Identification of Novel Polyfluorinated Ether Sulfonates as PFOS Alternatives in Municipal Sewage Sludge in China

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S Supporting Information

ABSTRACT: A 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFAES) with the trade name F-53B, is an alternative to perfluorooctanesulfonate (PFOS) in electroplating industry that is uniquely used in China. It was developed as a mist suppressant initially in the 1970s, but the environmental behaviors and potential adverse effects of the 6:2 Cl-PFAES have only recently been investigated. In this work, the occurrence and distribution of perfluoroalkyl sulfonate (PFSA), fluorotelomer sulfonate (FTSA), and PFAES analogues were investigated in municipal sewage sludge samples collected around China. Perfluorobutane, perfluorohexane, perfluorooctane, and perfluorodecanesulfonates, 6:2 and 8:2 FTSA, and the emerging 6:2 Cl-PFAES were detected. Moreover, 8:2 and 10:2 Cl-PFAESs were identified for the first time as new polyfluorinated contaminants using high resolution mass spectrometry. These fluorinated analytes were further quantified with the aid of commercial and laboratory-purified standards. PFOS was the predominant contaminant with a geometric mean (GM) value of 3.19 ng/g dry weight (d.w.), which was subsequently followed by 6:2 Cl-PFAES and 8:2 Cl-PFAES (GM: 2.15 and 0.50 ng/g d.w., respectively). Both 6:2 and 8:2 Cl-PFAES were positively detected as the major components in the F-53B commercial product, and discrete 6:2 Cl-PFAES/8:2 Cl-PFAES ratios in the product and sludge samples might suggest 8:2 Cl-PFAES had enhanced sorption behavior in the sludge due to the increase in hydrophobicity.

Emerging Environmental Contaminants: Polyfluorinated Ether Sulfonates (PFAESs)

INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a group of anthropogenic chemicals, which have been subjected to increased public scrutiny in recent decades. The unique chemical properties of PFASs such as thermal resistance and high surface activity have led to extensive industrial and household applications, such as fire-fighting foams, lubricants, surfactants, pesticides, and coating additives.^{1,2} The unintentional releases of PFAS from direct manufacture emissions and daily usages as well as transport and transformation of polyfluorinated precursors^{3,4} have resulted in their ubiquitous presence in the environment and wildlife.^{5,6} In particular, perfluorooctanesulfonate (PFOS) has received the most attention because of the persistency, long-range transport potential, and bioaccumulation properties.^{7–9} It displayed developmental, reproductive, and immunotoxic effects in both *in vitro* and *in vivo* studies.¹⁰ Thus, the production of PFOS was voluntarily phased out by major manufacturers in 2002 and has also been regulated under the Stockholm Convention on Persistent Organic Pollutants.¹¹

Metal plating is an important emission source of PFOS industrial uses in China, in which commercial products containing PFOS (e.g., FC-80 and FC-248) are applied as mist suppressant to protect workers from exposure to airborne

chromium(VI) spray mist in the electrolytic process.¹² Owing to the lack of cost-effective alternatives, use of PFOS and its derivatives is still permitted as a specific exemption in closed-loop systems of hard metal planting in certain countries.¹³ Nevertheless, additional polyfluorinated alternative substances, such as 6:2 fluorotelomer sulfonate (6:2 FTSA), are being tested at trial phase but with less efficiency in lowering the surface-tension and thus required more quantity/replenishment.¹⁴ China has been using polyfluoroalkyl ether sulfonates (PFAESs) as mist suppressant since the 1970s, even before the introduction of PFOS products, and is the only country with a documented usage of PFAESs.¹⁵ Potassium 1,1,2,2-tetrafluoro-2-(perfluorohexyloxy) ethanesulfonate (6:2 F-PFAES with trade name F-53, Figure 1) was first synthesized and demonstrated to have excellent performance in electroplating at small-scale attempts, but the market share has since been dominated by the commercialized chlorine-substituted analogue, potassium 2-(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexyloxy)-1,1,2,2-tetrafluoroethanesulfonate (6:2 Cl-PFAES

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Figure 1. Chemical name, acronym, and molecular structures of target Cl-PFAES, F-PFAES, PFAS, and FTSA analogues. Types of instrumental analysis for each analyte are presented as qualitative (Ql), semiquantitative (Sq), and quantitative (Qn), respectively.

with trade name F-53B), due to simplified production processes.^{15,16} Although PFAESs have been used for several decades, scientific studies on the emission, environmental occurrence, and potential environmental impacts are still very limited. Wang and co-workers were the first to evaluate the potential impacts of 6:2 Cl-PFAES to the environment.¹⁶ Release of 6:2 Cl-PFAES was found in electroplating plant wastewater (65–112 $\mu\text{g/L}$ in the influent and 43–78 $\mu\text{g/L}$ in the effluent). 6:2 Cl-PFAES showed moderate toxicity to *Brachydanio rerio* ($\text{LC}_{50}(96\text{ h})$ 15.5 mg/L) in acute toxicity assay and was resistant to biodegradation. Nevertheless, contamination trends and distribution patterns of 6:2 Cl-PFAES in different environmental compartments as well as the occurrence and fate of other Cl-PFAES and F-PFAES analogues are still unclear.

Wastewater treatment plants (WWTPs) are commonly considered as major sinks of various groups of PFASs and is also an important secondary point source of these compounds to both the aquatic and atmospheric environment.^{17,18} The sorption of perfluoroalkyl sulfonates (PFASs) onto suspended particulate matters indicates that sewage sludge could be used to track the presence, concentration, and fate of PFOS and analogous compounds due to human activities. The aims of this present work were (1) to establish a robust sample pretreatment and instrument quantification method for simultaneous analysis of PFASs, FTSAs, and 6:2 Cl-PFAES in sludge samples; (2) to screen for identification of potential PFOS alternatives by using high resolution mass spectrometry suspect screening strategy; (3) to measure the residue concentrations and investigate the composition profiles of PFOS and alternatives in sewage sludge from different regions in China. To our knowledge, this study is the first to report the presence of novel PFAES analogues (8:2 Cl-PFAES and 10:2 Cl-PFAES) in the environment.

MATERIALS AND METHODS

Materials. Chemical name, acronym, structure, and other information for the target compounds are shown in Figure 1. A native PFBS, PFHxS, PFOS, and PFDS standard mixture (PFAC-MXB, 2 $\mu\text{g/mL}$ for each analyte), native PFHpS, individual native fluorotelomer sulfonates (4:2, 6:2 and 8:2 FTSA, 50 $\mu\text{g/mL}$ for each analyte), and isotope-labeled internal

standards (MPFHxS, M3PFHxS, MPFOS, M8PFOS, and M2–6:2 FTSA, 50 $\mu\text{g/mL}$ for each analyte) were purchased from Wellington Laboratories (Ontario, Canada). 6:2 Cl-PFAES and 8:2 Cl-PFAES standards were laboratory-purified from the commercial F-53B mist suppressant product, and details on methodology and structural characterization procedures are given in the Supporting Information (SI). The purity of all chemicals were 95% or higher unless otherwise stated. HPLC-grade acetonitrile and methanol were supplied by J.T. Baker (Phillipsburg, NJ). Ultrapure water (18.3 $\text{M}\Omega\cdot\text{cm}$) was generated by a Milli-Q system (Millipore, Billerica, MA). ENVI-Carb powder (120–400 mesh, 100 m^2/g) was from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide, concentrated hydrochloric acid (37%), and ammonium acetate were obtained from Sinopharm Chemical Reagent, Inc. (Beijing, China), Merck (Darmstadt, Germany), and Dikma (Lake Forest, CA) separately.

Sampling and Extraction Procedures. Detailed sample collection processes have been published elsewhere,¹⁹ and the sampling locations are shown in Figure S1. In brief, a total of 56 grab sewage sludge samples were collected during October 2010 to May 2011 from individual wastewater treatment plants, covering 20 provinces and municipalities in China. Freshly digested sludge samples (approximately 500 g for each sample, wet weight, w.w.) from the WWTP dehydration process were packed in aluminum foil, sealed in polypropylene bags, and immediately express-delivered to our laboratory. The samples were then lyophilized, homogenized, and preserved at $-20\text{ }^{\circ}\text{C}$ until analysis. Relevant information on the WWTP characteristics, i.e., type of treatment and daily processing volume, is summarized in Table S1.

The sample pretreatment was based on the dispersive solid-phase extraction (DSPE) method for the analysis of PFASs in environmental matrices^{20,21} with slight modifications. In the primary DSPE procedure, approximately 0.5 g of individual sludge sample was placed in a 10 mL crimp neck glass vial with a rubber septa (CNW Technologies GmbH, Dusseldorf, Germany), and 2 ng of each surrogate standards (MPFHxS, MPFOS and M2–6:2 FTSA) was spiked to each sample. Alkaline organic solvent containing 3 mL of acetonitrile and 160 μL of 1 M sodium hydroxide solution were used to extract the analytes by shaking at $50\text{ }^{\circ}\text{C}$ for 120 min. After

Table 1. Descriptive Statistics of the Measured PFAS Concentrations (ng/g d.w. Sludge) in the Investigated Sludge Samples

analyte	GM ^a	median	95th percentage	range	detection ratio ^b (%)	average proportion (%)	
						in each analogue	in ΣFASs
Perfluoroalkyl Sulfonates (PFASs)							
PFBS	0.24	0.10*	1.53	N.D.—2.76	51.8	11.5	4.74
PFHxS	0.15	N.D.	1.10	N.D.—1.93	28.6	7.75	3.56
PFOS	3.19	3.21	154	N.D.—218	98.2	76.5	39.8
PFDS	0.09	N.D.	0.62	N.D.—0.94	19.6	4.30	1.87
ΣPFASs	4.38	4.42	154	N.D.—220	98.2	100	50.0
Chlorinated Polyfluoroalkyl Ether Sulfonates (Cl-PFAESs)							
6:2 Cl-PFAES	2.15	1.94	78.7	0.02*—209	100	75.9	30.1
8:2 Cl-PFAES	0.50	0.43	14.0	N.D.—31.8	89.3	19.8	7.17
10:2 Cl-PFAES	0.07	N.D.	0.56	N.D.—0.86	23.2	4.30	1.36
ΣCl-PFAESs	2.86	2.51	92.7	0.31—241	100	100	38.6
Fluotelomer Sulfonates (FTSAs)							
6:2 FTSA	0.13	0.13	2.56	N.D.—13.9	76.8	40.7	5.60
8:2 FTSA	0.23	0.28	1.52	N.D.—5.08	89.3	59.3	5.79
ΣFTSAs	0.49	0.58	2.83	N.D.—18.9	92.9	100	11.4
ΣFASs	10.5	8.07	167	1.12—413	100		100

^aGM: geometric mean. ^bDetection ratio: sample taken into account for each analyte when the instrument signal/noise >3.

centrifugation at 1600 g for 10 min, part of supernatant (600 μ L) was placed into a 2 mL microcentrifuge tube (Axygen, Union city, CA) and mixed with 400 μ L of acetonitrile, 15 μ L of 1 M sodium hydroxide solution and 10 mg of ENVI-Carb powder for further pretreatment. The mixture was then shaken at 50 °C for 120 min, while the supernatant was collected after centrifugation at 12 000g for 10 min and neutralized by addition of 15 μ L of 1 M hydrochloric acid and 4 μ L of 1 M ammonium acetate solution. In order to increase recovery of the target compounds in the residual sludge phase, a secondary DSPE cycle with the same operational steps was performed. All supernatant in the primary and secondary extraction was combined, concentrated to 200 μ L under a nitrogen stream, transferred into a LC vial, spiked with isotope-labeled injection standards (400 pg for M3PFHxS and M8PFOS, respectively), and shaken before instrument analysis.

Qualitative Analysis and Quantification Procedures.

In order to positively detect the target fluoroalkyl sulfonate analytes as well as to identify other potential analogues in the sludge extracts, a high resolution mass spectrometry (HRMS) suspect screening strategy was performed. An ultrahigh performance liquid chromatography-Orbitrap Fusion MS system (Thermo Fisher Scientific Inc., Waltham, MA) was operated in full scan mode (200–800 m/z) with a resolution of 120 000. The negative electrospray ionization (ESI) mode was chosen with a capillary voltage of 2500 V. Ion transfer tube and vaporizer temperatures were respectively 350 and 200 °C. The higher-energy collision dissociation (HCD) energy was optimized as 35%. Presence of fluoroalkyl sulfonates was confirmed by analysis of putative theoretical molecular ion masses in the full-scan spectrum data within a threshold of 5 part-per-million (ppm) accuracy (Table S2). Structural recognition was performed based on appropriate liquid chromatography retention time and characteristic MS² fragmentation patterns with the aid of the PFSA, FTSA, and PFAES standards.

The quantification of targeted fluoroalkyl sulfonates was performed on a API 5500 triple-quadrupole mass spectrometer (AB SCIEX Inc., Framingham, MA) interfaced with an Ultimate 3000 ultrahigh performance liquid chromatograph (Thermo Fisher Scientific Inc., Waltham, MA). An ACQUITY

BEH C18 analytical column (2.1 mm i.d. $\times$ 100 mm length, 1.7 μ m, Agilent) was used for separation. Column temperature was 35 °C, and flow rate was set at 0.3 mL/min. The flow gradient was initiated at a composition of 60:40 (methanol/water, v/v, 1 mM NH₄Ac additive in each phase), held for 1 min, linearly switched to 100% methanol in 5 min, and the composition was finally held for another 2 min. ESI ionization was operated in the negative mode with a capillary voltage of 3000 V. Ionization source temperature was optimized at 500 °C. Detailed multiple reaction monitoring (MRM) parameters, such as quantification ion transition, declustering potential and collision energy for each analyte, are summarized in Table S3.

Quality Assurance and Quality Control (QA/QC).

Extraction efficiencies of the target analytes were checked by a third basic acetonitrile DSPE extraction procedure of 10 randomly selected sludge samples, and these extracts were separately pretreated and quantified. Less than 4.0% of PFOS and 0.8% of 8:2 Cl-PFAES were found in the third extract, indicating maximum extraction of the target analytes during the primary and secondary extraction cycles. Two procedural blanks of 0.5 g of solvent-washed diatomaceous earth (Dionex, Sunnyvale, CA) were included in each batch of seven sludge samples. All analytes in the blanks were under the method quantification limits (MQLs, Table S3), which were in the range of 25.2 (6:2 FTSA) to 135 (PFBS) pg/g sludge. No target analyte was found in the solvents nor the LC system. The recoveries of matrix-spiked samples ($n = 3$, arithmetic mean $\pm$ standard deviation) were 112 ± 10 , 116 ± 13 , and 109 ± 14 for PFASs, FTSAs, and PFAESs at 2 ng spiked level, and were 110 ± 12 , 127 ± 16 , and 93 ± 6 for PFASs, FTSAs and PFAESs at 20 ng spiked level, respectively. Quantification was based on an internal calibration method. The recoveries of the surrogate standards ($n = 3$) were 91 ± 7 , 95 ± 3 , and 137 ± 10 for MPFHxS, MPFOS, and M2–6:2 FTSA at 2 ng spiked level, and were 92 ± 3 , 95 ± 5 , and 122 ± 7 for MPFHxS, MPFOS, and M2–6:2 FTSA at 20 ng spiked level, respectively. Ionization enhancement was observed in the quantification analysis, as the matrix interferences of the injection standards were 161 ± 18 and 158 ± 16 for M3PFHxS and M8PFOS (each spiked at 400 pg in the sample vials), separately. The dynamic range of the instrument responses were verified daily

(A) 20ppb-standard-MSn-35eV #2619-2651 RT: 6.69128-6.75097 AV: 5 NL: 1.02E6
F: FTMS - c ESI Full ms2 630.89@hcd35.00 [50.00-750.00]

(B) 6:2 Cl-PFAES standard:

(C) 6:2 Cl-PFAES in sludge:

Figure 2. Accurate mass measurement, LC retention time, and MS² fragmentation patterns of the identified Cl-PFAESs in the laboratory-purified standard and sludge samples. (A) MS² characteristic fragmentation ions of the 8:2 Cl-PFAES standard in the HCD mode (collision energy: 35%). (B) Reconstructed total ion chromatography of the molecular ion and fragmentation ion transits of individual 6:2 and 8:2 Cl-PFAES standards (spiked at 20 ng/mL for each analyte) with mass windows of theoretical mass ± 5 ppm. (C) Reconstructed total ion chromatography of the molecular ion and fragmentation ion transits of 6:2, 8:2, and 10:2 Cl-PFAES in a sludge sample with mass windows of theoretical mass ± 5 ppm.

using a nine-point calibration curve with concentrations in the range of 0.1–50 ng/mL. For samples with analyte concentrations beyond the quantification dynamic range, dilution was made by addition of acetonitrile containing both 2 ng/mL of M3PFHxS and M8PFOS injection standards, and the diluted

extract was then reanalyzed. All reported data in this study is presented on a dry weight (d.w.) basis.

Statistical Analysis. Geometric mean, median, and concentration ranges were used to describe the results (Table 1). Nonquantifiable analyte concentrations with signal-to-noise

less than 10 were set as MQL divided by the square root of 2. All data were log-transformed in the Spearman's test to assess correlations among the analyte residue levels as well as the WWTP characteristic (i.e., sludge total organic carbon content). Principal component analysis was performed using the varimax rotation method with Kaiser normalization. Statistical analyses were conducted using SPSS V17.0 for Windows Release (SPSS, Inc., 2009), and significant levels were determined at $p < 0.05$ level unless otherwise mentioned.

■ RESULTS AND DISCUSSION

Structure Confirmation of Novel PFAES Analogues.

Accurate molecular mass and characteristic structure fragmentation ion are both diagnostic parameters for the identification of unknown organic contaminants,²² information on which could be simultaneously obtained in retrospect analysis of HRMS MS² full scan spectrum. Thus, the fragmentation mechanisms of PFAESs were studied by using the 6:2 Cl-PFAES and 8:2 Cl-PFAES standards. The major fragmentation ions and the corresponding abundance of 6:2 Cl-PFAES in the HCD mode of the Orbitrap (Figure S2) were quite similar to those reported in the triple quadrupole (LC-MS/MS) instrument.¹⁶ For instance, cleavage of the ether bond in the molecular structure resulted in the most abundant characteristic ion $[M(^{35}\text{Cl})\text{-C}_2\text{F}_4\text{SO}_3]^-$, and the formation of $[\text{FSO}_3]^-$ and $[\text{FSO}_2]^-$ could also provide additional information on the sulfate functional group. Similar abundance of $[M(^{35}\text{Cl})\text{-C}_2\text{F}_4\text{SO}_3]^-$, $[\text{FSO}_3]^-$, and $[\text{FSO}_2]^-$ ions were found in 8:2 Cl-PFAES MS² spectrum (Figure 2A), which indicates that the observed fragmentation pattern could be suitable for identifying other PFAES analogues. As shown in Figure 2C, accurate molecular ion $[M(^{35}\text{Cl})]^-$ and characteristic fragmentation ion $[M(^{35}\text{Cl})\text{-C}_2\text{F}_4\text{SO}_3]^-$ for both 6:2 Cl-PFAES ($m/z = 530.8956$, $\Delta m = -0.021$ ppm, and $m/z = 350.9452$, $\Delta m = 0.017$ ppm, respectively) and 8:2 Cl-PFAES ($m/z = 630.8895$, $\Delta m = 0.485$ ppm, and $m/z = 450.9387$, $\Delta m = -0.171$ ppm, respectively) were discovered, indicating the presence of the analytes in the sludge extract. Moreover, 10:2 Cl-PFAES was also positively identified with lower instrument responses ($m/z = 730.8848$, $\Delta m = 2.754$ ppm, and $m/z = 550.9325$, $\Delta m = 0.218$ ppm for $[M(^{35}\text{Cl})]^-$ and $[M(^{35}\text{Cl})\text{-C}_2\text{F}_4\text{SO}_3]^-$ ions, respectively) in the sludge matrix, which was further supported by predicted delay in LC retention time (RT = 7.07 min) compared with those of 6:2 Cl-PFAES and 8:2 Cl-PFAES standards (RT = 6.18 and 6.65 min, respectively). The recognition of 8:2 and 10:2 Cl-PFAES analogues could imply the impact of impurity component release into the environment from the F-53B product usages. Nevertheless, the characteristic m/z of 4:2 Cl-PFAES was not observed in any of the commercial product or sludge samples, indicating no practical usage of the chemical in China. The identification method was also used to check the presence of F-PFAES analogues (4:2, 6:2, 8:2, and 10:2 F-PFAES, Figure 1 and Table S2) in the sludge extracts. However, no signal corresponding to the accurate molecular mass or MS² fragmentation ion was found for any of these analogues. This result confirms the suggestion in a previous report that F53 was replaced by F53B as the only commercial PFAES mist suppressant product in the market.¹⁶

PFSA Distribution. The quantification of PFSAs was performed by LC-MS/MS. PFBS, PFHxS, PFOS, and PFDS were positively detected in the sludge samples, while PFHpS was not found (method detection limit: 43 pg/g). Descriptive statistic data of the measured PFSA concentrations are

summarized in Table 1. PFOS was the dominant PFSA with a geometric mean (GM) concentration of 3.19 ng/g, which constitute 29–100% (average proportion: 76%) of total PFSA levels (ΣPFSAs). This was followed by PFBS (average 11%), PFHxS (average 7.8%) and PFDS (average 4.3%), with GM values of 0.24, 0.15, and 0.09 ng/g, respectively. The PFOS contamination levels in sewage sludge have been extensively studied in numerous countries, e.g. Switzerland (concentration range: 20–600 ng/g d.w.),²³ Denmark (4.8–71.4 ng/g d.w.),²⁴ Sweden (0.52–35 ng/g w.w.),²⁵ Canada (0.1–460 ng/g d.w.),²⁶ U.S. (14–444 ng/g d.w.)²⁷ and China (0.5–19.8 ng/g d.w.).^{28,29} A similar concentration range of <LOD–218 ng/g was also found in this study. The PFSA concentrations in sludge are influenced by environmental factors such as adsorption and biotransformation during the WWTP treatment processes as well as the residue amount in sewage influent released from industry and household applications.^{3,17,27,30} Yu and co-workers suggested that PFAS levels in sludge may be more relevant to the aqueous concentrations than sludge retention behaviors,³¹ even though more hydrophobic perfluorinated analogues have increased distribution coefficients (i.e., K_d values).³² Thus, the higher detection ratios of PFOS (98%) and PFBS (52%) in the sludge samples, compared with those of PFHxS (29%) and PFDS (20%), could indicate widespread usage of C8 and C4 PFSAs and relevant precursors in China. The finding was further supported by the reported uniform PFSA composition profiles in coastal rivers and marine sediments from Chinese territorial sea.³³

PFAES Distribution. The concentrations of 6:2 and 8:2 Cl-PFAES in the sludge samples were quantified using the laboratory-purified standards. As 10:2 Cl-PFAES was also identified in the HRMS suspect screening strategy, semi-quantification of 10:2 Cl-PFAES was further performed by applying the HRMS quantification strategy, which utilized the full MS scan spectrum and data-dependence MS² fragmentation ion for quantitative and qualitative purposes simultaneously.³⁴ The instrument response factor of 10:2 Cl-PFAES was assumed to be equal to that of an equimolar amount of 8:2 Cl-PFAES in full-scan HRMS spectrum since those of 6:2 and 8:2 Cl-PFAES were found to be quite similar (Figure S3). 6:2 and 8:2 Cl-PFAES were the main analogues with GM concentrations of 2.15 and 0.50 ng/g, which constitute 76% and 20% of total PFAES concentrations (ΣPFAESs), respectively. 10:2 Cl-PFAES was found in 23% of sludge samples with a GM concentration of 0.07 ng/g, which only contributed to a very small part (4.3%) of PFAES levels. The spatial concentrations and composition profiles of PFAES in different investigated regions are given in Figure S1. It is obvious that uniform composition profiles of 6:2 and 8:2 Cl-PFAES were found in almost all of the sampling locations, which could suggest common usage and/or sources. The PFAES levels in sludge showed geographical variations with the highest levels in the eastern coastal provinces, which coincided with areas of more intensive metal plating activities.¹² The limited knowledge on the environmental levels of PFAESs suggested considerable amounts of PFOS and 6:2 Cl-PFAES in electroplating plant wastewater and vicinity river samples.¹⁶ In this study, PFOS and 6:2 Cl-PFAES showed high detection ratio (98% and 100%, respectively) and similar maximum concentrations (218 and 209 ng/g, respectively), which indicate universal usage of both chemicals. Nevertheless, the greater PFOS average proportion in total fluoroalkyl sulfonate concentrations (ΣFASs , 40%) compared with that of the 6:2 Cl-PFAES

Table 2. Spearman's Correlation Matrix for the PFAS Concentrations and WWTP Characteristics (TOC Content and Daily Processing Volume)

	PFOS ^a	6:2 FTSA	8:2 FTSA	6:2 Cl-PFAES	8:2 Cl-PFAES	TOC content
6:2 FTSA ^b	−0.085					
8:2 FTSA	0.263** ^c	0.198				
6:2 Cl-PFAES	0.372**	−0.103	−0.001			
8:2 Cl-PFAES	0.266*	0.076	0.095	0.849**		
TOC content	0.032	0.108	−0.016	−0.240	−0.157	
processing volume	0.237	−0.095	0.178	0.110	0.110	0.272*

^aAnalytes with detection ratio less than 70% in the sludge samples were not taken into account. Concentrations under the MQLs were set as MQL divided by the square root of 2. ^bAll data were log-transformed. ^cThe symbols “*” and “**” represent the significant correlations at 0.05 and 0.01 levels, respectively.

(30% in Σ FASs), might imply more consumer usage amount and/or application purposes of PFOS in China regarding similar physical–chemical properties of the two compounds.¹⁶

FTSA Distribution. 6:2 and 8:2 FTSA were detected in most sludge samples with GM values of 0.13 and 0.23 ng/g, respectively. Specific 4:2 FTSA signals were not found in the monitored qualitative/quantitative transitions of the LC–MS/MS instrument. A high average proportion (59%) of 8:2 FTSA found in this study was consistent with those reported in surface water and human blood in China.³⁵ No specific regional difference in contamination levels was observed for FTSA among the sampling sites, and the small contribution of 6:2 and 8:2 FTSA to Σ FASs (5.6% and 5.8%, respectively), compared with those of PFSA and Cl-PFAES analogues (Table 1), imply relatively low usage of FTSA-based commercial products. FTSA residues in the environment were found to be mainly related with the aqueous film-forming foam (AFFF) applications instead of mist suppressant usage.³⁶ FTSA was found to be present in some AFFF formulations, and significant release levels (up to 14 600 ng/L) of 4:2, 6:2, and 8:2 FTSA were detected in groundwater where fire-training activities were conducted.³⁶ Degradation of fluorotelomer precursors is the primary FTSA source,³⁷ and the elevated abundance of 8:2 FTSA on surficial AFFF-impacted soil further suggest contributions of the C8 FTSA-based compounds in the environment.³⁸

Correlations between Fluorinated Sulfonates and Influencing Factors. The total fluoroalkyl sulfonate concentrations (Σ FASs, Table 1) were in the range of 1.12–413 ng/g, with a GM concentration of 10.5 ng/g. It consisted of $52 \pm 24\%$ in overall PFAS contamination levels (including the target fluoroalkyl sulfonates and C4–C14 perfluoroalkyl carboxylates, data not shown) in the sludge samples. Among the target fluoroalkyl sulfonate analogues, PFASs were the dominant chemicals with a relative proportion of $50 \pm 24\%$ in Σ FASs, which implied that the PFSA usages in China could result in a primary burden of fluoroalkyl sulfonate residues in the sludge samples. The relative abundance was subsequently followed by PFAESs and FTSAs, with relative proportions of $39 \pm 25\%$ and $11 \pm 12\%$ in Σ FASs, respectively. As also noted in Table 2, the log-transformed PFOS concentration was moderately correlated with those of the 8:2 FTSA, 6:2 Cl-PFAES, and 8:2 Cl-PFAES ($R = 0.263$ – 0.372 , $P < 0.05$). Significant correlations between the PFAS concentrations could indicate common release pathway and/or similar fate of the monitored compounds during the WWTP processes. Moreover, a strong correlation was also found between the residue levels of 6:2 Cl-PFAES and 8:2 Cl-PFAES ($R = 0.849$, $P < 0.01$), which further indicates the association between the two chemicals from

industrial manufacturing and/or commercial application. Nevertheless, no obvious relationship was observed between individual fluoroalkyl sulfonate concentration and total carbon content (TOC) in the sludge samples. Investigations on mass flows in wastewater treatment facilities around the world suggested that the fate of PFAS in WWTP processes is case dependent and that adsorption of PFAS onto the organic phase of sludge might not be the predominant removal mechanism.³⁰ Moreover, the impact of different WWTP sludge treatment techniques (anaerobic-anoxic-oxic, anoxic/oxic, oxidation ditch, and sequencing batch reactor) on the log-transformed PFSA concentrations was not observed in this study (Figure S4, one-way ANOVA, $P > 0.05$).

Relative Abundances of 6:2/8:2 Cl-PFAES in Commercial Product and Sludge Samples. Both 6:2 and 8:2 Cl-PFAES were positively identified as the major components in the purchased commercial F-53B product, and the 6:2/8:2 Cl-PFAES content ratios were measured by quantitative analysis of the diluted solution of the product powder at concentrations within the LC-MS/MS linearity range (spiked at 0.5, 1.0, 2.0, 5.0, 10, 20, and 50 $\mu\text{g/mL}$, $n = 5$ for each spiked level). The measured average 6:2/8:2 Cl-PFAES ratios were 12.9 ± 2.6 in the commercial product, which are significantly different (4.5 ± 2.7 , $n = 50$, Figure 3A) from those in the sludge samples with quantifiable levels (i.e., 6:2 and 8:2 concentration $> \text{MQLs}$). The trend was further confirmed by separately investigating the 6:2/8:2 Cl-PFAES ratio in sludge samples grouped by the 6:2 Cl-PFAES concentrations (Figure 3B). Compared with those in the commercial product, discrete 6:2/8:2 Cl-PFAES ratios were all obtained in the grouped sludge samples (2.4 ± 0.9 , 4.1 ± 1.6 , 4.9 ± 2.2 , and 6.7 ± 3.1 for 6:2 Cl-PFAES concentration groups <1 , 1 – 2 , 2 – 5 , and >5 ng/g, respectively). This phenomenon could partly be related to the differences in physical–chemical properties and fate of different fluoroalkyl sulfonates in the environment. For instance, PFDS was observed to have the highest sediment–water distribution coefficient among the investigated PFSA homologues (C4–C10),^{32,39} suggesting increased adsorption ability along with higher hydrophobic characteristic.

Environmental Implications. Both 6:2 Cl-PFAES and PFOS showed similar environmental behaviors such as biodegradation resistance and moderate toxicology effects.¹⁶ Thus, the identification of 8:2 and 10:2 Cl-PFAESs in the sludge samples calls for investigation on potential impacts of these emerging PFAES analogues. Compared with the molecular structures of PFASs, the additional unique chlorine (Cl[−]) and ether-substituted (C–O–C) functional groups in the Cl-PFAESs might contribute positively to their hydrophobic properties (Tables S4 and S5; EPI Suite V4.11⁴⁰). The specific

Figure 3. 6:2/8:2 Cl-PFAES concentration ratios in the commercial F-53B product and the sludge samples. (A) 6:2/8:2 Cl-PFAES ratios in product solution (spiked at 0.5, 1.0, 2.0, 5.0, 10, 20, and 50 $\mu\text{g/mL}$, $n = 5$ in each spiked level) and all sludge samples ($n = 50$, samples were taken into account only when both 6:2 and 8:2 Cl-PFAES concentrations surpass the corresponding MQLs). (B) 6:2/8:2 Cl-PFAES concentration ratios in the grouped sludge samples (samples were divided according to the 6:2 Cl-PFAES concentration, and the number of samples in the Conc. < 1, Conc. 1–2, Conc. 2–5, and Conc. > 5 groups were $n = 14, 9, 14$, and 13 , respectively).

BCF/BAF values of fluorinated substances could not be accurately estimated by current quantitative structure–property relationship models due to the nonlinear concentration-dependent adsorption mechanism.⁴¹ However, higher relative proportion of C10 fluorinated compounds such as PFDS and 8:2 FTSA in biota and human blood samples^{9,35,42} further substantiate the increasing bioaccumulation potential of long-chain fluoroalkyl sulfonates. Hence, biological uptake and accumulation of 6:2 and 8:2 Cl-PFAESs are highly possible.

Moreover, laboratory and field research on the environmental fate and toxicological effects of polyfluorinated chemicals also revealed discrepant behaviors among different PFSA analogues. For instance, increasing bioaccumulation factors were observed with increased carbon-chain lengths (i.e., PFBS, PFHxS, and PFOS) in aqueous and terrestrial species.^{8,9} Acute embryo toxicology on *Xenopus laevis*⁴³ and intercellular communication inhibition in subchronic bacterial exposure of PFOS⁴⁴ were more obvious compared with those

of PFBS and PFHxS. Multiple end point assays *in vitro* further suggested that the adverse effects are more influenced by the fluorinated carbon chain than the different functional groups.⁴⁵ Therefore, empirical studies on the fate and potential adverse effects of the newly identified Cl-PFAES analogues are warranted in order to better elucidate their behaviors in the environment.

■ ASSOCIATED CONTENT

● Supporting Information

Laboratory-purification procedures of the 6:2 and 8:2 Cl-PFAES standards from the commercial F-53B mist suppressant product; (Table S1) detailed information on the wastewater treatment plant characteristics; (Table S2) measured accurate mass and corresponding theoretical mass of the molecular ion and characteristic fragmentation ion for each PFAES analogues in the sludge samples; (Table S3) LC–MS/MS instrument parameters and method quantification limits for the separation and quantification of the target analytes; (Tables S4 and S5) details of the “fragment constant” methodology in the KOWWIN model; (Figure S1) sampling locations, spatial concentrations, and composition profiles of Cl-PFAES homologues in municipal sewage sludge samples in China; (Figure S2) MS² characteristic fragmentation ions of the 6:2 Cl-PFAES standard in the HCD mode; (Figure S3) instrument responses ($n = 3$) of $[\text{M}^{(35)\text{Cl}}]^-$ molecular ions for 6:2 and 8:2 Cl-PFAESs in the HRMS full scan spectrum; (Figure S4) the score plots (PC1 versus PC2) of analyte concentrations in the sewage sludge samples grouped by WWTP treatment techniques; (Figure S5) separation and collection of the elute fractions of the 6:2 and 8:2 Cl-PFAES in the Autopurification system; (Figures S6–S11) detailed information on the NMR (¹⁹F and ¹²C NMR) and purity results of the laboratory-purified 6:2 and 8:2 Cl-PFAES standards. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01010.

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Notes

The authors declare no competing financial interest.

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