Environment International 134 (2020) 105295

Contents lists available at ScienceDirect

Environment International

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

Distribution of novel and legacy per-/polyfluoroalkyl substances in serum and its associations with two glycemic biomarkers among Chinese adult men and women with normal blood glucose levels

Yishuang Duan[a,b,1], Hongwen Sun[a,*], Yiming Yao[a,1], Yue Meng[a,1], Yongcheng Li[a,1]

[a] Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin, China

[b] Institute of Environment and Health, Jianghan University, Wuhan, China

ARTICLE INFO

Handling Editor: Lesa Aylward

Keywords:
PFASs
6:2 Cl-PFAES
Fasting glucose
HbA1c
Glycemic biomarker

ABSTRACT

In recent years, the occurrence of novel per-/polyfluoroalkyl substances (PFASs) such as polyfluoroalkyl ether sulfonates (PFAESs) in human samples have aroused attention due to the change in PFASs production profile, however, the data are still lacking. Furthermore, epidemiological studies have examined the associations of PFAS exposure with glucose homeostasis, but with inconsistent results. Therefore, in this study, fasting serum samples from 252 participants with an age range from 19 to 87 years old were collected in Tianjin, China. A total of 21 target PFASs were determined to analyze the levels and distribution of novel and legacy PFASs in serum and to further evaluate the cross-sectional associations of serum PFAS concentrations with two glycemic biomarkers (i.e., fasting glucose and glycated hemoglobin (HbA1c)). 6:2 chlorinated PFAES (6:2 Cl-PFAES) and trifluoroacetic acid (TFA) were widely detected novel PFASs (greater than90%) with relatively high median concentrations (8.64 ng/mL and 8.46 ng/mL, respectively), which were second only to the two dominant legacy PFASs, i.e., perfluorooctanoic acid (PFOA, 14.83 ng/mL) and perfluorooctane sulfonic acid (14.24 ng/mL). The percentage contributions to the total known PFASs were separately 17.6% and 17.2% for 6:2 Cl-PFAES and TFA. The levels of 6:2 Cl-PFAES were significantly correlated with age and BMI, and the concentrations of TFA were also significantly correlated with age. Furthermore, 1% increase in serum PFOA and perfluorononanoic acid (PFNA) was separately significantly associated with 0.018% [95% confidence interval (CI): 0.004%, 0.033%] and 0.022% (95% CI: 0.007%, 0.037%) increment in fasting glucose levels. Similarly, 1% increase in serum perfluorohexanoic acid, PFNA, and perfluorohexane sulfonic acid was significantly associated with 0.030% (95% CI: 0.010%, 0.051%), 0.018% (95% CI: 0.003%, 0.033%), 0.007% (95% CI: 0.003%, 0.011%) increment in HbA1c levels, respectively. These findings suggested that 6:2 Cl-PFAES and TFA showed greater contributions to PFASs in serum and supported an association of exposure to PFASs with fasting glucose and HbA1c.

1. Introduction

Per-/polyfluoroalkyl substances (PFASs) are a group of synthetic chemicals. According to the differences in physicochemical properties, PFASs are mainly classified into ionizable PFASs including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), and neutral PFASs [e.g., flurotelomer alcohols (FTOHs)]. Due to their high hydrophobicity, lipophobicity, thermal and chemical stability, and surface activity, PFASs have been widely used in various industrial and consumer products, such as surfactants, textiles, and coating materials, which leads to their extensive detection in environmental and human samples (Xiao, 2017). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) have been reported to be the main PFASs in human serum as well as in other environmental matrices (Bao et al., 2011; Hurley et al., 2018; Li et al., 2011; Zhang et al., 2013a; Zhang et al., 2010). Due to their persistence, bioaccumulation, and toxicity on organisms and human, PFOS and PFOA were separately added to the Stockholm Convention in 2009 and 2019. As a consequence, some novel PFASs, such as polyfluoroalkyl ether sulfonates (PFAESs), perfluoroethylcyclohexane sulfonate (PFECHS, a cyclic PFAS) with similar physicochemical properties as PFOS, dodecafluoro-3H-4,8-dioxanonanoate (ADONA, one of the

* Corresponding author at: College of Environmental Science and Engineering, Nankai University, 38 Tongyan Road, Jinnan District, Tianjin 300350, China.
E-mail address: sunhongwen@nankai.edu.cn (H. Sun).
[1] Postal address: College of Environmental Science and Engineering, Nankai University, 38 Tongyan Road, Jinnan District, Tianjin 300350, China.

https://doi.org/10.1016/j.envint.2019.105295
Received 19 June 2019; Received in revised form 6 October 2019; Accepted 28 October 2019
Available online 11 November 2019

alternatives of PFOA), and FTOHs with short chain, have begun to be manufactured and used in the world, which leads to the wide detection of these novel PFASs in the environmental samples in recent years (Chen et al., 2019; De Silva et al., 2011; Pan et al., 2018; Xiao, 2017; Zhou et al., 2019).

However, in China, the production profile of PFASs is a little different from western countries. The total production PFASs has rapidly increased in China since the production has moved from western countries to Asian countries. Moreover, the use of PFAS containing products such as nonstick pans and sport cloth has increased recently (Bangma et al., 2019; Holmquist et al., 2016). It is reported that China has become one of the largest countries to produce PFOS and related chemicals and the annual production was estimated to increase from 30 tons to about 250 tons from 2001 to 2006 (Lim et al., 2011; Wang et al., 2015; Zhang et al., 2012). The production of PFOA has also been reported to increase in recent years due to the limitation on PFOS since 2009 (Wang et al., 2014). On the other hand, the detection frequency and level of short chain PFCAs, such as perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA), have increased both in the environment and human serum in China (Chen et al., 2018; Li et al., 2017; Tian et al., 2018; Zhang et al., 2013a). Specially, 6:2 chlorinated perfluoroalkyl ether sulfonate (6:2 Cl-PFAES), with the commercial name F-53B, has been used as chrome-fog depressant in the Chinese metal plating industry since 1970 s (Wang et al., 2013). Recently, several studies have reported 6:2 Cl-PFAES and its analogue 8:2 Cl-PFAES in pregnant women, and the levels of 6:2 Cl-PFAES were comparable to PFOA both in the maternal sera and cord sera (Chen et al., 2017; Pan et al., 2017). However, few studies have investigated the levels of Cl-PFAESs in serum of general population (Shi et al., 2016; Wang et al., 2018).

Besides, ultrashort-chain PFCAs, i.e., trifluoroacetic acid (TFA) and perfluoropropenoic acid (PFPrA), have varied sources such as the photodegradation of novel refrigerants in the atmosphere and the photodegradation or thermolysis of fluoropolymers, which have been increasingly used in recent years (Scheurer et al., 2017; Solomon et al., 2016). An increasing number of studies have shown the occurrence of TFA and PFPrA in various environmental samples, including air, surface water, sediment, and soil (Chen et al., 2018; Fang et al., 2018; Tian et al., 2018). However, to the best of our knowledge, TFA and PFPrA exposure levels in human have not yet been reported.

Diabetes, as a common metabolic disease, is characterized by chronic hyperglycemia. In 2015, the prevalence of diabetes in adults was estimated to be 8.8% in the world, rising to 10.4% by 2040 (Cavan et al., 2015). At present, animal studies have suggested that the gestational and lactational exposure to PFOS could lead to elevated fasting serum glucose and insulin, impaired glucose tolerance, and insulin resistance in both the offspring of rats and mice (Lv et al., 2013; Wan et al., 2014). A toxicological research has proven that exposure to perfluorononanoic acid (PFNA) could disturb glucose metabolism through inhibiting insulin signal pathway and increasing the output of glucose and glycogen synthesis in the rat liver (Fang et al., 2012). In addition, epidemiological studies have shown the associations of exposure to PFASs with glucose homeostasis, insulin resistance, and β cell function, but results are controversial (Cardenas et al., 2017; Koshy et al., 2017; Lind et al., 2014; Nelson et al., 2010; Timmermann et al., 2014). The inconsistent results may be owing to that people with diabetes or prediabetes who took antihyperglycemic medicines or changed their lifestyles were easily included in the study population based on the cross-sectional design in the most previous studies, which may interfere the associations of PFAS exposure with outcomes.

Therefore, in the present study, fasting serum were collected among the people with normal blood glucose levels and were measured for PFASs, including the novel and legacy PFASs. The major objectives of this study were to (1) report the human exposure levels of PFASs, especially novel PFASs, i.e., TFA, PFPrA, and the two main kinds of Cl-PFAESs (i.e., 6:2 Cl-PFAES and 8:2 Cl-PFAES); (2) analyze the distribution characteristics of PFASs; and (3) assess the associations of exposure to PFASs with two glycemic biomarkers, including fasting glucose and glycated hemoglobin (HbA1c), among Chinese adult men and women with normal blood glucose levels.

2. Material and methods

2.1. Study population

Two hundred and ninety four volunteers without self-reported diabetes or prediabetes who were the staff and support workers in Nankai University were randomly recruited by advertisement in Tianjin, June 2017. Tianjin is a seashore city along Bohai Bay, and it is also one of the China's four municipalities with heavy industry and dense population. Overnight fasting blood samples were drawn by professional nurses using plastic vacuum blood collection tubes and the levels of fasting glucose and HbA1c were measured in the school hospital of Nankai University. Afterwards, the serum samples were transported to the laboratory using polypropylene tubes on ice and stored at −80 °C before analysis for PFASs. The normal blood glucose levels were defined as fasting glucose between 3.89 and 6.11 mmol/L and HbA1c between 3.8% and 5.8% according to the normal reference ranges on the measurement reports in China (Guo et al., 2017). Finally, 252 subjects with normal blood glucose were selected for the study. All participants consented for the research and the study also received the ethical approval from Nankai University.

2.2. Demographic information collection

Data on age, sex, weight, height, education levels, alcohol consumption, exercising, smoking, and family history of diabetes were collected by questionnaires. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Education levels were classified into four groups, including primary school diploma or less, middle/high school diploma, undergraduate degree, and graduate degree. The status of smoking and alcohol consumption was both classified as usually (every day), sometimes (every few days), never, and ever (greater than1 year since quitting) depending on the frequencies of smoking and drinking. The status of exercising was categorized into three groups, including never, usually (every day), and sometimes (every few days).

2.3. Sample preparation and instrument analysis

A total of 21 target PFASs were measured in this study, including PFCAs with 2 to 12 carbon atoms [i.e., TFA, PFPrA, PFBA, perfluoropentanoic acid (PFPeA), PFHxA, perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA)], PFSAs with 4, 6, 8, and 10 carbon atoms [i.e., perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), PFOS, and linear-perfluorodecane sulfonic acid (L-PFDS)], fluorotelomer carboxylic acid [6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA) and 8:2 fluorotelomer unsaturated carboxylic acid (8:2 FTUCA)], and four other novel PFASs [including two main kinds of PFAESs (i.e., 6:2 Cl-PFAES and 8:2 Cl-PFAES), PFECHS, and ADONA]. Twelve isotopically-labeled PFASs ($^{13}C_4$-PFBA, $^{13}C_3$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, and $^{13}C_2$-8:2 FTUCA) were used as internal standards. Details on the standards were provided in Table S1 in Supporting Information (SI).

Serum samples were prepared with a modified method according to the previously described method for water samples (Taniyasu et al., 2005), which showed good recoveries for a wide range of PFASs. Briefly, a 0.5-mL serum sample was buffered with ammonium acetate buffer (pH 4.5) containing β-glucuronidase/sulfatase (glucuronidase

activity ≥ 100000 units/mL, sulfatase activity ≤ 7500 units/mL) and spiked with an internal standard solution containing twelve isotopically-labeled PFASs (500 ng/mL), and the mixture was incubated at 37 °C overnight. Then, solid phase extraction using Oasis WAX SPE cartridges (60 mg/3 mL, Waters, USA) and the Agilent 1200 Series high performance liquid chromatography tandem the Agilent G6460 triple quadrupole mass spectrometry (Agilent Technologies, USA) were performed to measure PFASs in serum. Further details on sample preparation and instrument analysis are available in the SI.

For each batch of serum samples (34 samples), two procedural blank samples (i.e., Milli-Q water) were prepared. For analytes that were detected in the procedural blanks, method detection limits (MDLs) were derived from three times the standard deviation of the procedural blank values. For analytes not detected or below the limits of quantitation in the procedural blanks, MDLs were derived from three times the limits of detection (LODs). LODs were derived from peak values with the signal-to-noise ratio (SNR) equaling three. Only PFHxS, PFOA, and PFNA were detected in the procedural blank samples (Table S2), and their concentrations in serum samples were subtracted with the blank values. Recoveries were validated by spiking standard solution containing 21 kinds of non-isotopically-labeled PFASs into the matrix before and after extraction, ranging from 73.4% for 6:2 FTUCA to 128.4% for TFA. The MDL values and recoveries are listed in Table S2.

2.4. Statistical analysis

All statistical analyses were performed with SPSS software (version 22.0; SPSS, IL, USA), and a two-sided $p < 0.05$ was considered significant. Due to the low detection rates of some PFASs (< 30%), only fifteen target chemicals (i.e., TFA, PFPrA, PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFHxS, PFOS, 6:2 Cl-PFAES, 8:2 Cl-PFAES, and 6:2 FTUCA) were included in the analysis. For the concentrations below MDLs, MDLs/were used to substitute for zero values. The concentrations of TFA and PFPrA were summed to represent the total concentrations of ultrashort-chain PFCAs ($\Sigma PFCAs_{C2\text{-}C3}$). The concentrations of PFBA, PFHxA, and PFHpA were summed to calculate the total concentrations of short-chain PFCAs ($\Sigma PFCAs_{C4\text{-}C7}$). And the concentrations of long-chain PFCAs ($\Sigma PFCAs_{C8\text{-}C12}$) were the sum of PFCAs with 8 to 12 carbon atoms. The four target chemicals with sulfonic acid groups (i.e., PFHxS, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES) were summed to represent the total concentrations of PFSAs (ΣPFSAs). The concentrations of $\Sigma PFCAs_{C2\text{-}C3}$, $\Sigma PFCAs_{C4\text{-}C7}$, $\Sigma PFCAs_{C8\text{-}C12}$, and 6:2 FTUCA were summed as the total concentrations of PFCAs (ΣPFCAs). All the fifteen target compounds were summed to calculate the total concentrations of PFASs (ΣPFASs). In the descriptive analyses, the concentrations of PFASs in serum are presented as geometric means, medians, 25th percentiles, and 75th percentiles. Differences in the concentrations of PFASs categorized by age, sex, and BMI were assessed using the nonparametric Mann-Whitney U test. Due to the concentrations of PFASs didn't conform to normal distributions ($p < 0.05$) depending on the Kolmogorov-Smirnov test, Spearman correlation coefficients were calculated for the levels of PFASs in serum. Pearson correlation analyses were conducted to explore the relationship of PFASs with age and BMI after the log transformations for PFASs, which ensured that PFASs conformed to normal distributions. Principal component analysis (PCA) was conducted to determine the similarity of sources of PFASs and the first three principle components with eigenvalue greater than 1 were extracted.

Linear regression models were conducted to assess the association of the levels of PFASs with fasting glucose and HbA1c in both crude and multi-adjusted models. The multiple linear regression models were used to adjust for potential confounders, including age (continuous), sex (male, female), BMI (continuous), exercising status (categorical), smoking and alcohol-drinking status (categorical), education level (categorical), and family history of diabetes (yes, no). The categorical variables were transformed into dummy variables for analysis. A stepwise method was used to judge which variables should be included in the models. Log transformation was performed for PFASs, fasting glucose, and HbA1c to ensure normal distribution of residuals. In addition, the interaction tests for covariates (i.e., age, BMI, and sex) and PFASs on two glycemic biomarkers were conducted. Due to significant interactions between age and nearly half of PFASs on HbA1c (Table S3), the associations of PFASs with HbA1c were analyzed in the population stratified by age (< 55 and ≥ 55 years of age) to explore whether associations differed between subgroups. Given that PFASs were regarded as endocrine disrupting chemicals and the associations of exposure to PFASs with glycemic biomarkers may be disturbed by the endogenous hormone levels, 55 years of age (paralleling the menopausal age) was selected as the boundary point.

Table 1
The characteristics of study population (n = 252).

Characteristics	Number (%)	Characteristics	Number (%)
Age categories (years)		Usually	33 (13.1)
19 to 39	35 (13.9)	Sometimes	16 (6.3)
40 to 54	136 (54.0)	Ever	17 (6.7)
55 to 69	73 (29.0)	Missing	3 (1.2)
70 to 87	8 (3.2)	**Status of drinking**	
Sex		Never	130 (51.6)
Male	95 (37.7)	Usually	20 (7.9)
Female	157 (62.3)	Sometimes	91 (36.1)
BMI categories (kg/m^2)		Ever	9 (3.6)
Underweight (< 18.5)	6 (2.4)	Missing	2 (0.8)
Normal (18.5 to 24.9)	160 (63.5)	**Status of exercising**	
Overweight (25 to 29.9)	76 (30.2)	Usually	70 (27.8)
Obese (≥30)	9 (3.6)	Sometimes	148 (58.7)
Missing	1 (0.4)	Never	34 (13.5)
Education		**Family history of diabetes**	
Primary school diploma or less	20 (7.9)	Yes	35 (13.9)
Middle/High school diploma	136 (54.0)	No	215 (85.3)
Undergraduate degree	60 (23.8)	Missing	2 (0.8)
Graduate degree	34 (13.5)	**Glycemic biomarkers**[a]	
Missing	2 (0.8)	Fasting glucose (mmol/L)	4.7 (4.4, 5.1)
Status of smoking		HbA1c (%)	4.5 (4.2, 4.8)
Never	183 (72.6)		

Note: BMI: body mass index.
[a] Data are medians (interquartile ranges) for fasting glucose and HbA1c.

3. Results

3.1. Population characteristics

The average age and BMI of the study population were 51 ± 10 years and 24.2 ± 3.0 kg/m^2, respectively. Demographic characteristics are shown in Table 1. Among the study population, females and people with normal weight separately accounted for 62.3% and 63.5%. And 54% of the participants were 40 to 54 years old or with middle/high school diploma. The people who never drank accounted for 51.6% and people who sometimes took exercise accounted for 58.7% of study population, respectively. A majority of people were never smokers (72.6%) and without family history of diabetes (85.3%).

3.2. Levels of PFASs in human serum

A total of 21 kinds of PFASs were determined in the study. The detection rates of PFPeA, PFBS, L-PFDS, 8:2 FTUCA, PFECHS, and ADONA were low (29.7%, 17.1%, 0%, 7.1%, 2.0%, and 16.7%, respectively), while, the remaining PFASs were detected above the MDLs in most serum samples. Table 2 shows the geometric means, medians, 25th percentiles, 75th percentiles, and detection rates of the PFASs.

Table 2
Concentrations (ng/mL) and detection rates of PFASs in serum of Chinese adult men and women[a].

	TFA	PFPrA	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	6:2 FTUCA
Geometric mean	7.21	0.46	0.34	0.54	0.06	14.82	3.14	1.82	2.31	0.10	0.09
25th percentile	5.36	0.24	0.31	0.41	n. d.	8.45	1.99	0.96	1.71	0.07	n. d.
Median	8.46	0.48	0.60	0.57	0.11	14.83	3.17	2.08	2.28	0.12	0.09
75th percentile	12.55	0.82	0.89	0.76	0.18	26.76	5.20	4.11	3.02	0.21	0.18
Detection rate (%)	97.0	77.0	85.7	99.6	66.7	100	100	99.2	100	88.9	70.2
	PFHxS	PFOS	6:2 Cl-PFAES	8:2 Cl-PFAES	$\Sigma PFCAs_{C2-C3}$	$\Sigma PFCAs_{C4-C7}$	$\Sigma PFCAs_{C8-C12}$	ΣPFCAs	ΣPFSAs	ΣPFASs	
Geometric mean	0.06	14.01	9.12	0.09	8.02	1.21	23.53	37.01	24.99	66.80	
25th percentile	n. d.	8.69	3.39	n. d.	5.66	0.90	14.48	25.36	13.23	40.68	
Median	0.33	14.24	8.64	0.06	8.98	1.28	23.28	36.31	23.81	62.22	
75th percentile	0.83	22.92	22.30	0.26	13.25	1.72	39.37	52.16	45.21	107.83	
Detection rate (%)	61.5	99.6	100	69.8	98.4	100	100	100	100	100	

Note:
[a] $\Sigma PFCAs_{C2-C3}$: the mass sum of the concentrations of TFA and PFPrA; $\Sigma PFCAs_{C4-C7}$: the mass sum of the concentrations of PFBA, PFHxA, and PFHpA; $\Sigma PFCAs_{C8-C12}$: the mass sum of the concentrations of PFOA, PFNA, PFDA, PFUnDA, and PFDoDA; ΣPFCAs: the mass sum of the concentrations of $\Sigma PFCAs_{C2-C3}$, $\Sigma PFCAs_{C4-C7}$, $\Sigma PFCAs_{C8-C12}$, and 6:2 FTUCA,; ΣPFSAs: the mass sum of the concentrations of PFHxS, PFOS, 6:2 Cl-PFAES, and 8:2CL-PFAES; ΣPFSAs: the mass sum of the concentrations of 15 PFAS concentrations. n. d. = Not detectable.

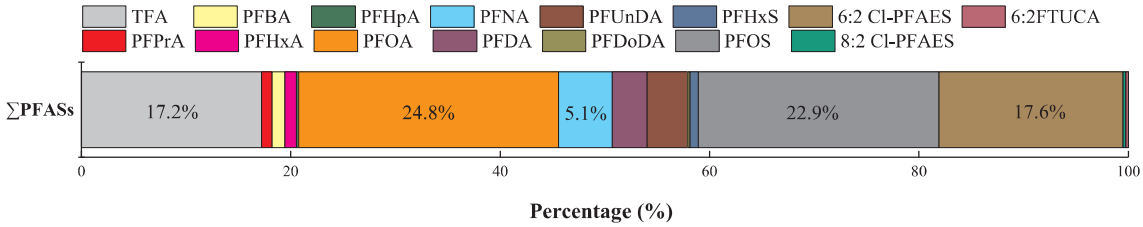

Fig. 1. The composition profiles of PFASs in serum among the total study population of Tianjin, China.

Among them, TFA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFOS, and 6:2 Cl-PFAES were detected in more than 90% of samples, followed by PFDoDA (88.9%), PFBA (85.7%), PFPrA (77.0%), 6:2 FTUCA (70.2%), 8:2 Cl-PFAES (69.8%), PFHpA (66.7%), and PFHxS (61.5%). PFOA was the primary compound with the median concentration at 14.83 ng/mL, followed by PFOS at 14.24 ng/mL, 6:2 Cl-PFAES at 8.64 ng/mL, TFA at 8.46 ng/mL, PFNA at 3.17 ng/mL, PFUnDA at 2.28 ng/mL, PFDA at 2.08 ng/mL, PFBA at 0.60 ng/mL, PFHxA at 0.57 ng/mL, and PFPrA at 0.48 ng/mL, whereas the concentrations of PFHpA, PFDoDA, 6:2 FTUCA, PFHxS, and 8:2 Cl-PFAES were two or three orders of magnitude lower than PFOA. The composition profiles of PFASs are shown in Fig. 1. PFOA, PFOS, 6:2 Cl-PFAES, and TFA accounted for 24.8%, 22.9%, 17.6%, and 17.2%, respectively, whereas other compounds constituted lower than 10%. Besides, the concentrations of long-chain PFCAs (i.e., $\Sigma PFCAs_{C8-C12}$) accounted for nearly 70% of ΣPFCAs, and the proportions of ultrashort-chain PFCAs (i.e., $\Sigma PFCAs_{C2-C3}$) and short-chain PFCAs (i.e., $\Sigma PFCAs_{C4-C7}$) relative to ΣPFCAs were 28.4% and 4.1%, respectively.

Spearman correlation analyses showed that PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES were significantly correlated with each other, with correlation coefficients (ρ) ranging from 0.452 to 0.878 (Table S4). Similarity, in the PCA, the first principal component (PC1) explained 39.3% of the total variance and PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES showed the high loading on PC1 (Fig. S1). For 6:2 FTUCA, there were significant positive correlations with PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS, and 6:2 Cl-PFAES (ρ = 0.126 to 0.616), but a negative correlation with PFHpA (ρ = -0.181) (Table S4). The second principal component in PCA explained 14.5% of the variability in PFASs and grouped 6:2 FTUCA, PFHxA, and PFHxS (Fig. S1). In addition, Spearman correlation analyses showed that TFA was significantly correlated with PFPrA (ρ = 0.455) and PFBA (ρ = 0.288) (Table S4). In similar, the third principal component (PC3) in PCA explained 10.0% of the variability in PFASs and TFA, PFPrA, and PFBA were found with high loading on PC3 (Fig. S1).

3.3. PFAS exposure with age, sex, and BMI

Table S5 shows the concentrations of PFASs categorized by age, sex, and BMI. Compared to people with age lower than 40 years, older people had higher concentrations of TFA, PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFHxS, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES. Similarly, Pearson correlations analyses showed that the concentrations of TFA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS, and 6:2 Cl-PFAES were significantly positively correlated with age ($r = 0.227$, $p < 0.001$ for TFA; $r = 0.220$, $p < 0.001$ for PFOA; $r = 0.296$, $p < 0.001$ for PFNA; $r = 0.157$, $p = 0.013$ for PFDA; $r = 0.240$, $p < 0.001$ for PFUnDA; $r = 0.318$, $p < 0.001$ for PFHxS; $r = 0.306$, $p < 0.001$ for PFOS; $r = 0.239$, $p < 0.001$ for 6:2 Cl-PFAES) (Fig. 2).

In general, no significant differences were observed between males and females for the concentrations of PFASs, except for that PFUnDA showed significant greater concentrations and PFBA showed significant lower concentrations in females than males (Table S5).

For BMI, compared to people with normal weight, there were lower concentrations of PFOA, PFNA, PFDA, PFDoDA, 6:2 Cl-PFAES, and 8:2 Cl-PFAES in the sera of underweight people. For overweight and obese people, the concentrations of PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES were greater than those in people with normal weight (Table S5). In similar, Pearson correlations analyses showed that the concentrations of PFOA, PFNA, PFDA, PFUnDA, PFDoDA, 6:2 FTUCA, PFHxS, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES were significantly positively correlated with BMI ($r = 0.275$, $p < 0.001$ for PFOA; $r = 0.313$, $p < 0.001$ for PFNA; $r = 0.327$, $p < 0.001$ for PFDA; $r = 0.202$, $p = 0.001$ for PFUnDA; $r = 0.177$, $p = 0.008$ for PFDoDA; $r = 0.160$, $p = 0.034$ for 6:2 FTUCA; $r = 0.200$, $p = 0.014$ for PFHxS; $r = 0.325$, $p < 0.001$ for PFOS; $r = 0.352$, $p < 0.001$ for 6:2 Cl-PFAES; $r = 0.163$, $p = 0.031$ for 8:2 Cl-PFAES) (Fig. 3).

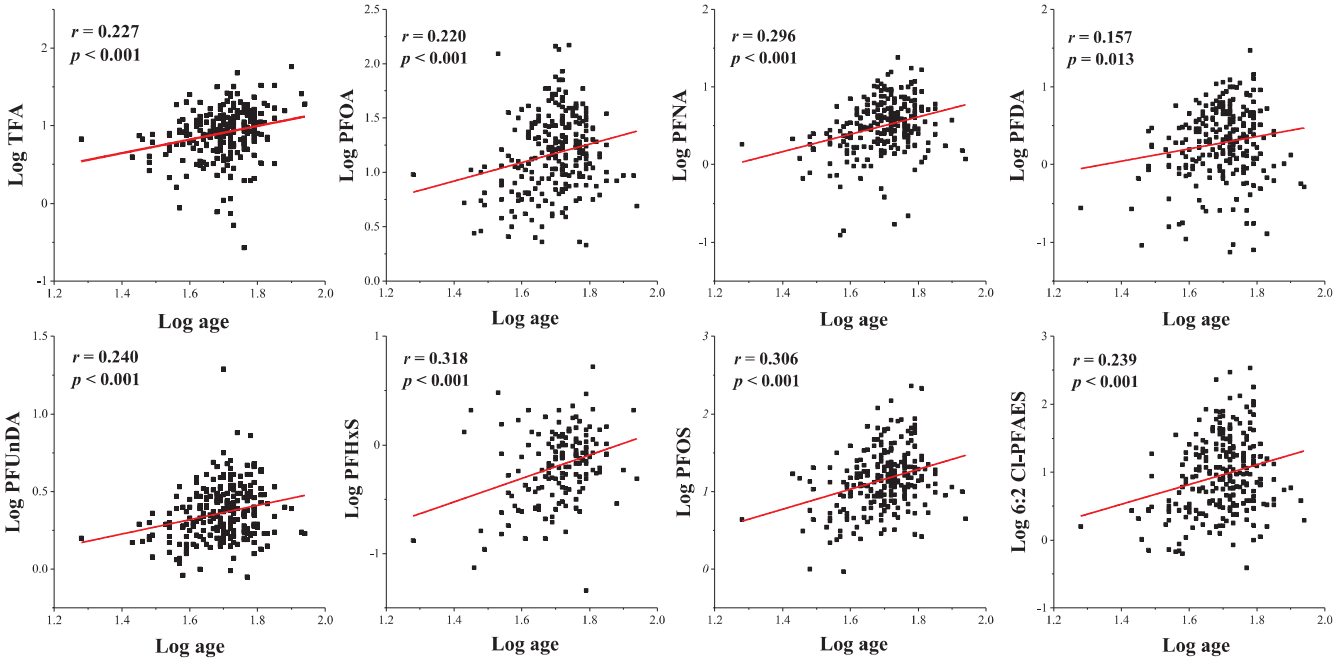

Fig. 2. Pearson correlations between serum several PFAS concentrations and age. Only results with significant correlations for PFASs with age are shown. Log transformation was performed for the concentrations of PFASs and age.

3.4. PFAS exposure with fasting glucose and HbA1c

Crude and adjusted associations between concentrations of PFASs and fasting glucose are presented in Table 3. Significant positive associations were observed for several PFASs with fasting glucose levels. Specifically, 1% increase in the concentrations of TFA, PFBA, PFHxA, PFOA, PFNA, PFHxS, ΣPFCAs$_{C2\text{-}C3}$, ΣPFCAs$_{C4\text{-}C7}$, ΣPFCAs$_{C8\text{-}C12}$, ΣPFCAs, and ΣPFASs was associated with 0.024% [95% confidence interval (CI): 0.012%, 0.035%], 0.013% (95% CI: 0.005%, 0.021%), 0.031% (95% CI: 0.008%, 0.054%), 0.017% (95% CI: 0.002%, 0.033%), 0.022% (95% CI: 0.007%, 0.038%), 0.011% (95% CI: 0.007%, 0.014%), 0.025% (95% CI: 0.012%, 0.039%), 0.056% (95% CI: 0.031%, 0.081%), 0.018% (95% CI: 0, 0.035%), 0.036% (95% CI: 0.014%, 0.058%), and 0.018% (95% CI: 0, 0.036%) increment in fasting glucose levels, respectively. After adjustment for covariates, only 1% increase in the concentrations of PFOA, PFNA, ΣPFCAs$_{C4\text{-}C7}$, ΣPFCAs$_{C8\text{-}C12}$, ΣPFCAs, and ΣPFASs was associated with elevated levels of fasting glucose [0.018% (95% CI: 0.004%, 0.033%) for PFOA, 0.022% (95% CI: 0.007%, 0.037%) for PFNA, 0.027% (95% CI: 0.002%, 0.053%) for ΣPFCAs$_{C4\text{-}C7}$, 0.021% (95% CI: 0.004%, 0.038%) for ΣPFCAs$_{C8\text{-}C12}$, 0.029% (95% CI: 0.008%, 0.050%) for ΣPFCAs, and 0.020% (95% CI: 0.002%, 0.038%) for ΣPFASs].

Associations between PFAS concentrations and HbA1c levels are shown in Table 4. In the crude models, although the concentrations of PFPrA and 8:2 Cl-PFAES were inversely associated with HbA1c levels, 1% increase in the concentrations of PFBA, PFHxA, PFHxS, and ΣPFCAs$_{C4\text{-}C7}$ was associated with 0.010% (95% CI: 0.002%, 0.018%), 0.047% (95% CI: 0.025%, 0.069%), 0.013% (95% CI: 0.009%, 0.017%), and 0.052% (95% CI: 0.027%, 0.076%) increment in HbA1c levels, respectively. After adjusted for covariates, only 1% increase in

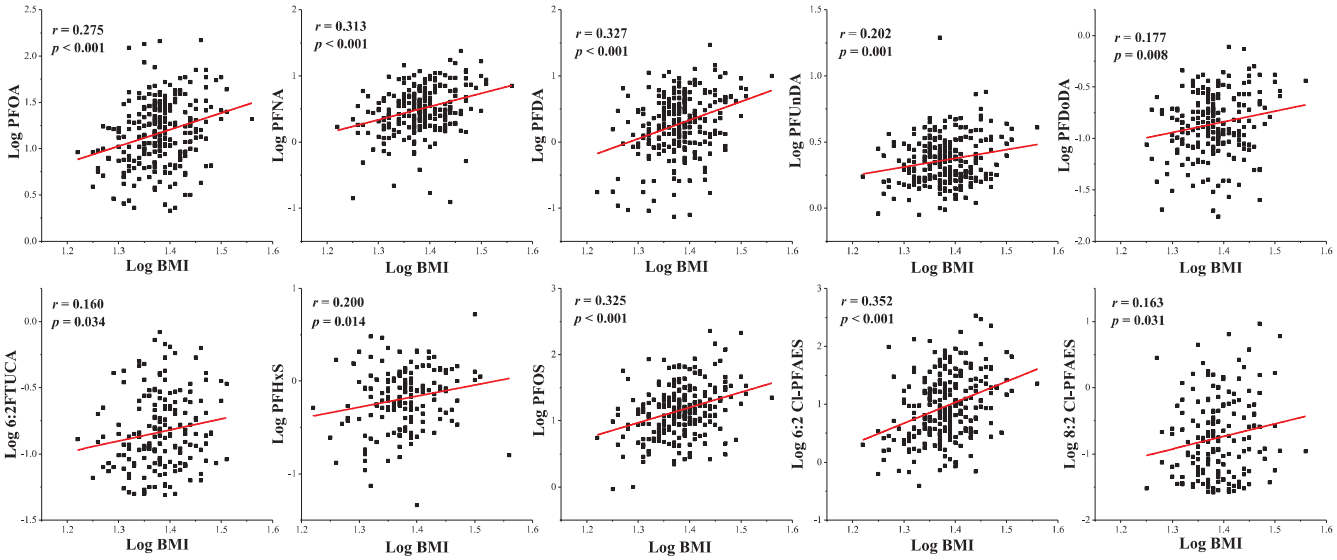

Fig. 3. Pearson correlations between serum several PFAS concentrations and BMI. Only results with significant correlations for PFASs with BMI are shown. Log transformation was performed for the concentrations of PFASs and BMI.

Table 3
Percentage increments (95% CI) in fasting glucose levels with 1% increase in serum PFAS concentrations in linear regression models.

Fasting glucose	Unadjusted		Multiple adjusted[a]	
	% increment (95% CI)	*p*	% increment (95% CI)	*p*
TFA	**0.024 (0.012, 0.035)**	**< 0.001**	0.010 (−0.002, 0.021)	0.092
PFPrA	−0.009 (−0.026, 0.007)	0.253	−0.007 (−0.021, 0.008)	0.380
PFBA	**0.013 (0.005, 0.021)**	**0.001**	0.005 (−0.003, 0.013)	0.232
PFHxA	**0.031 (0.008, 0.054)**	**0.009**	0.013 (−0.008, 0.035)	0.226
PFHpA	−0.003 (−0.012, 0.006)	0.494	−0.001 (−0.010, 0.007)	0.752
PFOA	**0.017 (0.002, 0.033)**	**0.028**	**0.018 (0.004, 0.033)**	**0.014**
PFNA	**0.022 (0.007, 0.038)**	**0.005**	**0.022 (0.007, 0.037)**	**0.005**
PFDA	0.002 (−0.009, 0.013)	0.751	0.009 (−0.002, 0.020)	0.116
PFUnDA	0.031 (0.000, 0.062)	0.053	0.025 (−0.004, 0.054)	0.096
PFDoDA	0.002 (−0.010, 0.014)	0.763	0.006 (−0.006, 0.017)	0.319
6:2 FTUCA	0.001 (−0.014, 0.015)	0.905	0.001 (−0.013, 0.014)	0.913
PFHxS	**0.011 (0.007, 0.014)**	**< 0.001**	0.004 (−0.001, 0.009)	0.087
PFOS	0.012 (−0.001, 0.026)	0.077	0.013 (−0.001, 0.026)	0.062
6:2 Cl-PFAES	0.002 (−0.008, 0.012)	0.665	0.008 (−0.003, 0.018)	0.144
8:2 Cl-PFAES	−0.007 (−0.015, 0.001)	0.103	−0.001 (−0.010, 0.007)	0.721
$\Sigma PFCAs_{C2\text{-}C3}$	**0.025 (0.012, 0.039)**	**< 0.001**	0.010 (−0.004, 0.023)	0.154
$\Sigma PFCAs_{C4\text{-}C7}$	**0.056 (0.031, 0.081)**	**< 0.001**	**0.027 (0.002, 0.053)**	**0.033**
$\Sigma PFCAs_{C8\text{-}C12}$	**0.018 (0.000, 0.035)**	**0.047**	**0.021 (0.004, 0.038)**	**0.014**
ΣPFCAs	**0.036 (0.014, 0.058)**	**0.001**	**0.029 (0.008, 0.050)**	**0.007**
ΣPFSAs	0.007 (−0.006, 0.019)	0.291	0.010 (−0.002, 0.022)	0.112
ΣPFASs	**0.018 (0.000, 0.036)**	**0.049**	**0.020 (0.002, 0.038)**	**0.029**

[a] Adjusted for sex, age, body mass index, smoking and alcohol-drinking status, exercising status, education level, and family history of diabetes.

the concentrations of PFHxA, PFNA, PFHxS, $\Sigma PFCAs_{C4\text{-}C7}$ was associated with elevated levels of HbA1c [0.030% (95% CI: 0.010%, 0.051%) for PFHxA, 0.018% (95% CI: 0.003%, 0.033%) for PFNA, 0.007% (95% CI: 0.003%, 0.011%) for PFHxS, and 0.026% (95% CI: 0.002%, 0.050%) for $\Sigma PFCAs_{C4\text{-}C7}$]. In addition, there was a significant negative association between $\Sigma PFCAs_{C2\text{-}C3}$ and HbA1c [-0.014% (95%CI: −0.027%, −0.001%)]. In the stratified analyses by age, the concentrations of PFHxA and $\Sigma PFCAs_{C4\text{-}C7}$ were only significantly positively related to HbA1c levels in the age ≥55 years subgroup (Fig. S2). Different from the age < 55 years subgroup, PFNA, PFDA, PFOS, $\Sigma PFCAs_{C8\text{-}C12}$, ΣPFSAs, and ΣPFASs showed significant positive associations with HbA1c only in the age ≥55 years subgroup (Fig. S2).

4. Discussion

Our study for the first time reported the levels and the contributions of novel PFASs, i.e., ultrashort-chain PFCAs, two Cl-PFAESs, and 6:2 FTUCA to the total known PFASs in the serum of Chinese adult men and women with a moderate sample size. 6:2 Cl-PFAES and TFA showed great contributions to PFASs in serum, second only to the legacy PFOA and PFOS. Most PFASs (including TFA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, 6:2 FTUCA, PFHxS, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES) in serum showed increasing levels with age or BMI. In addition, significant positive associations were observed for several serum PFASs (including PFHxA, PFOA, PFNA, PFHxS) with two glycemic biomarkers (i.e., fasting glucose and HbA1c) among the people with normal blood glucose levels.

Table 4
Percentage increments (95% CI) in HbA1c levels with 1% increase in serum PFAS concentrations in linear regression models.

HbA1c	Unadjusted		Multiple adjusted[a]	
	% increment (95% CI)	*p*	% increment (95% CI)	*p*
TFA	0.005 (−0.007, 0.017)	0.402	−0.009 (−0.021, 0.002)	0.098
PFPrA	**−0.018 (−0.033, −0.002)**	**0.026**	−0.012 (−0.026, 0.002)	0.099
PFBA	**0.010 (0.002, 0.018)**	**0.013**	0.001 (−0.007, 0.009)	0.817
PFHxA	**0.047 (0.025, 0.069)**	**< 0.001**	**0.030 (0.010, 0.051)**	**0.004**
PFHpA	−0.001 (−0.009, 0.008)	0.868	0.002 (−0.006, 0.010)	0.557
PFOA	0.009 (−0.006, 0.024)	0.248	0.013 (−0.001, 0.027)	0.074
PFNA	0.015 (−0.001, 0.030)	0.062	**0.018 (0.003, 0.033)**	**0.022**
PFDA	−0.006 (−0.016, 0.005)	0.279	−0.003 (−0.014, 0.007)	0.542
PFUnDA	0.015 (−0.016, 0.045)	0.346	0.013 (−0.016, 0.041)	0.388
PFDoDA	−0.007 (−0.019, 0.005)	0.249	−0.002 (−0.013, 0.010)	0.777
6:2 FTUCA	0.001 (−0.013, 0.015)	0.905	0.003 (−0.010, 0.016)	0.681
PFHxS	**0.013 (0.009, 0.017)**	**< 0.001**	**0.007 (0.003, 0.011)**	**0.002**
PFOS	0.008 (−0.005, 0.022)	0.209	0.012 (−0.001, 0.025)	0.076
6:2 Cl-PFAES	−0.001 (−0.011, 0.009)	0.838	0.003 (−0.008, 0.013)	0.624
8:2 Cl-PFAES	**−0.008 (−0.016, 0.000)**	**0.047**	−0.003 (−0.011, 0.005)	0.459
$\Sigma PFCAs_{C2\text{-}C3}$	0.004 (−0.010, 0.017)	0.599	**−0.014 (−0.027, −0.001)**	**0.037**
$\Sigma PFCAs_{C4\text{-}C7}$	**0.052 (0.027, 0.076)**	**< 0.001**	**0.026 (0.002, 0.050)**	**0.031**
$\Sigma PFCAs_{C8\text{-}C12}$	0.008 (−0.009, 0.025)	0.342	0.015 (−0.001, 0.032)	0.071
ΣPFCAs	0.011 (−0.011, 0.032)	0.333	0.008 (−0.013, 0.028)	0.462
ΣPFSAs	0.004 (−0.008, 0.016)	0.548	0.010 (−0.002, 0.022)	0.114
ΣPFASs	0.005 (−0.012, 0.023)	0.563	0.010 (−0.007, 0.028)	0.244

[a] Adjusted for sex, age, body mass index, smoking and alcohol-drinking status, exercising status, education level, and family history of diabetes.

Most previous studies, which were conducted before or around the year of 2009 when PFOS was phased out, have reported that PFOS was the dominant PFAS, whereas the levels of PFOA were 2–60 times lower than those of PFOS in the general population in China (Guo et al., 2011; Yeung et al., 2006; Zhang et al., 2011; Zhang et al., 2010; Zhang et al., 2013b). However, in recent years, studies have reported that the levels of PFOA have increased and were comparable with those of PFOS in human serum (Wang et al., 2018), which is consistent with the results of the present study. Compared to another study collecting human blood samples at 2009 in Tianjin (Zhang et al., 2011), the present investigation showed slightly increased levels of PFOS (14.24 ng/mL vs. 12.2 ng/mL) and sharply increased levels of PFOA (14.83 ng/mL vs. 0.22 ng/mL) in general population. This could be due to that in recent 10 years, the living standard of Chinese people has raised, which leads to much more using of commercial products including Telflon pans and sport coats that contain PFASs. The sharp increase in PFOA is a result that PFOS and its salts were added to the Stockholm Convention in 2009 and as an alternative, the production of PFOA and its precursors has increased in recent years. In the present study, the concentrations of most legacy PFASs, including PFOA, PFOS, PFNA, PFUnDA, PFDA, PFHpA, and PFDoDA, were higher than those in human serum samples collected in Nanjing between 2017 and 2018 (Wang et al., 2018), suggesting a higher exposure to PFASs for people in Tianjin. The differences of PFAS levels in serum from the two cities may be due to the different diet habits, and people in Tianjin, which is on the seashore of Bohai Sea, regularly eat fish and seafoods that have been regarded as the major pathway of human exposure to PFOS (Zhang et al., 2011).

Moreover, high concentrations of 6:2 Cl-PFAES and TFA were detected in human serum, with median concentrations of 8.64 ng/mL and 8.46 ng/mL, second only to PFOA and PFOS. The composition profiles showed that 6:2 Cl-PFAES and TFA separately accounted for 17.6% and 17.2%, suggesting a relatively larger contribution to the total PFASs. The levels of 6:2 Cl-PFAES in the present study were 2–4 times greater than those reported in pregnant women from China (Chen et al., 2017; Pan et al., 2017), indicating that there may be a higher exposure risk for the general people in Tianjin. 6:2 Cl-PFAES, with the commercial name F-53B, has been used as a commercial mist suppressant for over 40 years in China (Wang et al., 2013). The long time usage led to the accumulation of 6:2 Cl-PFAES in Chinese people. To our knowledge, this is the first study to report the levels of TFA in human, which suggested widespread exposure of TFA to people. TFA has a wide range of sources. On one hand, TFA itself is used in the chemical industry and as a laboratory reagent; on the other hand, aside from as by-product in the chemical synthesis process, there are some increased indirect sources for TFA in recent years, including atmospheric oxidation of novel refrigerants, the photodegradation or thermolysis of fluoropolymers, and degradation of pharmaceuticals and plant protecting agents with trifluoromethyl moieties (Boutonnet et al., 1999; Scheurer et al., 2017). These indirect sources could increase the release of TFA to the environment and further result in widespread human exposure.

8:2 Cl-PFAES, usually regarded as the impurity of F-53B, was also detected in 69.8% of the serum samples, although the median concentration was more than 100 times lower than that of 6:2 Cl-PFAES. Besides TFA, another ultrashort-chain PFCA, PFPrA, was widely detected in the human serum, and the concentration of PFPrA were approximately 20 times lower than TFA. Previous studies have reported that the photodegradation of neutral PFAS precursors such as FTOHs and perfluorooctane sulfonamides/ethanols, could produce PFPrA in the atmosphere (D'Eon et al., 2006; Ellis et al., 2004; Martin et al., 2006). In particular, the levels of 6:2 FTUCA were for the first time reported among general population in the present study, with a median concentration of 0.09 ng/mL and a detection rate of 70.2%. Pharmacokinetic modeling and metabolism studies on rodents and hepatocytes have demonstrated that 6:2 FTOH was first metabolized to form some transient intermediates, including 6:2 fluorotelomer aldehyde, 6:2 fluorotelomer saturated carboxylic acid, and 6:2 FTUCA, in the Phase I metabolism process, and then were combined with glucuronide, sulfate, and glutathione by Phase II conjugation reactions (Kabadi et al., 2018). In addition, a small fraction of transient intermediates may be metabolized to terminal products such as PFBA, PFPeA, PFHxA, and PFHpA, via another metabolic pathway (Kabadi et al., 2018). In the present study, a significant positive correlation between 6:2 FTUCA and PFHxA and a significant negative correlation between 6:2 FTUCA and PFHpA were observed, which agreed with the metabolic pathways of 6:2 FTOH, suggesting that 6:2 FTUCA, in part at least, may have come from the metabolism of 6:2 FTOH in human body. Some studies have reported the occurrence of 6:2 FTUCA in cold cuts and microwave popcorn bags, indicating that there may also be direct human exposure sources for 6:2 FTUCA (Ostertag et al., 2009; Zabaleta et al., 2016). Toxicological investigations have found that fluorotelomer acids were more toxic than PFCAs to aquatic organisms (Mitchell et al., 2011; Phillips et al., 2007). Therefore, more studies are needed to verify the occurrence of 6:2 FTUCA in human serum and explore the toxic effects on human health.

According to the percentage composition profiles, long-chain PFCAs were in the majority of PFCAs, which accounted for approximately 70% of the total PFCAs, whereas the proportions of ultrashort-chain PFCAs and short-chain PFCAs relative to the total PFCAs were only 28.4% and 4.1%. Compared to the ultrashort-chain PFCAs and short-chain PFCAs, the greater proportion of long-chain PFCAs in serum was related to their stronger lipophilic property (Sanchez Garcia et al., 2018). In the PCA, PFOS, 6:2 Cl-PFAES, and 8:2 Cl-PFAES and long-chain PFCAs showed similar loading on PC1 (Fig. S1), which was consistent with the significant positive correlations among them in Spearman correlation analyses (Table S4), indicating a common source for them (e.g., food or indoor dust due to degradation of fluoropolymers). Different form the long-chain PFCAs, the ultrashort-chain PFCAs (i.e., TFA and PFPrA) and PFBA showed higher loading in PC3 (Fig. S1), indicating additional sources such as atmospheric oxidation of novel refrigerants mentioned above.

Both the nonparametric Mann-Whitney U test and Pearson correlations analyses presented increased concentrations of PFCAs (mainly long-chain PFCAs), PFSAs, and Cl-PFAESs with age and BMI (Table S5, Figs. 2 and 3), which was consistent with previous studies (Fu et al., 2014; Li et al., 2011; Seo et al., 2018; Zhang et al., 2010). These results suggested the bioaccumulation and long half-lives of PFASs in human body. Specially, although TFA had high water solubility and low K_{ow} ($\log K_{ow} = -2.1$), there was a significant positive correlation between TFA and age (Fig. 2). Previous studies have reported that TFA, with the similar structure to acetate, which is a major biosynthetic molecule, could be incorporated into biomolecule fractions such as protein, lipid, and so on, which results in the bioaccumulation of TFA in microorganisms, invertebrates, and plants (Boutonnet et al., 1999). However, the bioaccumulation mechanisms of TFA in human are worthy to further explore. In addition, the differences in the concentrations of PFBA and PFUnDA between females and males may be due to different patterns in exposure sources or routes of PFASs.

Several epidemiological studies have investigated the associations of several PFASs with fasting glucose and HbA1c levels, mostly focusing on PFOA, PFNA, PFUnDA, PFHxS, and PFOS, but with inconsistent results (Cardenas et al., 2017; Karnes et al., 2014; Lin et al., 2009; Lin et al., 2011; Liu et al., 2018; Su et al., 2016). For example, in a cross-sectional study on the general American people from National Health and Nutrition Examination Survey (NHANES) 1999–2000 and 2003–2004, a positive association was observed for the concentrations of PFNA with hyperglycemia in adolescents, but not for PFOA, PFHxS, and PFOS (Lin et al., 2009). However, an analysis on the NHANES 2013–2014 cycle found that PFOA was inversely associated with fasting glucose and HbA1c (Liu et al., 2018). In a combined cohort study on the community residents living in places with drinking water contaminated by high levels of PFOA in the C8 Health Project and DuPon employees from a previous retrospective research, no association was observed for

the estimated PFOA concentrations with fasting glucose levels (Karnes et al., 2014). Similarly, a study on the young people in Taiwan showed no cross-sectional associations between the concentrations of PFOA, PFNA, PFOS, and PFUnDA with glucose homeostasis (Lin et al., 2011). Inversely, in another study on working-aged Taiwanese adults, which excluded the people with clinically diagnosed diabetes, the concentrations of PFOS were associated with impaired glucose homeostasis and increased HbA1c levels, whereas PFOA, PFUnDA, and PFNA showed opposite trends for the associations with fasting glucose and HbA1c (Su et al., 2016). Interestingly, a study on the U.S. people with prediabetes from the Diabetes Prevention Program found that at baseline, plasma PFOS, PFOA, and PFNA concentrations were cross-sectionally related to increased fasting glucose and the concentrations of PFOS and PFOA were positively associated with HbA1c. However, during the follow-up period when people accepted intensive lifestyle intervention and placebo treatment, no associations were observed for plasma PFAS concentrations with prospective change in glycemic biomarkers (Cardenas et al., 2017), which may result from that the changes of lifestyle could confound the associations of PFAS exposure with glycemic indicators. Therefore, in the present study, we systematically evaluated the associations of various PFASs, including PFCAs, PFSAs, and Cl-PFAESs, with two glycemic biomarkers (i.e., fasting glucose and HbA1c) in people with normal blood glucose levels, which avoided the potential interference from people with prediabetes or diabetes who may control their blood sugar by changing their lifestyle or taking antihyperglycemic medicines. The results of the multivariate linear regression analyses showed significant positive associations for the concentrations of PFOA, PFNA, $\Sigma PFCAs_{C4\text{-}C7}$, $\Sigma PFCAs_{C8\text{-}C12}$, ΣPFCAs, and ΣPFASs with fasting glucose levels, but not for PFSAs and Cl-PFAESs (Table 3), which suggested that compared to PFASs with sulfonic acid group, the PFASs with carboxylic acid group might be more tightly associated with fasting glucose. In addition, serum PFHxA, PFNA, PFHxS, and $\Sigma PFCAs_{C4\text{-}C7}$ concentrations were positively related to HbA1c, but $\Sigma PFCAs_{C2\text{-}C3}$ showed inverse association with HbA1c (Table 4). The reasons for the inverse association between $\Sigma PFCAs_{C2\text{-}C3}$ and HbA1c were unknown, which need to be confirmed and elucidated in the prospective epidemiological studies and mechanism research. At present, several studies have evaluated the toxicity effect of TFA on aquatic organisms, and this compound shows relatively high no observed effect concentrations (NOEC) for water fleas, zebra fish, duckweed, and several algae, mostly higher than 100 mg/L, except for *Selenastrum capricornutum* with a NOEC of 0.12 mg/L (Berends et al., 1999; Hanson and Solomon, 2004). In addition, mechanism studies have reported that exposure to PFNA in rats could result in increased serum glucose levels and hepatic glycogen through changing the expression levels of genes involved in hepatic glycogen metabolism and the protein levels related to insulin signal pathway in the liver (Fang et al., 2012), which was consistent with the present study that the concentrations of PFNA in serum were significantly positively associated with fasting glucose and HbA1c levels.

In the stratified analyses by age, significant positive associations of serum PFAS concentrations with HbA1c mainly were observed in the people with age ≥55 years (Fig. S2), which suggested that older subgroup may be more susceptible to the effect of PFAS exposure on HbA1c due to the increased levels of PFASs with age. Few studies have reported the age difference for the associations of PFAS exposure with glycemic biomarkers. The cross-sectional study basing on the data from NHANES 1999–2000 and 2003–2004 only showed a significant positive association for serum PFNA with hyperglycemia in adolescents, but not in adults (Lin et al., 2009). Further studies are needed to confirm the difference by age to explore the underlying mechanisms.

It should be noted that there were some limitations in this study. First, due to the nature of cross-sectional study, our study only could conclude that PFAS exposure were cross-sectionally related to glycemic biomarkers, but causality could not be inferred. Therefore, prospective studies are needed to be conducted to verify the influence of exposure to PFASs on the glucose homeostasis. Moreover, we included the people with fasting glucose between 3.89 and 6.11 mmol/L and HbA1c between 3.8% and 5.8% as the study population. Therefore, the scopes of fasting glucose and HbA1c were narrow, which resulted in weak correlation coefficients though they were statistically significant ($p < 0.05$). Further, although many demographic variables were included in the adjusted analyses, residual confounders may remain.

In conclusion, the occurrence of 6:2 Cl-PFAES and TFA with relatively high levels and percentage compositions in serum suggested a widespread human exposure to these novel PFASs among general population in China. The presence of 6:2 FTUCA in human serum was also for the first time reported in our study. Our results provided support for future studies which need to further explore human exposure pathways and toxic effects on human health for these compounds. Furthermore, significant positive associations between serum PFAS concentrations and glycemic biomarkers were observed among the people with normal blood glucose levels in the present study. Prospective epidemiological and toxicological studies are necessary to verify and evaluate the potential effects of PFASs on glucose homeostasis.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by the Tianjin “131” innovative group program and 111 program, Ministry of Education, China (T2017002). We are grateful to all the recruited people for participating in this study and staff in the hospitals for contributing to the research.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105295.

References

Bao, J., Liu, W., Liu, L., Jin, Y., Dai, J., Ran, X., Zhang, Z., Tsuda, S., 2011. Perfluorinated compounds in the environment and the blood of residents living near fluorochemical plants in Fuxin, China. Environ. Sci. Technol. 45 (19), 8075–8080.

Bangma, J.T., Ragland, J.M., Rainwater, T.R., Bowden, J.A., Gibbons, J.W., Reiner, J.L., 2019. Perfluoroalkyl substances in diamondback terrapins (*Malaclemys terrapin*) in coastal South Carolina. Chemosphere 215, 305–312.

Berends, A.G., Boutonnet, J.C., de Rooij, C.G., Thompson, R.S., 1999. Toxicity of trifluoroacetate to aquatic organisms. Environ. Toxicol. Chem. 18 (5), 1053–1059.

Boutonnet, J.C., Bingham, P., Calamari, D., Rooij, C.d., Franklin, J., Kawano, T., Libre, J.-M., McCul-loch, A., Malinverno, G., Odom, J.M., Rusch, G.M., Smythe, K., Sobolev, I., Thompson, R., Tiedje, J.M., 1999. Environmental risk assessment of trifluoroacetic acid. Hum. Ecol. Risk Assess. 5 (1), 59–124.

Cardenas, A., Gold, D.R., Hauser, R., Kleinman, K.P., Hivert, M.F., Calafat, A.M., Ye, X., Webster, T.F., Horton, E.S., Oken, E., 2017. Plasma concentrations of per- and polyfluoroalkyl substances at baseline and associations with glycemic indicators and diabetes incidence among high-risk adults in the Diabetes Prevention Program Trial. Environ. Health Perspect. 125 (10), 107001.

Cavan, D., da Rocha Fernandes, J., Makaroff, L., Ogurtsova, K., Webber, S., 2015. International Diabetes Federation (IDF) Diabetes Atlas, seventh ed. IDF, Brussels.

Chen, F., Yin, S., Kelly, B.C., Liu, W., 2017. Chlorinated polyfluoroalkyl ether sulfonic acids in matched maternal, cord, and placenta samples: a study of transplacental transfer. Environ. Sci. Technol. 51 (11), 6387–6394.

Chen, H., Yao, Y., Zhao, Z., Wang, Y., Wang, Q., Ren, C., Wang, B., Sun, H., Alder, A.C., Kannan, K., 2018. Multimedia distribution and transfer of per- and polyfluoroalkyl substances (PFASs) surrounding two fluorochemical manufacturing facilities in Fuxin, China. Environ. Sci. Technol. 52 (15), 8263–8271.

Chen, H., Zhang, L., Li, M., Yao, Y., Zhao, Z., Munoz, G., Sun, H., 2019. Per- and polyfluoroalkyl substances (PFASs) in precipitation from mainland China: contributions of unknown precursors and short-chain (C2–C3) perfluoroalkyl carboxylic acids. Water Res. 153, 169–177.

De Silva, A.O., Spencer, C., Scott, B.F., Backus, S., Muir, D.C., 2011. Detection of a cyclic perfluorinated acid, perfluoroethylcyclohexane sulfonate, in the Great Lakes of North America. Environ. Sci. Technol. 45 (19), 8060–8086.

D'Eon, J.C., Hurley, M.D., Wallington, T.J., Mabury, S.A., 2006. Atmospheric chemistry of N-methyl perfluorobutane sulfonamidoethanol, $C_4F_9SO_2N(CH_3)CH_2CH_2OH$: Kinetics

and mechanism of reaction with OH. Environ. Sci. Technol. 40 (6), 1862–1868.
Ellis, D.A., Martin, J.W., De Silva, A.O., Mabury, S.A., Hurley, M.D., Andersen, M.P.S., Wallington, T.J., 2004. Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids. Environ. Sci. Technol. 38 (12), 3316–3321.
Fang, X., Gao, G., Xue, H., Zhang, X., Wang, H., 2012. Exposure of perfluorononanoic acid suppresses the hepatic insulin signal pathway and increases serum glucose in rats. Toxicology 294 (2–3), 109–115.
Fang, X., Wang, Q., Zhao, Z., Tang, J., Tian, C., Yao, Y., Yu, J., Sun, H., 2018. Distribution and dry deposition of alternative and legacy perfluoroalkyl and polyfluoroalkyl substances in the air above the Bohai and Yellow Seas, China. Atmos. Environ. 192, 128–135.
Fu, Y., Wang, T., Wang, P., Fu, Q., Lu, Y., 2014. Effects of age, gender and region on serum concentrations of perfluorinated compounds in general population of Henan, China. Chemosphere 110, 104–110.
Guo, F., Zhong, Y., Wang, Y., Li, J., Zhang, J., Liu, J., Zhao, Y., Wu, Y., 2011. Perfluorinated compounds in human blood around Bohai Sea, China. Chemosphere 85 (2), 156–162.
Guo, X., Shen, L., Dou, J., Lv, Y., Zhang, A., Shi, F., Xue, Z., Yu, Y., Jin, L., Yao, Y., 2017. Associations of fasting blood glucose with influencing factors in Northeast China: a quantile regression analysis. Int. J. Environ. Res. Public Health 14, 1368.
Hanson, M.L., Solomon, K.R., 2004. Haloacetic acids in the aquatic environment. Part II: ecological risk assessment. Environ. Pollut. 130 (3), 385–401.
Holmquist, H., Schellenberger, S., van der Veen, I., Peters, G.M., Leonards, P.E., Cousins, I.T., 2016. Properties, performance and associated hazards of state-of-the-art durable water repellent (DWR) chemistry for textile finishing. Environ. Int. 91, 251–264.
Hurley, S., Goldberg, D., Wang, M., Park, J.S., Petreas, M., Bernstein, L., Anton-Culver, H., Nelson, D.O., Reynolds, P., 2018. Time trends in per- and polyfluoroalkyl substances (PFASs) in California women: Declining serum levels, 2011–2015. Environ. Sci. Technol. 52 (1), 277–287.
Kabadi, S.V., Fisher, J., Aungst, J., Rice, P., 2018. Internal exposure-based pharmacokinetic evaluation of potential for biopersistence of 6:2 fluorotelomer alcohol (FTOH) and its metabolites. Food Chem. Toxicol. 112, 375–382.
Karnes, C., Winquist, A., Steenland, K., 2014. Incidence of type II diabetes in a cohort with substantial exposure to perfluorooctanoic acid. Environ. Res. 128, 78–83.
Koshy, T.T., Attina, T.M., Ghassabian, A., Gilbert, J., Burdine, L.K., Marmor, M., Honda, M., Chu, D.B., Han, X., Shao, Y., Kannan, K., Urbina, E.M., Trasande, L., 2017. Serum perfluoroalkyl substances and cardiometabolic consequences in adolescents exposed to the World Trade Center disaster and a matched comparison group. Environ. Int. 109, 128–135.
Li, X., Zhang, J., Liu, W., Li, X., Zhang, X., Jiang, Y., Zhou, J., Jin, Y., 2011. Serum levels of perfluorinated compounds in the general population in Shenzhen, China. Chinese Sci. Bull. 56 (28–29), 3092–3099.
Li, Y., Cheng, Y., Xie, Z., Zeng, F., 2017. Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. Sci. Rep. 7, 43380.
Lim, T.C., Wang, B., Huang, J., Deng, S., Yu, G., 2011. Emission inventory for PFOS in China: Review of past methodologies and suggestions. Scientific World J. 11, 1963–1980.
Lin, C.-Y., Chen, P.-C., Lin, Y.-C., Lin, L.-Y., 2009. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32 (4), 702–707.
Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Chen, C.Y., Hsu, S.H., Chien, K.L., Sung, F.C., Chen, P.C., Su, T.C., 2011. Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. Environ. Sci. Technol. 45 (24), 10691–10698.
Lind, L., Zethelius, B., Salihovic, S., van Bavel, B., Lind, P.M., 2014. Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 57 (3), 473–479.
Liu, H.S., Wen, L.L., Chu, P.L., Lin, C.Y., 2018. Association among total serum isomers of perfluorinated chemicals, glucose homeostasis, lipid profiles, serum protein and metabolic syndrome in adults: NHANES, 2013–2014. Environ. Pollut. 232, 73–79.
Lv, Z., Li, G., Li, Y., Ying, C., Chen, J., Chen, T., Wei, J., Lin, Y., Jiang, Y., Wang, Y., Shu, B., Xu, B., Xu, S., 2013. Glucose and lipid homeostasis in adult rat is impaired by early-life exposure to perfluorooctane sulfonate. Environ. Toxicol. 28 (9), 532–542.
Martin, J.W., Ellis, D.A., Mabury, S.A., Hurley, M.D., Wallington, T.J., 2006. Atmospheric chemistry of perfluoroalkanesulfonamides: Kinetic and product studies of the OH radical and Cl atom initiated oxidation of N-ethyl perfluorobutanesulfonamide. Environ. Sci. Technol. 40 (3), 864–872.
Mitchell, R.J., Myers, A.L., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2011. Toxicity of fluorotelomer carboxylic acids to the algae *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, and the amphipod *Hyalella azteca*. Ecotoxicol. Environ. Saf. 74 (8), 2260–2267.
Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general US population. Environ. Health Perspect. 118 (2), 197–202.
Ostertag, S.K., Chan, H.M., Moisey, J., Dabeka, R., Tittlemier, S.A., 2009. Historic dietary exposure to perfluorooctane sulfonate, perfluorinated carboxylates, and fluorotelomer unsaturated carboxylates from the consumption of store-bought and restaurant foods for the Canadian population. J. Agric. Food Chem. 57 (18), 8534–8544.
Pan, Y., Zhu, Y., Zheng, T., Cui, Q., Buka, S.L., Zhang, B., Guo, Y., Xia, W., Yeung, L.W., Li, Y., Zhou, A., Qiu, L., Liu, H., Jiang, M., Wu, C., Xu, S., Dai, J., 2017. Novel chlorinated polyfluorinated ether sulfonates and legacy per-/polyfluoroalkyl substances: Placental transfer and relationship with serum albumin and glomerular filtration rate. Environ. Sci. Technol. 51 (1), 634–644.
Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L.W.Y., Sun, Y., Guo, Y., Dai, J., 2018. Worldwide distribution of novel perfluoroether carboxylic and sulfonic acids in surface water. Environ. Sci. Technol. 52 (14), 7621–7629.
Phillips, M.M.M., Dinglasan-Panlilio, M.J.A., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2007. Fluorotelomer acids are more toxic than perfluorinated acids. Environ. Sci. Technol. 41 (20), 7159–7163.
Sanchez Garcia, D., Sjodin, M., Hellstrandh, M., Norinder, U., Nikiforova, V., Lindberg, J., Wincent, E., Bergman, A., Cotgreave, I., Munic Kos, V., 2018. Cellular accumulation and lipid binding of perfluorinated alkylated substances (PFASs) - A comparison with lysosomotropic drugs. Chem. Biol. Interact. 281, 1–10.
Scheurer, M., Nödler, K., Freeling, F., Janda, J., Happel, O., Riegel, M., Müller, U., Storck, F.R., Fleig, M., Lange, F.T., Brunsch, A., Brauch, H.-J., 2017. Small, mobile, persistent: Trifluoroacetate in the water cycle – Overlooked sources, pathways, and consequences for drinking water supply. Water Res. 126, 460–471.
Seo, S.H., Son, M.H., Choi, S.D., Lee, D.H., Chang, Y.S., 2018. Influence of exposure to perfluoroalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects. Environ. Int. 113, 149–161.
Shi, Y., Vestergren, R., Xu, L., Zhou, Z., Li, C., Liang, Y., Cai, Y., 2016. Human exposure and elimination kinetics of chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs). Environ. Sci. Technol. 50 (5), 2396–2404.
Solomon, K.R., Velders, G.J., Wilson, S.R., Madronich, S., Longstreth, J., Aucamp, P.J., Bornman, J.F., 2016. Sources, fates, toxicity, and risks of trifluoroacetic acid and its salts: Relevance to substances regulated under the Montreal and Kyoto Protocols. J. Toxicol. Environ. Health 19 (7), 289–304.
Su, T.C., Kuo, C.C., Hwang, J.J., Lien, G.W., Chen, M.F., Chen, P.C., 2016. Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environ. Int. 88, 15–22.
Taniyasu, S., Kannan, K., So, M.K., Gulkowska, A., Sinclair, E., Okazawa, T., Yamashita, N., 2005. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. J. Chromatogr. A 1093 (1–2), 89–97.
Tian, Y., Yao, Y., Chang, S., Zhao, Z., Zhao, Y., Yuan, X., Wu, F., Sun, H., 2018. Occurrence and phase distribution of neutral and ionizable per- and polyfluoroalkyl substances (PFASs) in the atmosphere and plant leaves around landfills: A case study in Tianjin, China. Environ. Sci. Technol. 52 (3), 1301–1310.
Timmermann, C.A., Rossing, L.I., Grontved, A., Ried-Larsen, M., Dalgard, C., Andersen, L.B., Grandjean, P., Nielsen, F., Svendsen, K.D., Scheike, T., Jensen, T.K., 2014. Adiposity and glycemic control in children exposed to perfluorinated compounds. J. Clin. Endocrinol. Metab. 99 (4), E608–E614.
Wan, H.T., Zhao, Y.G., Leung, P.Y., Wong, C.K., 2014. Perinatal exposure to perfluorooctane sulfonate affects glucose metabolism in adult offspring. PLoS One 9 (1), e87137.
Wang, J., Pan, Y., Cui, Q., Yao, B., Dai, J., 2018. Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. Environ. Sci. Technol. 52 (22), 13553–13561.
Wang, T., Wang, P., Meng, J., Liu, S., Lu, Y., Khim, J.S., Giesy, J.P., 2015. A review of sources, multimedia distribution and health risks of perfluoroalkyl acids (PFAAs) in China. Chemosphere 129, 87–99.
Wang, Z., Cousins, I.T., Scheringer, M., Buck, R.C., Hungerbuhler, K., 2014. Global emission inventories for C4–C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. Environ. Int. 70, 62–75.
Wang, Z., Cousins, I.T., Scheringer, M., Hungerbühler, K., 2013. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. Environ. Int. 60, 242–248.
Xiao, F., 2017. Emerging poly- and perfluoroalkyl substances in the aquatic environment: A review of current literature. Water Res. 124, 482–495.
Yeung, L.W.Y., So, M.K., Jiang, G.B., Taniyasu, S., Yamashita, N., Song, M.Y., Wu, Y.N., Li, J.G., Giesy, J.P., Guruge, K.S., Lam, P.K.S., 2006. Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. Environ. Sci. Technol. 40 (3), 715–720.
Zabaleta, I., Bizkarguenaga, E., Bilbao, D., Etxebarria, N., Prieto, A., Zuloaga, O., 2016. Fast and simple determination of perfluorinated compounds and their potential precursors in different packaging materials. Talanta 152, 353–363.
Zhang, L., Liu, J., Hu, J., Liu, C., Guo, W., Wang, Q., Wang, H., 2012. The inventory of sources, environmental releases and risk assessment for perfluorooctane sulfonate in China. Environ. Pollut. 165, 193–198.
Zhang, T., Sun, H., Lin, Y., Qin, X., Zhang, Y., Geng, X., Kannan, K., 2013a. Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ. Sci. Technol. 47 (14), 7974–7981.
Zhang, T., Sun, H., Lin, Y., Wang, L., Zhang, X., Liu, Y., Geng, X., Zhao, L., Li, F., Kannan, K., 2011. Perfluorinated compounds in human blood, water, edible freshwater fish, and seafood in China: Daily intake and regional differences in human exposures. J. Agric. Food Chem. 59 (20), 11168–11176.
Zhang, T., Wu, Q., Sun, H.W., Zhang, X.Z., Yun, S.H., Kannan, K., 2010. Perfluorinated compounds in whole blood samples from infants, children, and adults in China. Environ. Sci. Technol. 44 (11), 4341–4347.
Zhang, Y., Beesoon, S., Zhu, L., Martin, J.W., 2013b. Isomers of perfluorooctanesulfonate and perfluorooctanoate and total perfluoroalkyl acids in human serum from two cities in North China. Environ. Int. 53, 9–17.
Zhou, J., Li, Z., Guo, X., Li, Y., Wu, Z., Zhu, L., 2019. Evidences for replacing legacy per- and polyfluoroalkyl substances with emerging ones in Fen and Wei River basins in central and western China. J. Hazard. Mater. 377, 78–87.