Identification of Novel Hydrogen-Substituted Polyfluoroalkyl Ether Sulfonates in Environmental Matrices near Metal-Plating Facilities

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ABSTRACT: Environmental occurrence and behaviors of 6:2 chlorinated polyfluoroalkyl ether sulfonate (Cl-6:2 PFESA, with trade name F-53B) have been receiving increased attention recently. Nevertheless, its potential fates under diversified conditions remain concealed. In this study, susceptibility of Cl-6:2 PFESA to reductive dehalogenation was tested in an anaerobic super-reduced cyanocobalamin assay. A rapid transformation of dosed Cl-6:2 PFESA was observed, with a hydrogen-substituted polyfluoroalkyl ether sulfonate (1H-6:2 PFESA) identified as the predominant product by a nontarget screening workflow. With the aid of laboratory-purified standards, hydrogen-substituted PFESA analogues (i.e., 1H-6:2 and 1H-8:2 PFESA) were further found in river water and sediment samples collected from two separate regions near metal-plating facilities. Geometric mean concentrations of 560 pg/L (river water) and 11.1 pg/g (sediment) for 1H-6:2 PFESA and 11.0 pg/L (river water) and 7.69 pg/g (sediment) for 1H-8:2 PFESA were measured, and both analytes consisted average compositions of 1% and 0.1% among the 18 monitored per- and polyfluoroalkyl sulfonate and carboxylate pollutants, respectively. To our knowledge, this is the first to report existence of polyfluoroalkyl sulfonates with both hydrogen and ether functional group in the environment.

1. INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are considered as a group of ubiquitous xenobiotic contaminants. In particular, perfluoroalkyl sulfonates (PFSSAs) and perfluoroalkyl carboxylates (PFCAs) were detected in various environmental compartments and biological species.1−4 Their persistent, bioaccumulative and toxic behaviors have triggered restrictions on usages and voluntary phase-out initiatives.5,6 Increasing attention has been paid on the identification of novel fluorinated chemicals, because analogues with diverse functional groups were found in the environment. For instance, a series of hydrogen- and chlorine-substituted PFCAs were discovered in wastewater from combined fluorochemical manufacturing origins.7 Perfluoroalkyl ether carboxylates and sulfonates were noticed in natural water downstream the locations where wastewater treatment plant and industrial effluent streams occurred.8,9

6:2 Chlorinated polyfluoroalkyl ether sulfonate (Cl-6:2 PFESA, trade name: F-53B) is used as an alternative of perfluorooctanesulfonate (PFOS) products in metal plating industry.10 Much concern has been specially focused on this chemical recently, due to discoveries of its widespread presence in riverine water,11 sewage sludge,12 aquatic organism13,14 and human serum.15 Longer-chain analogues, that is, Cl-8:2 and Cl-10:2 PFESA, were also recognized, which were considered as impurity components released from the F-53B product usages.12 Based on up-to-date knowledge, the residue levels, persistent and bioaccumulative properties, and elimination kinetics in human exposure of Cl-6:2 PFESA were comparable with those of PFOS in the investigation scenarios.10,13,15 Nevertheless, environmental behaviors associated with the unique chlorine atom in the Cl-PFESA molecular structure were yet uninvestigated.

A diversity of analytical techniques were applied for the identification of unknown fluorinated compounds, in which liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) emerged as a powerful tool.7,16−18 With the aid of fast atom bombardment and quadrupole-time-of-flight HRMS instruments, accurate masses and elemental formulas of ions with suspected functional groups and carbon chain length were screened, which resulted in discovery of
zwitterionic, cationic and anionic fluorotelomer sulfonates in commercial aqueous film-forming foam products. Mass defect filtering could be used as an effective protocol to exclude quantity of irrelevant information in MS spectrum. Polyfluorinated carboxylates and sulfonates were distinguished in manufacturing wastewater by visualizing existing series of homologues in horizontal lines in the CF2 adjusted Kendrick mass defect (AKMD) plot. Meanwhile, a case-control strategy using LC-quadrupole time-of-flight tandem mass spectrometry and statistical analysis was developed to elucidate analytes with significantly different abundances, which led to recognition of chlorine- and ketone-substituted PFOS deriva-
tives in sera of firefighters. A combination of these techniques is also requisite for illumination of PFAS precursor transformation processes in the environment.

Reductive dehalogenation is vital in microbial transformation of chlorinated and brominated pollutants in anaerobic environ-
ment. The in vitro assay using corrinoid macro-cycles, i.e. cyanocobalamin, as catalysts is a simplified biomimetic system to imitate the anaerobic biotransformation behaviors. Super-reduced cyanocobalamin (CCA, Vitamin B12) was confirmed functional to decompose branched isomers of PFOS and technical product under certain incubation conditions. Similar assays were further applied for prediction and confirmation of potential microbial degradation metabolites. For instance, tri- and tetra-ortho substituted congeneres generated from incuba-
tion of polybrominated biphenyls (PBBS) in super-reduced CCA assay could match the composition pattern of PBB residues in Baltic cod liver. Coincidence on major debromination pathways of decabromodiphenyl ether was observed by comparison of characteristic metabolites in the super-reduced CCA assay and in sediment.

In this study, a LC-HRMS nontarget screening workflow was established with the aim (1) to test transformation feasibility of Cl-6:2 PFESA in anaerobic environment by using a super-
reduced CCA assay; (2) to screen for major terminal transformation products; (3) to check the existence and behaviors of relevant chemicals in the environment.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. The Cl-6:2 PFESA and Cl-
6:2 PFESA standards were laboratory-purified from commercial F-53B mist suppressant product, with purification methodology and structural characterization described elsewhere. A native PFASs and PFCAs standard mixture (PFAC-MXB, 2 μg/mL for each analyte), native fluorotelomer sulfonates (4:2, 6:2 and 8:2 FTSA, 50 μg/mL for each analyte), and isotope-labeled standards (M3PFHxA, MPFOA, MPFUdA, MPFDoA, MPFHxS, MPFOS, M2−6:2 FTSA, M5PFHxA, M6PFDA, M3PFHxS, M8PFOS, 50 μg/mL for each standard) were obtained from Wellington Laboratories (Ontario, Canada). Cyanocobalamin, ENVI-Carb powder (120–400 mesh, 100 m2/g) and sodium citrate tribasic dehydrate were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (NH4OH, 50%, v/v) and sodium carbonate were from Alfa Aesar. HPLC grade acetic acid and ammonium acetate were obtained from DikmaPure (LakeForest, CA). Sodium hydroxide, concentrated hydrochloric acid (37%), and titanium(III) chloride (TiCl3, 20% w/v solution in 2N hydrochloric acid) were acquired from Sinopharm Chemical Reagent, Inc. (Beijing, China), Merck (Darmstadt, Germany) and Acros Organics (Belgium, Germany) respectively. The purities of all chemicals were 95% or higher unless otherwise stated. HPLC-grade methanol was supplied by J.T. Baker (Phillipsburg, NJ). Ultrapure water (18.3 MΩ-cm) was generated by a Milli-Q system (Millipore, Billerica, MA).

2.2. In-Vitro Reductive Transformation Assay. Super-
reduced CCA assay was prepared according to literature with minor modifications, and detailed procedures were given in the Supporting Information (SI). Transformation process was performed by mixing 400 μL of 1.0 mg/mL Cl-6:2 PFESA (in methanol) and 1 mL of super-reduced CCA solution in 15 mL polypropylene centrifugation tubes at room temperature (25 ± 2 °C). Each sample was operated individually at designated time intervals, with a maximum incubation time of 240 s. At each appointed incubation time, 3.6 mL of methanol was added to effectively dilet and retard transformation process (SI Figure S1). The samples were then transferred outside the anaerobic chamber, and purged with ambient air to finally quench reactions. Control samples containing the same amounts of dosed analytes, solvent and agents except for CCA (CCA-lacking assay) were also performed. Supernatants were obtained by centrifugation at 6600g for 10 min, which were diluted both 2- and 2000-fold in methanol. One milliliter of each diluted supernatant was transferred to LC vials for instrumental analysis, with 20 ng of isotope-labeled standard mixture (M3PFHxS, MPFOS, M8PFOS, and M2−6:2 FTSA, 2 μg/mL in methanol for each standard) added in order to monitor matrix effects.

2.3. Sampling and Pretreatment Procedures. A total of 60 river water and sediment samples were collected in two separate sampling regions (Fenghuajiang River in Zhejiang Province, and Pan River in Shandong Province) in May 2016, in order to verify whether potential transformation products found in the super-reduced CCA assay could be identified in real environment. Sampling sites were located upstream and downstream of electroplating factories. River water samples were collected ~0.4 m below the water surface using 1 L polypropylene bottles, which were precleaned by methanol and ultrapure water. Sediment samples with a depth of 3−5 cm were gathered using a grab sampler (Wildco Ekman Grab, Buffalo, NY), packed in aluminum foil and stored in polypropylene zip bags. All samples were preserved at zero Celsius placing on ice in the field and transported back to laboratory immediately. Water samples were kept at 4 °C, and sediment samples were freeze-dried, homogenized and stored at −20 °C until analysis.

River water samples were pretreated by solid phase extraction (SPE) according to literature. In brief, 200 mL of water was filtered through a glass microfiber filter (0.7 μm, Whatman Inc., Pittsburgh, PA), and the filter was washed by 5 mL of methanol to prevent possible analyte loss. The combined sample was spiked with 1 ng of each surrogate standard including M3PFHxS, MPFOA, MPFUdA, MPFDoA, MPFHxS, MPFOS, M2−6:2 FTSA. It was then loaded onto a HLB cartridge (Waters Inc., Milford, MA) at a flow rate of 5−6 mL/min, which was preconditioned by 4 mL of methanol and 4 mL of ultrapure water. The cartridge was then dried under vacuum and eluted by 3 × 2 mL methanol, and the eluent was concentrated to a final volume of 200 μL by a gentle nitrogen flow. For sediment samples, an alkaline extraction method was used with minor modifications. Approximately 1.0 g of sediment was included in a 15 mL polypropylene centrifugation tube, spiked with 1 ng of each surrogate standard, and soaked in 1 mL of 100 mM NaOH in methanol/H2O (v/v, 4:1) for 1 h. Three milliliters of methanol was then added into the tube,
which was ultrasonic extracted (600 W at room temperature) for 30 min and shaken at 250 rotations per minute for another 1 h. Supernatant was collected after centrifugation at 1500 g for 10 min, and the extraction process was repeated. All supernatants were combined, concentrated to ~2 mL, and diluted with 100 mL of ultrapure water. The same SPE procedure as described for the river water samples was then additionally applied to enrich potential fluorinated chemicals. All samples were spiked with 1 ng of each isotope-labeled injection standard (M5PFHxS, M6PFDA, M3PFHxS, and M8PFOS) before quantification analysis.

2.4. Qualitative and Quantitative Analysis. An ultrahigh performance liquid chromatograph-Orbitrap Fusion mass spectrometer system (Thermo Fisher Scientific Inc., Waltham, MA) was operated in negative electrospray ionization mode, which was applied for both qualitative and quantitative purposes. An ACQUITY C18 column (Waters, 1.7 μm, 2.1 × 100 mm) was used for analyte separation. Column temperature was 35 °C, and flow rate was set as 0.3 mL/min. The flow gradient initiated at a composition of 25:75 (methanol/water, v/v, 1 mM NH₄Ac additive in each phase), held for 1 min, linearly switched to 80% methanol in 5 min, and finally reached 100% methanol in another 3 min. Full-scan mass spectrum (MS³, scan range of 150~1500 m/z) was obtained with a full width at half-maximum (fwhm, at m/z = 200) resolution of 120000 for accurate mass measurement and retrospective quantification of identified PFAS chemicals. Information of both precursor ions and characteristic daughter ions was generated in high resolution MS fragmentation (MS³) mode for chemical structure elucidation. MS³ precursors selected by quadrupole with an isolation window of 1 m/z were transferred to the ion routing multipole (IRM) for MS³ fragmentation at higher-energy collisional dissociation (HCD) energy of 20−60%. For MS³ fragmentation, the ions in IRM were transported to the ion trap, filtered at an isolation window of 2 m/z, and transferred back for fragmentation at HCD energy of 30% with an resolution of 60,000 (fwhm at m/z = 200). HRMS instrumental performance was checked every week by using Pierce ion calibration solution kit (Thermo Fisher Scientific Inc., Waltham, MA) to ensure accurate mass precision of reference materials in the range of 2 ppm.

For identification of potential Cl-6:2 PFESA transformation products in reductive environment, a nontarget screening workflow was established (SI Figure S3). Full-scan MS³ spectrum of 10 samples during the first 10−100s incubation time in super-reduced CCA assay were used as the data set of metabolite-transformation group, and those of another 10 samples from the CCA-lacking assay at the same 10−100s incubation time were selected as the data set of dosed-control group in order to maximum eliminate irrelevant information in the LC vials. All raw data was introduced in the XCMS processing package for peak detection, with further feature filtering and statistical ranking protocols performed mainly constituted of the 80% Rule. Kendrick mass defect, intensity threshold, and orthogonal partial least-squares-discriminant analysis. Chemical structure confirmation was based on deprotonated monoisotopic mass (MIM), retention time (RT), isotope distribution, and MS⁵ fragmentation ion analysis. The identified PFAS compounds were additionally searched in the river water and sediment samples by a suspect screening procedure (SI Table S3). Occurrence of novel fluorinated contaminants was verified by comparison of MIM, RT, and MS⁵ with laboratory-purified standards. More detailed information on data analysis was summarized in the SI.

2.5. Quality Assurance and Quality Control (QA/QC). For evaluation of instrumental performance in the super-reduced CCA assay, one blank injection (pure methanol) was included in each batch of two 2-fold diluted samples as well as in each batch of five 2000-fold diluted samples to monitor residue levels of Cl-6:2 PFESA and metabolites in LC-MS instrumental system. Few Cl-6:2 PFESA was found, with no metabolites observed. No subtraction was thus made for analyte quantification because maximum Cl-6:2 PFESA residue was <0.14% of the amount dosed in each sample. Quantified results of isotope-labeled injection standards based on external calibration curves were 98%, 97%, 94%, and 97% for M3PFHxS, MPFOS, M8PFOS, and M2−6:2 FTSA, suggesting negligible matrix effect in the super-reduced CCA assay. Quality control samples (QCs, n = 10) were acquired by mixing a certain amount (200 μL) of supernatant from each sample after transformation process, which were 2-fold diluted by methanol and injected in every batch of five injections. Significant correlations of instrumental responses (IRs, R = 0.914−0.964, p < 0.05) were observed for all the 3613 features coexisted in the QCs, suggesting sufficient performance of replication in the qualitative analysis procedure.

For quantification of PFAS analytes in the environmental samples, two procedure blanks of 200 mL of ultrapure water or 1.0 g of methanolic prewashed diatomaceous earth (Dionex, Sunnyvale, CA) were added in each batch of eight river water and sediment samples, respectively. Most of the target analytes were not detectable except for trace amounts (~0.15 pg on column) of Cl-6:2 PFESA, which contributed to <5% of averaged concentrations in the same batch of samples. Instrumental drift in sensitivity was verified daily by continuous injection of 0.01−1.0 ng/mL (n = 7, R = 0.989−0.999, p < 0.05) and 1.0−50 ng/mL (n = 6, R = 0.991−0.999, p < 0.05) methanolic standard solution. Method quantification limits (MQLs, SI Table S5) calculated by a signal/noise ratio of ten ranged from 14 pg/L (1H-8:2 PFESA) to 218 pg/L (PFHxS) in river water and 9 pg/g (1H-8:2 PFESA) to 37 pg/g (PFOS) in sediment samples, respectively. Average recoveries of isotope-labeled surrogate standards (n = 30, 1 ng for each standard, SI Table S7) were 71% (MPPFxHA) to 108% (MPFOS) in river water and 70% (MPFUDA) to 86% (M2−6:2 FTSA) in sediment samples, individually, indicating internal calibration quantification was applicable for the target analytes. Minor ionization suppression was observed, as matrix interferences of the isotope-labeled injection standards (n = 30, spiked at 1 ng for each standard, SI Table S6) were in the range of 86% (M6PFDA) to 95% (M8PFOS) in river water and 84% (M6PFDA) to 90% (M5PFHxS) in sediment samples. No blank contamination subtraction nor matrix effect correction was made for all measured analyte concentrations.

2.6. Statistical Analysis. Feature identification including data deconvolution, peak detection, retention alignment, and scaling was performed by the XCMS software. All processed information (e.g., accurate mass, intensity, retention behavior of each feature) was then aligned together in an Excel file (SI Table S1). Orthogonal partial least-squares-discriminant analysis (OPLS-DA, SIMCA-P 13.0, Umetrics, Umeå, Sweden) was carried out to rank all features with respect to variations in transformation process. Xcalibur software (Thermo Scientific) was used for both qualitative and quantitative analysis. Correlations of IRs among coexisted features in QC samples, DOI: 10.1021/acs.est.7b02961

and correlations in concentrations between quantified PFAS analogues in river water samples were assessed by Spearman’s test using SPSS V17.0 for Windows Release (SPSS Inc. 2009). Significant level was set as p < 0.05 unless otherwise mentioned. Geometric mean, concentration range, detection frequency and average composition were used to describe the quantification results of target analytes in river water and sediment samples.

3. RESULTS AND DISCUSSION

3.1. Changes of Chemical Profiles in the in Vitro Assay. Proper case-control sampling could be an effective data-mining strategy, which was applied to emphasize differences in chemical profiles between experimental and control samples in investigated conditions. A total of 3389 features were recognized in all raw data of both metabolite-transformation and dosed-control groups. Twenty-six features were finally discerned after filtering and ranking protocols, which illustrated Cl-6:2 PFESA and potential metabolite candidates in elimination (DOWN) and generation (UP) trends with high constituent abundances, respectively.

Among the features shown in SI Table S1, accurate MIM = 530.8949 was present with a mass error of Δm = −1.28 ppm compared with exact mass of Cl-6:2 PFESA ([M(35Cl)-H]−, MIM = 530.8956). It could be easily recognized as the dosed compound into the in vitro assay, as reproducible LC retention behaviors (RT = 7.85 min, SI Figure S7) were observed in sample supernatant and the Cl-6:2 PFESA standard. MS² fragmentation pattern in HCD mode was further employed for analyte structure confirmation. Characteristic daughter ion [CIC6F12O]− (MIM = 350.9451, mass error: −0.15 ppm) in SI Figure S6-A clearly showed cleavage of ether bond, and formation of [FSO3]− (MIM = 98.9555, mass error: −2.69 ppm) and [FSO2]− (MIM = 82.9606, mass error: −3.03 ppm) also verified existence of sulfonate functional group in the molecular structure. The 2.2 folds of average concentration in DOWN trend in metabolite-transformation group revealed Cl-6:2 PFESA elimination in the transformation process.

The same identification protocol (SI Figure S3) was applied for screening of other important features. The feature with the most abundant averaged IRs and isotopic peak clusters was noticed (MIM = 496.9338, RT = 7.19 min). Fragmentation pattern of this feature was quite similar to that of Cl-6:2 PFESA (SI Figure S6-B). For instance, occurrence of [FSO3]− and [FSO2]− indicated the presence of sulfonate group in the molecular structure, and MIM = 198.9492 ([CIC6F12O]−, mass error: −0.90 ppm) and MIM = 316.9841 ([CIC6F12O]−, mass error: −0.08 ppm) were a pair of daughter ions generated by ether bond cleavage. Interestingly, characteristic [CIC6F12O]− (MIM = 296.9779, mass error: 0.01 ppm) ion found both in this MS³ spectrum and in MS² spectrum of [CIC6F12O]− (SI Figure S9-A) suggested a neutral loss of HF in collision process, which was further unzipped by dropping an additional CF2O carbon skeleton to form [CIC6F10]− (MIM = 230.9861, mass error: −0.33 ppm). Thus, it could be identified as one hydrogen-substituted metabolite (1H-6:2 PFESA). A minor mass error of Δm = −1.51 ppm was observed between the measured MIM and exact mass of the deprotonated [CIC6F10SO4]− ion, and a 1.4-fold higher of averaged IRs in UP trend in the metabolite-transformation group still showed its generation in the reductive assay. This chemical structure was further confirmed (Figure 1) by laboratory purified 1H-6:2 PFESA as described in the SI, which was supported by 1H and 19F nuclear magnetic resonance spectrum. Therefore, a confidence level (CL) of Level 1 could be reached for both Cl-6:2 PFESA and 1H-6:2 PFESA according to structure identification communicating criteria proposed by Schymanski et al.31

It is interesting to find that several deprotonated ions with molecular mass >1000 Da were present in the recognized features (SI Table S1). The LC retention behaviors (RT = 7.17 min) and MS² fragmentation patterns (SI Figure S9-B and C) were almost the same with that of 1H-6:2 PFESA. Formation of noncovalent homodimers has been reported for a number of PFASs such as PFCAs, perfluorinated alkyl phosphates and perfluoroalkyl ether carboxylates, due to facility of per- and polyfluorinated surfactants to aggregate in gas phase in the MS source. 8,32 Therefore, these deprotonated ions could be assigned to isotopic clusters of [2M-2H+NH4]+ and [2M-2H+Na]+ (SI Table S2, M representing 1H-6:2 and Cl-6:2 PFESA) adducts, with mass errors in the range of −0.39 to −1.39 ppm between measured MIMs and exact masses. [2M-H]+ adducts also appeared in the full-scan MS³ spectrum with significant contents, that is, IRs > 10². These adducts are indicators of parent ions but not irrelevant interferent formed by in-source collision in MS instrument, as reaction probability of solvent (refer to NH₄Ac additive in this study) and metal adducts was discussed to be low. 32

Other transformation products, including an unsaturated transit intermediate (1H-6:2 PFUESA, CL: Level 3) and a 2H-substituted metabolite (2H-6:2 PFESA, CL: Level 2b), and possible standard impurities (Cl-5:2 PFESA and 1H-5:2 PFESA, CL: Level 2b) were also found in the reductive assay, with detailed information provided in SI Table S1. Thus, a mass balance analysis of dosed Cl-6:2 PFESA and proposed
transformation products during the whole 10–240s incubation time was used to assess the reductive transformation process. Except for Cl-6:2 and 1H-6:2 PFESA with laboratory purified standards, instrumental responses of other proposed transformation products were assumed equal to that of an equimolar amount of 1H-6:2 PFESA. A rapid transformation of dosed Cl-6:2 PFESA in the super-reduced CCA assay was surprisingly observed in all samples, including Cl-5:2 and Cl-7:2 PFESA, 1H-5:2, and 1H-7:2 PFESA, 2H-6:2 PFESA and 1H-6:2 PFUESA. Figure 4 showed spatial distribution of Cl-6:2 and 1H-6:2 PFESA from Fenghuajiang River, Zhejiang Province. 1H-6:2 PFESA was found in most of the river water samples (detection frequency: 88%) with concentrations in a range of not detected to 3.31 × 10^3 pg/L (geometric mean: 799 pg/L). A decreasing trend of spatial concentrations for 1H-6:2 PFESA was observed, which was quite similar to that of Cl-6:2 PFESA. Significant correlation in concentrations between Cl-6:2 PFESA and 1H-6:2 PFESA (SI Table S8, R = 0.914, p < 0.01) was also found, which might indicate potential impact of metal plating activities in this area. Low residue levels of 1H-6:2 PFESA and 1H-8:2 PFESA were also quantified in sediment samples with concentrations in the range of not detected to 503 pg/g dry weight (d.w.; detection frequency: 18%) and not detected to 165 pg/g d.w. (detection frequency: 6%), respectively. Apart from the 1H-PFESAs, residue levels of Cl-6:2 and Cl-8:2 PFESA, 4:2, 6:2 and 8:2 FTSAs, C₆⁻C₁₀ PFCA and C₆⁻C₁₀ PFAS were monitored as well. Perfluorooctanoate (PFOA) was the most predominant PFAS contaminant (GM: 6.36 × 10^3 pg/L), which constituted 40–52% (mean: 46%, n = 17) of the total quantified PFAS concentrations (∑PFASs). It was followed by perfluorohexanesulfonate (PFHxS) and Cl-6:2 PFESA (GM: 3.05 × 10^4 pg/L and 1.93 × 10^3 pg/L, respectively) with mean proportions of 24% and 14% in ∑PFASs, respectively. Our results were in accordance with previous study by Lu et al. [34] that PFOA was also the most significant PFAS pollutant in Fenghuajiang River with a concentration of 53 ng/L. Among all the identified PFAS compounds, 1H-6:2 PFESA accounted for 0–1% (mean: 0.8%, n = 17) in the total quantified PFAS concentrations.

Different composition profiles of PFAS analogues were observed in sediment samples, where 1H-6:2 PFESA and 1H-8:2 PFESA constituted 0–8% (mean: 1%, n = 17) and 0–4% (mean: 0.3%, n = 17) in ∑PFASs, respectively. The most abundant PFAS pollutant was Cl-6:2 PFESA (GM: 1.77 × 10^3 pg/g d.w.), which comprised proportions ranged from 8% to 77% (mean: 52%, n = 17) in ∑PFASs. The other dominant PFAS analogues were Cl-8:2 PFESA (GM: 379 pg/g d.w.) and PFOA (GM: 177 pg/g d.w.), with a mean proportion of 16% and 13% in ∑PFASs, separately.

Significant abundances of the hydrogen-substituted analogues were also found in Pan River in Shandong Province (SI Figure S2). 1H-6:2 PFESA was one of the abundant fluoroalkyl substances in river water, with concentrations in the range of 97.3 pg/L to 1.25 × 10^3 pg/L (GM: 352 pg/L). Other analogues with comparable residue levels included PFOA (GM: 5.87 × 10^3 pg/L), PFOS (GM: 3.85 × 10^3 pg/L), PFHxS (GM: 1.52 × 10^3 pg/L), Cl-6:2 PFESA (GM: 1.64 × 10^3 pg/L), 6:2 FTSAs (GM: 807 pg/L) and Cl-8:2 PFESA (GM: 33.3 pg/L). Similar composition was observed in sediment samples that the geometric mean concentration of 1H-6:2 PFESA was 10.0 pg/g, compared with those of PFOS (GM: 254 pg/g), Cl-6:2 PFESA (GM: 149 pg/g), PFOA (GM: 55.3 pg/g), and Cl-8:2 PFESA (GM: 37.2 pg/g), individually. The proportion of 1H-6:2 PFESA were 0–7% (mean: 2%, n = 26) of total quantified C₆⁻C₁₀ fluoroalkyl sulfonates and carboxylates in the river water and sediment samples, and that of 1H-8:2 PFESA were 0–2% (mean: 0.3%, n = 26), separately.

Origins of the hydrogen-substituted analogues could partly be elucidated by comparison of 1H-6:2 PFESA and Cl-6:2 PFESA residue levels in the commercial F-53B mist suppressant.
product and in the investigated environmental compartments. Existence of all possible PFESA analogues (SI Table S3) was first screened in the F-53B product. Besides Cl-6:2 PFESA, Cl-8:2 PFESA (<0.05%), Cl-10:2 PFESA (<0.05%), Cl-7:2 PFESA (<0.05%), Cl-5:2 PFESA (1%), and 1H-8:2 PFESA (0.4%) were found, but none of 2H-PFESA nor 1H-PFUESA compound was detectable. Different mass ratios of 1H-6:2 PFESA to Cl-6:2 PFESA were measured in river water and sediment samples from Fenghuajiang River, which were in a range of 4−11% (mean: 6%, n = 15) and 2−18% (mean: 6%, n = 6), respectively. Similar abundances were also observed in Pan River that the mass ratios were in a range of 9−111% (mean: 27%, n = 13) and 2−18% (mean: 6%, n = 6) in river water and sediment samples, separately, which were relatively higher than that in F-53B commercial product (mean: 1%). It implied additional influencing factors such as reductive dechlorination process and/or transport behaviors might be responsible for 1H-6:2 PFESA contamination in the environment, besides pollution caused by plausible emission from industry usage.

4. ENVIRONMENTAL IMPLICATION

This manuscript described the identification of major transformation products (i.e., 1H-6:2 PFESA, 2H-6:2 PFESA, and 1H-6:2 PFUESA) of Cl-6:2 PFESA in the super-reduced CCA assay. Hydrogen-substituted PFESA analogue (i.e., 1H-6:2 PFESA and 1H-8:2 PFESA) were further observed in water and sediment samples for the first time. The results indicated that dechlorination of CI-PFESAs might be an indirect source of H-PFESAs. Moreover, the established nontarget screening workflow would provide an effective tool for recognition of emerging fluorinated transformation products in the environment.

Chlorine- and hydrogen-substituted polyfluoroalkyl substances were continuously discovered in water,35 fish,36,37 and human sera18 recently, which have distinct molecular functional groups compared with legacy PFCAs or PFSAs. The hydrogen might have an influence on the behaviors of 1H-PFESAs in the environment. For instance, the substitute of hydrogen makes the molecule more hydrophilic than Cl-PFESAs and PFSAs with the same carbon-chain length, because weaker retention on reversed-phase C18 HPLC column was observed (SI Figure S15, RT = 7.87 and 7.20 for Cl-6:2 PFESA and 1H-6:2 PFESA, respectively). Less hydrophobic PFASs were generally easier to transport in aquatic environment.38 The presence of hydrogen in the fluorinated carbon backbone may provide potential sites for microbial degradation. Fluorine removal with the aid of hydrogen attached to the adjacent carbon is a vital step for telomerized fluorochemicals, especially fluorotelomer alcohols and X:3 fluorotelomer carboxylates.35,59 While, defluorination process for perfluoroalkyl sulfonates are difficult, which often product and in the investigated environmental compartments.
requires extreme physical-chemical conditions.\textsuperscript{21} Biodegradability and atmospheric degradation of ether-containing functional groups in perfluoropolyethers (such as ADONA and GenX) were not found based on currently incomplete information.\textsuperscript{40,41}

A quick transformation of Cl-6:2 PFESA described in this manuscript was observed in an anoxic reductive environment, which might not be contradictory to its persistence reported in literature.\textsuperscript{10,13,15} The slow transformation of Cl-6:2 PFESA in the aerobic closed bottle test did not meet readily degradability criteria according to OECD 301D, and only minor loses (<10\%) under various abiotic oxidation conditions might suggest its stability in photodegradation and Fenton reactions in the environment.\textsuperscript{10} Estimated human total elimination half-lives in the range of 10.1–56.4 years in fishery employees\textsuperscript{15} and a median log BAF\textsubscript{(whole body)} factor value of 4.322 found in crucian carp\textsuperscript{75} further indicated strong biopersistence and bioaccumulation propensity of this eight-carbon polyfluoroether sulfonate. Thus, more detailed investigation is warranted to reveal impacts of chlorine and hydrogen atoms on the fate of novel polyfluorinated substances in diverse environmentally relevant conditions.

\section*{ASSOCIATED CONTENT}

\begin{itemize}
\item Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02961.
\end{itemize}

Preparation of CCA assay; nontarget screening workflow; laboratory purification of 1H-6:2 and 1H-8:2 PFESA standards; and spike recovery experiments in water and sediment samples; (Table S1) features with VIP > 1 generated in the nontarget screening workflow; (Table S2) homodimers of Cl-6:2 and 1H-6:2 PFESA; (Table S3) detailed information on PFAs in suspect screening procedure; (Table S4) instrumental parameters on chromatographic separation and mass spectrometry; (Table S5) MQLs of PFAS analytes in water and sediment samples; (Table S6 and S7) recoveries of PFAS analytes and surrogate standards in water and sediment samples; (Table S8 and S9) descriptive statistics and correlation analysis of quantified PFAS concentrations; (Figure S1) methanol dilution effectively retarded transformation; (Figure S2) sampling map in Pan River; (Figure S3) nontarget screening workflow; (Figure S4 and S5) PCA and OPLS-DA analysis; (Figure S6–S9) total ion chromatography and MS\textsuperscript{n} spectrum of Cl-6:2 PFESA, transformation products and homodimers; (Figure S10) tentative molecular structures of 2H-PFESA and 1H-6:2 PFUESA; (Figure S11–S13) LC separation and structure characterization of 1H-6:2 and 1H-8:2 PFESA laboratory-purified standards; (Figure S14) extraction efficiency in sediment samples; (Figure S15) retention behaviors of linear-PFOS, Cl-6:2 and 1H-6:2 PFESA on reversed C18 column (PDF)
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REFERENCES


