

3. Results and discussion

3.1. Analytical method performance

Calibration curves were obtained by dilution of PFOS and PFOA mixed standard solutions. Calibrated concentrations of PFOS and PFOA ranging from 0.05 to 10 $\mu g\,L^{-1}$ were prepared in 50:50 (v/v) methanol/ultrapure water and 40:60 (v/v) acetonitrile/ultrapure water – solutions which matched the final preparation of extracted samples. The different component in PFOS and PFOA mixed standard solution between the 50:50 (v/v) methanol/ultrapure water solution and 40:60 (v/v) acetonitrile/ultrapure water solution could confirm the different component of standard solution by observing and determining the slope of a standard curve. If the solution effect was not present, both slopes of the standard curve and the spiked sample calibration should be the same. Table 2 shows the standard and the spiked sample calibration equations. Their slopes were not much different, indicating that no interference occurred from the solution compositions. The calibration curves of PFOS and PFOA in 50:50 (v/v) methanol and ultrapure water solution were linear response. Correlation of determination (R^2) was 0.9999 for both PFOS and PFOA. In the case of the calibration curves of PFOS and PFOA in 40:60 (v/v) acetonitrile and ultrapure water solution, R^2 of PFOS and PFOA were 0.9998 and 0.9999, respectively. R^2 values obtained from this study were greater than 0.9995, which is the required level for the accepted accuracy to verify linearity. A chromatogram of PFOS and PFOA at 10 $\mu g\,L^{-1}$ is shown in Fig. 1, with retention times for PFOS and PFOA of 10.9 and 4.8 min, respectively. Ions selected of PFOS and PFOA were 80 and 369, respectively.

3.2. PFOS and PFOA concentration in food packaging

The 34 samples of food packaging were analyzed with LC–MS/MS after PLE. The samples extracted by methanol solvent had average concentrations of PFOS and PFOA of 8.57 and 5.03 $ng\,dm^{-2}$, respectively; while the average concentrations of PFOS and PFOA were 4.80 and 4.55 $ng\,dm^{-2}$, respectively, for the samples extracted by saliva simulant. PFOS and PFOA were detected in almost all food packaging samples made of paper. A detailed overview of the results is given in Table 3.

Of the samples extracted by methanol, the highest concentration of PFOS was found in fried-chicken box, at a level of 92.48 $ng\,dm^{-2}$. For PFOA, the highest concentration in samples extracted by methanol was found in ice cream cup sample, at a level of 16.91 $ng\,dm^{-2}$. Tests for PFOS and PFOA migration from food packaging samples using saliva simulant showed that the highest concentration of PFOS was found in beverage cup on the hot cup sample (10.26 $ng\,dm^{-2}$); for PFOA, the highest concentration was in french-fried box (41.71 $ng\,dm^{-2}$). The data from this study showed generally low levels of PFOS and PFOA (<100 $ng\,dm^{-2}$) presented in the food packaging samples, indicating that not all types of food packaging included PFOS and PFOA as coating materials on the food-contact side, or as additives in the paper material. A previous study of paper products from the USA showed the highest levels of PFOA in microwave-popcorn bags (290 $\mu g\,kg^{-1}$); but PFOA was not detected in some paper products such as sandwich wrappers, hamburger wrappers and french-fry boxes [8]. In this study the highest concentration of PFOA was in ice cream cup, at 16.91 $ng\,dm^{-2}$ (~7.27 $\mu g\,kg^{-1}$), as shown in Table 3; the concentration in the samples was around 40 times less than in the microwave-popcorn bags in the previous study. Some food packaging samples were bought from small restaurants/cafes, and (for reasons of cost savings by the owner when buying food packaging from industrial or wholesale suppliers) did not necessarily contain coated/added fluorochemicals; this may explain the absence of detectable PFOS and PFOA levels in some samples. Jogsten et al. [12] investigated the concentrations of PFCs in food and packaged food in Spain. Their report showed that PFOS was the compound most frequently detected, ranging from 0.01 to 0.33 $ng\,g^{-1}$, while PFOA was only found in one sample at 0.675 $ng\,g^{-1}$. Meanwhile the average concentrations of PFOS and PFOA in food packaging in this study were ~10.11 and ~5.94 $ng\,g^{-1}$ (Table 3), respectively, which was higher than in the packaged food in the report by Jogsten et al. [12]. Concentrations of PFOS and PFOA found in both packaged food and food packaging items suggested that food packaging might play

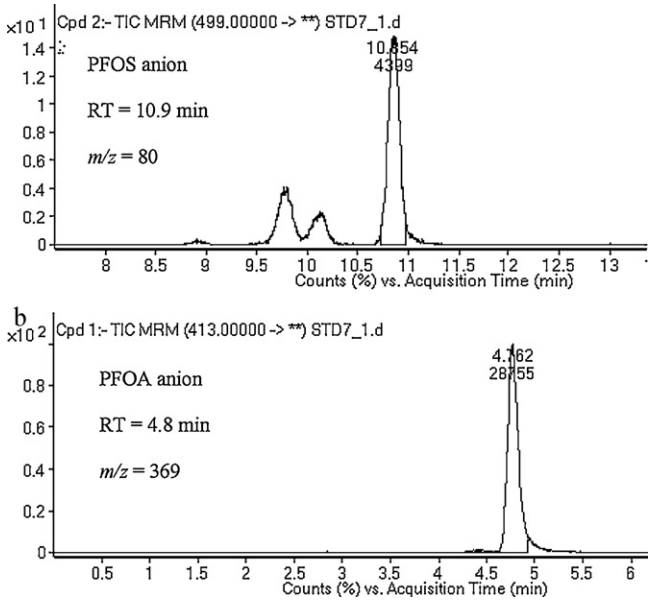

Fig. 1. Example chromatograms of PFOS (a) and PFOA (b) at 10 $\mu g\,L^{-1}$.

Table 2
Linear regression equations of standard PFOS and PFOA.

Calibration curves of PFOS and PFOA	PFOS (C8-S)		PFOA (C8-A)	
	Linear regression equation	R^2	Linear regression equation	R^2
In methanol:water, 50:50 (v/v)	$y = 427.8319x$	0.9999	$y = 1379.0101x$	0.9999
In acetonitrile:water, 40:60 (v/v)	$y = 436.7081x - 42.80$	0.9998	$y = 928.7694x + 44.35$	0.9999

y is the response and x is the concentration ($\mu g\,L^{-1}$).

a key role as a source of human exposure to PFOS and PFOA through the diet.

3.3. Comparison of PFOS and PFOA concentration with areas of paper packaging

Comparison of PFOS and PFOA concentrations in food packaging samples using an area of 1 g of paper sample ranged in value between ≤ 1 and 5 $dm^2\,g^{-1}$. For a 1 g paper sample, the area size was the inverse of the thickness of the paper sample. (If 1 g samples have greater area, it means that they have less thickness.) As seen in Fig. 2, the results show that the concentrations of PFOS and PFOA extracted from paper samples by methanol and saliva simulant were comparable. The results demonstrate a significant relationship of PFOS and PFOA concentrations to the paper area. The concentrations increased as the area of the paper samples decreased. The highest concentration was found in paper samples with an area $\leq 1\,dm^2\,g^{-1}$. Food packaging samples with areas $\leq 1\,dm^2\,g^{-1}$ were instant food cups, ice cream cups, and some kinds of fast-food containers such as fried-chicken boxes and french-fry boxes. This group of paper samples had the greatest thickness. The average concentrations of PFOS and PFOA in a 10 cm × 10 cm paper sample were 12.38 and 7.25 ng, respectively. In testing of the saliva simulant, PFOS and PFOA were leached from the group of the thickest paper samples at levels of 7.01 and 6.41 ng, respectively. The results indicated that 1 g paper samples with less area size (more thickness) might have larger quantities of coated/added PFOS or PFOA. Moreover, this group of paper samples are widely use in food and beverage packaging. Any chemical additives can easily leach into food and beverages from the paper packaging. Saliva simulant can initiate the migration of high concentrations of PFOS and PFOA from paper samples, at almost the same levels as from paper samples extracted by methanol solvent (Fig. 2). Therefore, there is a potentially significant negative impact on human health from the consumption of food and beverages contained in paper packaging.

Table 3
Concentrations ($ng\,dm^{-2}$) of PFOS and PFOA in various food packaging samples.[a]

Categories/#trademark	Detail	Area, $dm^2\,g^{-1}$	PFOS, $ng\,dm^{-2}$		PFOA, $ng\,dm^{-2}$	
			Methanol	Saliva simulant	Methanol	Saliva simulant
Instant food cup (10)						
#1	Noodle cup (5)	0.35	7.47	8.48	8.82	4.95
	Instant rice porridge cup (1)	0.35	8.25	*ND*	9.55	*ND*
#2	Noodle cup (1)	0.40	7.22	8.72	4.21	1.10
	Instant rice porridge cup (1)	0.40	7.37	7.44	6.11	4.17
#3	Instant rice porridge cup (1)	0.40	7.53	7.44	2.36	3.44
#4	Instant rice porridge cup (1)	0.30	9.83	*ND*	6.75	*ND*
Microwave-popcorn bag (3)						
#5	Microwave-popcorn bag (2)	1.20	2.54	2.48	1.71	1.44
#6	Microwave-popcorn bag (1)	1.30	*ND*	2.26	0.12	2.90
Beverage cup (3)						
#7	Hot cup (1)	0.40	11.45	7.44	8.11	1.51
#8	Hot cup (1)	0.30	9.62	10.26	3.01	2.06
#9	Cool cup (1)	0.35	8.43	*ND*	10.29	*ND*
Ice cream cup (2)						
#10	Ice-cream cup (1)	0.40	7.37	7.35	16.91	1.91
#11	Ice-cream cup (1)	0.45	6.41	6.59	3.39	1.23
Fast food container (8)						
#12	Fried-chicken box (1)	0.40	12.56	9.15	16.07	15.15
	French-fried bag (1)	2.00	8.60	2.23	4.36	3.28
	French-fried wrapper (1)	2.40	9.16	1.24	4.16	3.72
	Fried-chicken Wrapper (1)	2.50	2.94	1.41	2.36	1.77
#13	Fried-chicken box (1)	0.40	92.48	7.52	3.86	17.74
	French-fried bag (1)	2.20	1.37	1.45	0.65	2.66
	French-fried box (1)	0.50	6.90	6.02	9.41	41.71
	Hamburger wrapper (1)	3.50	0.86	0.86	0.42	0.99
Dessert container (7)						
#14	Pretzels box (1)	0.35	8.79	8.60	5.79	5.69
	Pretzels wrapper (1)	2.10	1.40	*ND*	0.52	0.72
#15	Donut box (1)	0.35	8.25	8.40	1.16	1.86
	Donut wrapper (1)	4.40	0.66	*ND*	0.41	*ND*
#16	Donut box (1)	0.30	10.04	9.92	1.15	12.34
	Donut wrapper (1)	4.65	*ND*	*ND*	0.09	0.34
	Donut bag (1)	2.25	*ND*	1.42	0.90	0.79
Baking paper (1)						
#17	Wrapper (1)	2.45	1.33	*ND*	1.23	*ND*
Average of 34 food packaging samples		1.18	8.57	4.80	5.03	4.55

Sample (n): number of sample. ND: non detection.
[a] Two composite samples were analyzed for each food packaging.

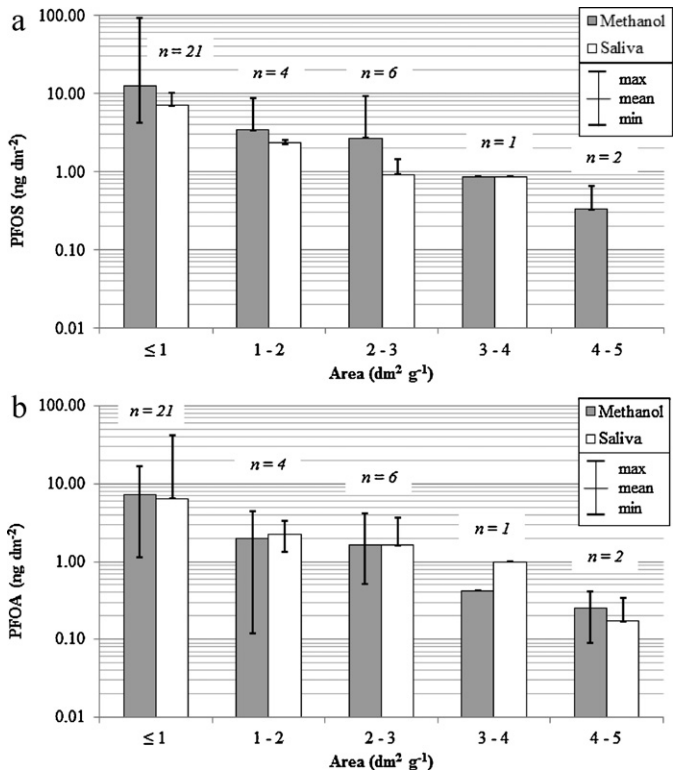

Fig. 2. PFOS (a) and PFOA (b) leached from the difference area size in 1 g of food packaging samples.

4. Conclusions

The results from the analysis of 34 food packaging products made of paper material indicated that they were contaminated with PFOS and PFOA, which could be leached by saliva simulant. Almost all target analytes were detected in the food packaging samples. The data presented in this paper showed that food packaging appears to be a significant source of human exposure to PFOS and PFOA. These contaminants may enter the body through consumption of food which is contained in paper packaging. Moreover, PFOS and PFOA may also enter the environment from landfill sites where paper products and materials that contain these chemicals are transferred for disposal. The present results can serve as a source of data for product-specific exposure assessment of PFOS and PFOA contamination in paper food packaging. The toxicity and migration behavior of PFOS and PFOA are not completely understood, and require further study. To ensure the safety of food packaging made of paper, these compounds should also be regulated.

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