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Methodology for studying biotransformation of polyfluoroalkyl precursors in the environment

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ABSTRACT

Biotransformation of polyfluoroalkyl precursors contributes in part to the perfluoroalkyl carboxylates and sulfonates detected in the global environment and biota. Robust sample preparation and sensitive analytical techniques for maximum analyte recovery are essential to identify and to quantify biotransformation products often present at low levels in environmental matrices and experimental systems. This critical review covers current sample-preparation and analytical methods, including extraction, concentration, clean-up and derivatization, mass spectrometry coupled to gas or liquid chromatography, and radioisotope labeling and tracking techniques. We also critically review methodologies for molecular structural elucidation and *in-silico* prediction of potential transformation products. We describe current knowledge gaps and challenges in studying novel alternative polyfluoroalkyl substances. We discuss future trends on utilizing advanced analytical techniques.

Contents

Abbreviations: APPI, Atmospheric pressure photoionization; ARC, Accurate radioisotopic counting; DNPH, 2,4-Dinitrophenylhydrazine; DSPE, Dispersive solid-phase extraction; ECD, Electron-capture detector; ECF, Electrochemical fluorination; EI, Electron impact; ESI, Electrospray ionization; FT, Fluorotelomer; GC-MS, Gas chromatography mass spectrometry; HRMS, High-resolution mass spectrometry; IDL, Instrument detection limit; IPE, Ion-pair extraction; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LSC, Liquid-scintillation counting; MRM, Multiple-reaction monitoring; MTBE, Methyl *tert*-butyl ether; NCI, Negative chemical ionization; PCI, Positive chemical ionization; PFASs, Per- and poly-fluoroalkyl substances; PFCA, Perfluoroalkyl carboxylate; PFP, Pentafluorophenyl; PFSA, Perfluoroalkyl sulfonate; QqQ, Triple-quadupole mass spectrometry; QSAR, Quantitative structure-activity relationship; Q-TOF, Quadrupole-time of flight; TBAS, Tetrabutyl ammonium hydrogen sulfate; WMSE, Water-miscible solvent extraction; WWTP, Wastewater-treatment plant.

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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals that have been used in the manufacture of additives and polymers for the past 60 years [1]. Due to their chemical inertness and surfactant properties, PFASs are used in household and industry applications, including surfactants, lubricants, paints, pesticides and coatings [2,3]. The broad use of PFASs has resulted in the wide distribution of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs) in environmental matrices and biological species, including humans [4–6]. The behavior of PFCAs and PFSAs, such as persistency, long-range transport propensity and potential toxicity, has attracted considerable attention to their possible adverse effects in the environment and biota [7,8].

The origins of PFASs entering into the environment are complex. Wang et al. [3] calculated the mass flux of PFCAs in the global transport processes and suggested that historical direct emissions from manufacture and consumer usages were the major contributors of PFCAs detected in the environment. However, the indirect contributions via the biodegradation and biotransformation of polyfluoroalkyl precursors and atmospheric oxidation have also been confirmed by model prediction [9], laboratory experiments [10,11] and environmental monitoring [12]. Laboratory systems utilizing microbial-consortium and animal models to study polyfluoroalkyl-precursor biotransformation provided insight to understand the extent of contributions of such precursors to PFCAs and PFSAs detected in the environment and biota.

Table 1 presents PFCA and PFSA precursors studied so far from telomerization, manufacturing and electrochemical fluorination (ECF) processes [1]. The potential precursors from telomerization include fluorotelomer alcohols (FTOHs), iodides (FTIs), olefins (FTOs), sulfonates (FTSAs), stearate monoesters (FTSs), citrate triester (TBC), polyfluoroalkyl phosphates (PAPs), polyethoxylated 2-perfluoroaklyl ethanols (FTEOs), and FTOH-based acrylate and urethane-based polymers. The potential precursors from ECF include mixtures of linear and branched isomers of perfluorooctane sulfonamide (FOSA), sulfonamido ethanol (FOSE), FOSE-based phosphate diester (SAmPAP diester) and sulfonamide-based acrylate polymers.

The design of biotransformation studies is associated with the occurrence and the partitioning of the above precursors in different environmental compartments. Both fluorotelomer-based (FT) precursors (e.g., FTOHs and FTIs) and perfluoroalkane sulfonamido derivatives, including N-ethyl perfluorooctane sulfonamide (N-EtFOSA) and N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE), were found in ambient air particles [13–15]. Fluorotelomer alcohols, monosubstituted and disubstituted PAPs, and perfluorooctane sulfonamidoacetic acid (FOSAA) analogues have frequently been detected in wastewater-treatment plant (WWTP) effluent, activated sludge and marine sediments [15–17]. Elevated PFAS concentrations found in effluents compared with those in influents may further indicate contributions of polyfluorinated-precursor biodegradation in the WWTPs [18]. Covalent binding of certain intermediate transformation products (e.g., fluorotelomer unsaturated aldehydes or carboxylic acids) to nucleophilic amino acids and proteins, and formation of conjugated Phase II metabolites are also possible biological fates of polyfluoroalkyl precursors in mammals and fish [19–21].

Biotransformation processes of polyfluoroalkyl precursors in microbial and animal models generally initiate from the hydrocarbon part of the molecular structures, due to the high carbon-fluorine bond energy (450 KJ/mol) [22]. For example, biotransformation of fluorotelomer-based substances often involves cleavage of functional groups (e.g., ester, ether, urethane) to form FTOHs, which are then further converted to FT aldehyde (FTAL), FT unsaturated carboxylic acids (FTUCAs), FT ketone and FT secondary alcohol (sFTOH), and x:3 fluorotelomer acids ($F(CF_2)_nCH_2CH_2COOH$, $n = 3–7$) [23,24]. Sediment/soil-bound residues formed from precursors and related transformation products with highly absorptive properties (e.g., FTOHs and x:3 acids) are not quantifiable without optimized sample-extraction methods and proper analytical procedures [25,26]. Furthermore, sophisticated analytical instrumentation, such as high-resolution mass spectrometry (HRMS), is often needed to identify low levels of potential novel transformation products.

The aim of this review is to provide information from peer-reviewed literature on comprehensive sample preparation and effective analytical methods for quantitative recovery and identification of transient intermediates and terminal polyfluorinated acids. We also discuss knowledge gaps and future trends in the analysis of novel alternative polyfluoroalkyl substances.

2. Sample-preparation strategy

2.1. Test system

A well-established test system should take into account the physical and chemical properties of the target polyfluoroalkyl precursors, and biotransformation studies should simulate close to what happens in different environmental matrices. Biotransformation of PFASs involves the defluorination process, which is usually a rate-limiting reaction, often leading to incomplete mineralization of polyfluoroalkyl precursors. Thus, measurements of the endpoints, such as biochemical oxygen demand and CO_2 production, in standardized ready and inherent biodegradability tests are not suitable for studying the biotransformation of polyfluoroalkyl precursors [24]. Instead, rigorous sample preparation and instrumental analysis methodologies in simulation tests were used to recover, to identify and to quantify specific polyfluoroalkyl precursors and transformation products to achieve understanding of biodegradation kinetics and pathways.

Generally, an aerobic test system consists of semi-static closed bottles, containing both headspace and liquid/solid phases. Aeration is achieved by pumping ambient air into the headspace when the oxygen content is below 10%. One or two C_{18} SPE cartridges were inserted into the headspace as the conduit and to capture volatile parent and potential volatile transformation products [25,27]. To mimic continuous exchange of PFASs in surface soil or active sludge with surrounding air, flow-through systems using purge-and-trap methods were used to study 6:2 FTOH and 6:2 polyfluoroalkyl-phosphate biotransformation [26,28]. Wang et al. [29] found that the molar yield of perfluorooctanoic acid (PFOA) in the flow-through system was only half of that in semi-static closed bottles. The lower yield was due to the partitioning of ^{14}C-labeled 8:2 FTOH [$F(CF_2)_7{}^{14}CF_2CH_2CH_2OH$] and 7:2 sFTOH [$F(CF_2)_7{}^{14}CH(OH)CH_3$], a direct PFOA precursor, from soil to the headspace in the flow-through system, significantly reducing their availability for degradation to PFOA.

To avoid residual contamination and to reduce potential surface adsorption of the precursor and transformation products, test vessels and caps should be made of non-fluorinated materials. Polyfluoroalkyl substances in the narrow-necked glass bottles sealed with butyl-rubber stoppers and C_{18} cartridges are extractable and can be quantitatively recovered using a conventional solvent-extraction method (see sub-section 2.2) to reduce adsorption losses on the laboratory ware.

Various kinds of environmental matrices and animal models were chosen as the investigating matrices, depending on the presence and the behavior of polyfluoroalkyl precursors in the real environment. Soil is the primary deposit medium of volatile precursors through atmospheric deposition, while sewage sludge and river sediment are important sinks of PFASs in aqueous systems from household and industry emissions [15–17]. To obtain knowledge on the specific microorganisms responsible for the polyfluoroalkyl precursor biotransformation processes, microbial consortium or pure bacterial cultures from soil and sewage sludge were isolated and

Table 1
Acronyms, chemical names, and molecular structures of polyfluoroalkyl precursors, potential major or stable biotransformation products, and polyfluoroalkyl alternatives with novel functional groups

Acronym	Chemical name	Molecular structure
Polyfluoroalkyl precursors reported in literature		
X:2 FTOHs	Fluorotelomer alcohols	$F(CF_2)_xCH_2CH_2OH$
X:2 FTIs	Fluorotelomer iodides	$F(CF_2)_xCH_2CH_2I$
X:2 FTOs	Fluorotelomer olefins	$F(CF_2)_xCH{=}CH_2$
X:2 FTSAs	Fluorotelomer sulfonates	$F(CF_2)_xCH_2CH_2SO_3H$
X:2 FTSs	Fluorotelomer stearate monoesters	$F(CF_2)_xCH_2CH_2OOCC_{17}H_{35}$
X:2 TBCs	Fluorotelomer citrate triester	$F(CF_2)_xCH_2CH_2OOCC(OH)[CH_2COO\ CH_2CH_2(CF_2)_xF]_2$
X:2 mono-PAPs	Polyfluoroalkyl phosphoric monoester	$[F(CF_2)_xCH_2CH_2O]P(=O)(OH)_2$
X:2 di-PAPs	Polyfluoroalkyl phosphoric diester	$[F(CF_2)_xCH_2CH_2O]_2P(=O)(OH)$
FTEO	Fluorotelomer ethoxylate	$F(CF_2CF_2)_x(CH_2CH_2O)_yH$
X:2 FTAcs	Fluorotelomer acrylates	$F(CF_2)_xCH_2CH_2OC(O)CH{=}CH_2$
N-FOSAs	N-Alkyl perfluorooctane sulfonamides	$F(CF_2)_8SO_2NH(C_mH_{2m+1})$
N-FOSEs	Perfluooctane sulfonamidoethanols	$F(CF_2)_8SO_2N(C_mH_{2m+1})CH_2CH_2OH$
SAmPAP diester	Perfluooctane sulfonamidoethanol-based phosphate	$[F(CF_2)_8SO_2N(C_2H_5)CH_2CH_2O]_2P(=O)(OH)$
Major or stable biotransformation products		
X:2 FTAL	Fluorotelomer aldehyde	$F(CF_2)_xCH_2CHO$
X:2 FTCA	X:2 Fluorotelomer saturated carboxylic acids	$F(CF_2)_xCH_2COOH$
X:2 FTUCA	Fluorotelomer unsaturated carboxylic acids	$F(CF_2)_{x-1}CF{=}CHCOOH$
X:2 FT Ketone	Fluorotelomer ketone	$F(CF_2)_xC(O)CH_3$
5:3 ketone aldehyde	5:3 Fluorotelomer ketone aldehyde	$F(CF_2)_5C(O)CH_2CHO$
X:2 sFTOH	Polyfluorinated secondary alcohol	$F(CF_2)_xCH(OH)CH_3$
X:3 acid	X:3 Fluorotelomer saturated carboxylic acids	$F(CF_2)_xCH_2CH_2COOH$
3-Fluoro 5:3 acid	3-Fluoro 5:3 FT saturated carboxylic acid	$F(CF_2)_5CFHCH_2COOH$
PFCAs	Perfluoroalkyl carboxylic acids	$F(CF_2)_xCOOH$
PFOA	Perfluorooctanoic acid	$F(CF_2)_7COOH$
X:2 FTOH-Gluc	FTOH glucuronide conjugate	$F(CF_2)_xCH_2CH_2O$-Gluc
X:2 FTOH-Sulf	FTOH sulfate conjugate	$F(CF_2)_xCH_2CH_2OSO_3^-$
X:2 uFTOH-GS	Unsaturated FTOH glutathione conjugate	$F(CF_2)_{x-1}C(SG) = CHCH_2OH$
6:2 FTUI	6:2 unsaturated FT iodide	$F(CF_2)_6CH = CH_2I$
8:1 FT olefin	8:1 fluorotelomer olefin	$F(CF_2)_7CF = CH_2$
N-EtFOSE	N-ethyl perfluorooctane sulfonamidoethanol	$F(CF_2)_8SO_2N(C_2H_5)CH_2CH_2OH$
N-EtFOSE aldehyde	N-ethyl perfluorooctane sulfonamidoethanol aldehyde	$F(CF_2)_8SO_2N(C_2H_5)CH_2CHO$
N-EtFOSAA	N-ethyl-perfluorooctane sulfonamide acetic acid	$F(CF_2)_8SO_2N(C_2H_5)CH_2COOH$
N-EtFOSA	N-ethyl-perfluorooctane sulfonamide	$F(CF_2)_8SO_2NC_2H_5$
EtFOSA alcohol	Perfluorooctane sulfonamido alcohol	$F(CF_2)_8SO_2NHCH_2CH_2OH$
EtFOSA aldehyde	Perfluorooctane sulfonamido aldehyde	$F(CF_2)_8SO_2NHCH_2CHO$
FOSAA	Perfluorooctane sulfonamido acetic acid	$F(CF_2)_8SO_2NHCH_2COOH$
FOSA	Perfluorooctane sulfonamide	$F(CF_2)_8SO_2NH_2$
PFOSI	Perfluorooctane sulfinic acid	$F(CF_2)_8SO_2H$
PFSAs	Perfluoroalkyl sulfonic acids	$F(CF_2)_xSO_3H$
PFOS	Perfluorooctane sulfonic acid	$F(CF_2)_8SO_3H$
Polyfluoroalkyl alternatives with novel functional groups		
Zwitterionic, cationic and anionic fluoroalkyl aqueous film-forming foams:		
X:2 FtTAoS	Fluorotelomer thioamido sulfonates	$F(CF_2)_xCH_2CH_2SCH_2CH_2CONHCC(CH_3)_2\ CH_2SO_3^-$
X:2 FtTHN$^+$	Fluorotelomer thiohydroxy ammonium	$F(CF_2)_xCH_2CH_2SCH_2CH(OH)CH_2N(CH_3)_3^+$
X:2 FtSaB	Fluorotelomer sulfonamido betaines	$F(CF_2)_xCH_2CH_2SO_2NH(CH_2)_3N(CH_3)_2CH_2COO^-$
X:2 FtSaAm	Fluorotelomer sulfonamido amines	$F(CF_2)_xCH_2CH_2SO_2NH(CH_2)_3NH(CH_3)_2^+$
X:1:2 FtB	X:1:2 Fluorotelomer betaines	$F(CF_2)_xCFH(CH_2)_2N(CH_3)_2CH_2COO^-$
X:3 FtB	X:3 Fluorotelomer betaines	$F(CF_2)_x(CH_2)_3N(CH_3)_2CH_2COO^-$
PFASaAm	Perfluoroalkyl sulfonamido amines	$F(CF_2)_xSO_2NH(CH_2)_3NH(CH_3)_2^+$
PFASaAmA	Perfluoroalkyl sulfonamido amino carboxylates	$F(CF_2)_xSO_2N(CH_2CH_2COO^-)(CH_2)_3NH(CH_3)_2^+$
Functionalized perfluoropolyethers:		
ADONA (3M)	CAS No. 958445-44-8	$CF_3OCF_2CF_2CF_2OCFHCF_2COO^-$
GenX (DuPont)	CAS No. 62037-80-3	$CF_3CF_2CF_2OCF(CF_3)COO^-$
Asahi's product	CAS No. 908020-52-0	$CF_3CF_2OCF_2CF_2OCF_2COO^-$
Solvay's product	CAS No. 329238-24-6	$ClF_6C_3O[CF_2CF(CF_3)O]_m[CF(CF_3)O]_n\ CF_2COO^-$

used [30–32]. To elucidate the metabolism mechanism and possible adverse effects due to covalent binding of unsaturated polyfluoroalkyl intermediate metabolites to biological macromolecules, *in-vitro* bioassays were performed using hepatocytes, cytosol fractions and liver microsomes from mammalian and fish species [19,21]. *In-vivo* dietary exposure studies were also conducted to evaluate uptake, metabolism and elimination dynamics of PFASs in Sprague-Dawley rats and juvenile rainbow trout [20,33,34].

2.2. Sample-extraction method

The selection of extraction methods is mainly based on the complexity of the investigated medium in the incubation systems (Table 2 and references therein). For a test system with simple components and weak matrix interference, such as a pure bacterial culture, a straightforward water-miscible solvent extraction (WMSE) plus centrifugation and filtration procedures would be sufficient to obtain a representative sample extract for further instrumental analysis [31]. This was done by adding an equal or slightly larger volume of a solvent (e.g., acetonitrile) to the bacterial liquid culture medium to extract precursors and transformation products.

Bound residues or conjugates between fluorinated transformation products and natural organic components were frequently found in more complex environmental samples, such as living soil and sediment, requiring multiple extraction procedures to improve analyte recovery [25,26]. Dispersive solid-phase extraction (DSPE) methods,

Table 2
Literature information on analytical and sample-preparation methodologies for studying biotransformation of polyfluoroalkyl precursors in environmental and biological matrices

Matrix	Compounds	Extraction [a]	Clean-up/ Concentrate[b]	Derivatization[c]	Quantification		Isotopic tracking[d]	Structure elucidation	Ref.
					Instrument	Chromatographic separation			
Environmental matrices:									
Activated sludge	[3-^{14}C] 8:2 FTOH	WMSE (MTBE) WMSE (ACN, bottle & septa)	N	TMS	GC-MS LC-MS/MS	DB-5 MS (30m × 0.25 mm × 1 μm), Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	LSC, LC-ARC	LC-Q-TOF, GC-TOF (RT, accurate mass, MS^2)	[10]
Sediment	6:2 FTOH	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	N	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	N	[25]
Forest soil	[1,2-^{14}C] 6:2 FTOH	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	N	LC-MS/MS	Fluorous PF-C8 (150 × 4.6 mm, 5 μm)	LSC, LC-ARC, TC	N	[26]
Forest soil	6:2 FTI	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	N	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	LC-LTQ-Orbitrap MS, GC-TOF (mass defect, accurate mass, MS^2)	[27]
Sewage sludge	Mono-PAPs (4:2, 6:2, 8:2, 10:2) Di-PAPs (6:2)	WMSE (MeOH), IPE (TBAS, MTBE)	XAD-2 (EA)	N	GC-MS LC-MS/MS	ZB-WAX (30m × 0.25 mm × 0.25 μm), Gemini C18 (50 × 4.6 mm, 3 μm)	N	N	[28]
Forest & Agricultural soil	[3-^{14}C] 8:2 FTOH	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	N	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), DB-5 MS (30m × 0.25 mm × 1 μm)	LC-ARC, TC	LC-Q-TOF, GC-TOF, GC-MS (suspect ion screening, MS^2)	[29]
Forest soil	6:2 FTOH	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	DNPH	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), Zorbax SB-C18 (150 × 2.1 mm, 5 μm)	N	LC-LTQ-Orbitrap MS (non-target screening, MS^2, in-source CID)	[30]
Activated sludge	6:2 FTSA	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	DNPH	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	N	[35]
Marine sediment	N-EtFOSE & SAmPAP Diester	DSPE (MeOH, ACN)	Envi-Carb	N	LC-MS/MS	xTerra C18 (30 × 4.6 mm, 5 μm)	N	N	[36]
Agricultural soil	8:2 FTS	DSPE (EA, ACN, NaOH)	C18 (ACN)/ Envi-Carb	N	GC-MS LC-MS/MS	DB-5 MS (30m × 0.25 mm × 0.25 μm) Synergi Max-RP (150 × 2 mm, 4 μm)	N	N	[37]
Activated sludge	5:3 acid	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	N	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), Zorbax SB-C18 (150 × 2.1 mm, 5 μm)	N	LC-LTQ-Orbitrap MS (non-target screening, accurate mass, mass defect, suspect ion screening)	[38]
Anaerobic sludge	6:2 FTOH, [3-^{14}C] 8:2 FTOH	DSPE (ACN, NaOH, bottle & septa)	C18 (ACN)/ Envi-Carb	DNPH	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), Zorbax SB-C18 (150 × 2.1 mm, 5 μm)	TC	LC-LTQ-Orbitrap MS (non-target screening, accurate mass, MS^2)	[39]
Microbial Isolates:									
Mixed sludge bacterial culture	[3-^{14}C] 8:2 FTOH	WMSE (ACN, MTBE, bottle & septa)	C18 (ACN)	N	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), Fluorous PF-C8 (150 × 4.6 mm, 5 μm) DB-5 MS (30m × 0.25 mm × 1 μm)	LSC, LC-ARC	LC-Q-Orbitrap MS, GC-TOF, GC-MS (RT, accurate mass, precursor ion scan)	[11]
Mixed sludge bacterial culture	6:2 FTOH	WMSE (ACN, bottle & septa)	N	DNPH	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	LC-Q-Orbitrap MS, (non-target screening, suspect ion screening, MS^2)	[30]
Pure *Pseudomonas* strains	FTOHs (4:2, 6:2, 8:2)	WMSE (ACN, hexane)	N	N	GC-MS, GC-ECD, LC-MS/MS	DB-5 (30m × 0.25 mm × 0.25 μm), DB-1 MS (30m × 0.25 mm × 0.25 μm), Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	N	[31]
Pure soil bacterial isolates	8:2 FTOH	WMSE (ACN)	N	N	LC-MS/MS	Luna C8 (100 × 2 mm, 3 μm), Synergi Max-RP (100 × 4.6 mm, 5 μm) Zorbax Rx-C8 (150 × 2.1 mm, 5 μm)	N	N	[32]
Mixed culture (sediment & wastewater)	8:2 FTOH	WMSE (MeOH)	PDMS	N	GC-ECD, GC-MS, LC-MS/MS	DB-35 (30m × 0.25 mm × 0.25 μm), DB-WAX (30m × 0.25 mm × 0.25 μm), Zorbax Rx-C8 (250 × 4.6 mm, 5 μm)	N	N	[40]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Compounds	Extraction [a]	Clean-up/ Concentrate[b]	Derivatization[c]	Quantification		Isotopic tracking[d]	Structure elucidation	Ref.
					Instrument	Chromatographic separation			
In-vitro assays:									
Isolated rat hepatocytes	FTOHs (4:2, 6:2, 8:2, 10:2)	IPE (TBAS, MTBE) WMSE (MeOH, ACN)	N	DNPH	LC-MS/MS	Genesis C8 (50 × 2.1 mm)	N	LC-QqQ (neutral loss scan, precursor ion scan, suspect ion screening)	[19]
Isolated cytosol hepatocytes, microsome (rat, mouse, human, trout)	[3-^{14}C] 8:2 FTOH	WMSE (ACN)	N	DNPH	LC-MS/MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), Zorbax SB-C18 (150 × 2.1 mm, 5 μm)	LC-ARC, LSC	LC-QqQ, GC-MS, GC-TOF (suspect ion screening, MS^2)	[20]
Human microsome & recombinant human CYP2C9, C19	N-EtFOSA	WMSE (MTBE), WMSE (MeOH)	PDMS	N	GC-ECD, LC-MS/MS	DB-35 MS (80m × 0.25 mm × 0.25 μm), FluoroSep RP C8 (150 × 2.1 mm, 3 μm)	N	N	[41]
Pooled rat liver microsome	6:2 FTI, [1,2-^{14}C] 6:2 FTOH	WMSE (ACN, bottle & septa)	C18 (ACN)	N	LC-MS/MS, GC-MS	Zorbax Rx-C8 (150 × 2.1 mm, 5 μm), DB-5 MS (30m × 0.25 mm × 1 μm)	LSC	N	[42]
In-vivo studies:									
Sprague-Dawley rats	[3-^{14}C] 8:2 FTOH	WMSE (ACN, plasma), ASE (ACN/water, MeOH/water, Feces)	PDMS	DNPH	GC-MS, LC-MS/MS	DB-5 MS (30m × 0.25 mm × 1 μm) Synergi Max-RP (20 × 2 mm, 2 μm) Zorbax SB-C18 (150 × 2.1 mm, 5 μm)	LC-ARC, LSC, TC	LC-Q-TOF (suspect ion screening, MS^2)	[20]
Juvenile Rainbow Trout	PFPAs (C6, C8, C10); PFPiAs (C6-6, C6-8, C8-8)	IPE (TBAS, MTBE)	N	N	LC-MS/MS	Kinetex C18 (50 × 4.6 mm, 2.6 μm)	N	N	[33]
Sprague-Dawley rats	Mono-PAPs, Di-PAPs (4:2, 6:2, 8:2, 10:2)	Sonication (ACN, blood, urine), Sonication (ACN/water, feces)	N	N	LC-MS/MS	Gemini NX C18 (50 × 4.6 mm, 3 μm)	N	N	[34]
Sprague-Dawley rats	8:2 Mono-PAPs, 8:2 Di-PAPs	IPE (TBAS, MTBE)	N	N	LC-MS/MS	Genesis C18 (50 × 2.1 mm, 4 μm), Allure PFP propyl (50 × 2.1 mm, 4 μm)	N	N	[43]
Rainbow Trout	8:2 FTAc	WMSE (EA)	N	N	GC-MS, LC-MS/MS	RTX-WAX (30m × 0.25 mm × 0.25 μm) ACE C18 (50 × 2.1 mm, 3 μm)	N	LC-QqQ (suspect ion screening, MS^2)	[44]

[a] WMSE, Water-miscible solvent extraction (including centrifugation and filtration); DSPE, Dispersive solid-phase extraction; IPE, Ion-pair extraction; ASE, Accelerated solvent extraction; ACN, Acetonitrile; MeOH, Methanol; MTBE, Methyl *tert*-butyl ether; EA, Ethyl acetate; TBAS, Tetrabutyl ammonium hydrogen sulfate;
[b] N, Not applied; PDMS, Polydimethylsiloxane;
[c] TMS, Trimethyl silyl; DNPH, 2;4-dinitrophenylhydrazine;
[d] LSC, Liquid-scintillation countering; LC-ARC, Liquid chromatography accurate radioisotope counting; TC, Thermal combustion.

initially established for the determination of PFCAs in environmental matrices, were widely adapted in soil, sediment and activated sludge-incubation systems [26,35–37,45]. Specifically, solid samples were first soaked in an organic solvent (methanol, acetonitrile, or methyl *tert*-butyl ether), then constantly shaken at 150–200 rpm for from several hours up to 5 days and then centrifuged to obtain supernatant. If methyl *tert*-butyl ether (MTBE) was used for extraction, the soil was first saturated with deionized water before adding MTBE to avoid soil aggregation during extraction. The supernatant was then mixed with Envi-Carb graphitized carbon adsorbent to reduce matrix effects, which may enhance or suppress instrumental signals of analytes [33,46]. Liu et al. [30] developed the procedure by including an alkaline treatment (25 mM NaOH final concentration, Fig. 1) for the soil extract to release ^{14}C-labeled transformation products from dissolved soil-component conjugates. This led to enhancement of 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$] recovery by 6–38 times.

For biological matrices, such as animal tissue and serum samples, extraction methods based on ion-pair extraction (IPE), initially developed by Hansen et al. [47], were more applicable. Briefly, sodium carbonate buffer (pH = 10) and tetrabutyl ammonium hydrogen sulfate (TBAS) were mixed with solid samples, which were extracted by MTBE. The MTBE aliquots were then combined, brought to dryness under a stream of nitrogen, and the polyfluorinated analytes were reconstituted in methanol. The methods were successfully applied to recover FOSAs, mono-PAPs, di-PAPs, perfluoroalkyl phosphonic acids (PFPAs) and corresponding biotransformation products in both *in-vitro* and *in-vivo* exposure studies [33,43,46,47]. Washington et al. [48] also developed multi-step extraction procedures for PFCAs in soils by combining both DSPE and IPE pretreatment procedures, achieving quantitative recoveries with minimized matrix effects.

Despite the well-established DSPE and IPE methods that could be applied to other novel polyfluoroalkyl precursor biotransformation studies, optimization of the detailed sample-pretreatment procedures is still essential. For example, hydrolysis of ester bonds in 8:2 fluorotelomer citrate triester (8:2 TBC) and substantial formation of 8:2 FTOH occurred when dosed ^{60}Co-γ-irradiated sterile soil samples were extracted using the DSPE method [49]. The ester hydrolysis was enhanced by solvents with high dielectric constants (i.e., methanol and acetonitrile) compared with less polar solvents (e.g., MTBE and ethyl acetate). An additional 10–20% of ^{14}C-labeled polyfluoroalkyl transformation products was recovered from soil initially dosed with ^{14}C-labeled 8:2 FTOH when the DSPE extraction temperature was elevated to 50°C [29]. D'eon et al. [16] also found that the removal of sodium-carbonate buffer in the IPE procedures minimized 8:2 diPAP blank contamination in the analysis of mono-PAP and di-PAP concentrations in human-serum samples with adequate extraction efficiencies (85 ± 22%).

2.3. Sample concentration and clean-up

The starting concentrations of polyfluoroalkyl precursors were generally in the part-per-million range (ppm, mg/kg of soil, sediment, or activated sludge) in most studies, without the need for sample concentration and allowing known transformation products to be quantified with either gas chromatography MS (GC-MS) or liquid chromatography-tandem MS (LC-MS/MS) instruments. However, identification of low levels of novel transformation products requires HRMS analysis in the full-scan mode. An elevated precursor concentration up to 10–100 mg/kg soil or sludge helped reduce false positives and obtain clean mass spectra of several novel transformation products [30]. For volatile precursors, such as FTOHs, FOSAs and FOSEs, with the tendency to move into the headspace, concentration procedures were performed over long-term incubation periods [40,41]. In semi-static systems, the headspace was usually purged through C_{18} SPE cartridges at the beginning of sample preparation to trap volatile precursors and transformation products [25,27]. XAD adsorbents were also chosen to monitor the formation of FTOHs from the degradation processes of mono-PAPs and di-PAPs by aerobic microbes from a WWTP in flow-through systems [28]. Meanwhile, polydimethylsiloxane solid-phase microextraction materials were expediently used in enrichment and identification of novel transformation intermediates and products in headspace and to quantify the gas-fraction concentrations of analytes [27,40,41].

To reduce matrix effects caused by complex organic components in environmental and biological samples, treatment used Envi-Carb graphitized carbon powder or cartridges as an effective clean-up approach [45]. Organic interferents with a degree of aromaticity will be strongly adsorbed on the surface of graphitized carbon, while interactions between Envi-Carb and PFASs are implausible. The selectivity is not influenced by the presence of organic solvents, such as methanol, acetonitrile and MTBE, which made the purification procedure easily coupled to both DSPE and IPE methods, as mentioned in sub-section 2.2. The protocol was simple and robust for quantitative recovery of fluorotelomers- and EtFOSE-based precursors and transformation products [29,30,35–37]. Zhang et al. [46] demonstrated low matrix effects for PFCAs (C_5–C_{10}), PFSAs ($C_{4,6,8}$), FOSAs and FOSAAs in the range -13% to +24% in digested sewage-sludge extract using the Envi-Carb clean-up procedure.

2.4. Derivatization step

The derivatization step improves PFAS analysis through formation of stable derivatives . Fluorotelomer aldehydes (FTALs) are short-lived initial intermediates of fluorotelomer-based product transformation, and could be quickly oxidized into fluorotelomer carboxylic acids (FTCAs). 2,4-Dinitrophenylhydrazine (DNPH) was frequently used as the derivatization reagent for the identification of FTALs in *in-vitro* animal studies by mixing organic-sample extracts with DNPH solution in hydrochloric acid prior to LC-MS analysis [19–21]. The derivatization step was also applied to microbial incubation systems to identify 8:2 FTAL and 5:3 ketone aldehyde in 8:2 FTOH dosed soil and 6:2 FTOH dosed mixed bacterial culture, respectively [29,30].

Recently, Peng et al. [50] developed a novel derivatization technique using the dansyl-chloride (5-(dimethylamino)naphthalene-1-sulfonyl chloride) reaction with FTOHs, allowing sub-ppb (part per billion, μg/kg) quantitation in LC-MS analysis. Also, Wang et al. [10] used trimethyl silyl (TMS) to increase the volatility of metabolites derived from an 8:2 FTCA biodegradation study in GC-MS analysis, and 7:3 FTCA was firstly recognized as the proposed structure of an unidentified peak.

3. Instrumental analysis

3.1. LC/GC-MS quantitative analysis

3.1.1. LC-MS

The majority of polyfluoroalkyl precursors and transformation products are in ionic form under environmentally relevant pH conditions [51]. LC separation coupled with triple-quadupole MS (QqQ) detection is the most common method to analyze anionic PFASs because of its good compatibility with aqueous samples and polar extraction solvents. Perfluorinated carbon chains of PFASs ($C \geq 6$) ensure appropriate retention behavior on reversed-phase stationary phases, such as C_8, C_{18} and pentafluorophenyl (PFP) [10,29,30,43], whereas ion-exclusion chromatography was also used to separate perfluoroalkylsulfonic acids and perfluoroaklylsulfinic acids with short alkyl-chain lengths up to C_4 and C_6, respectively [52].

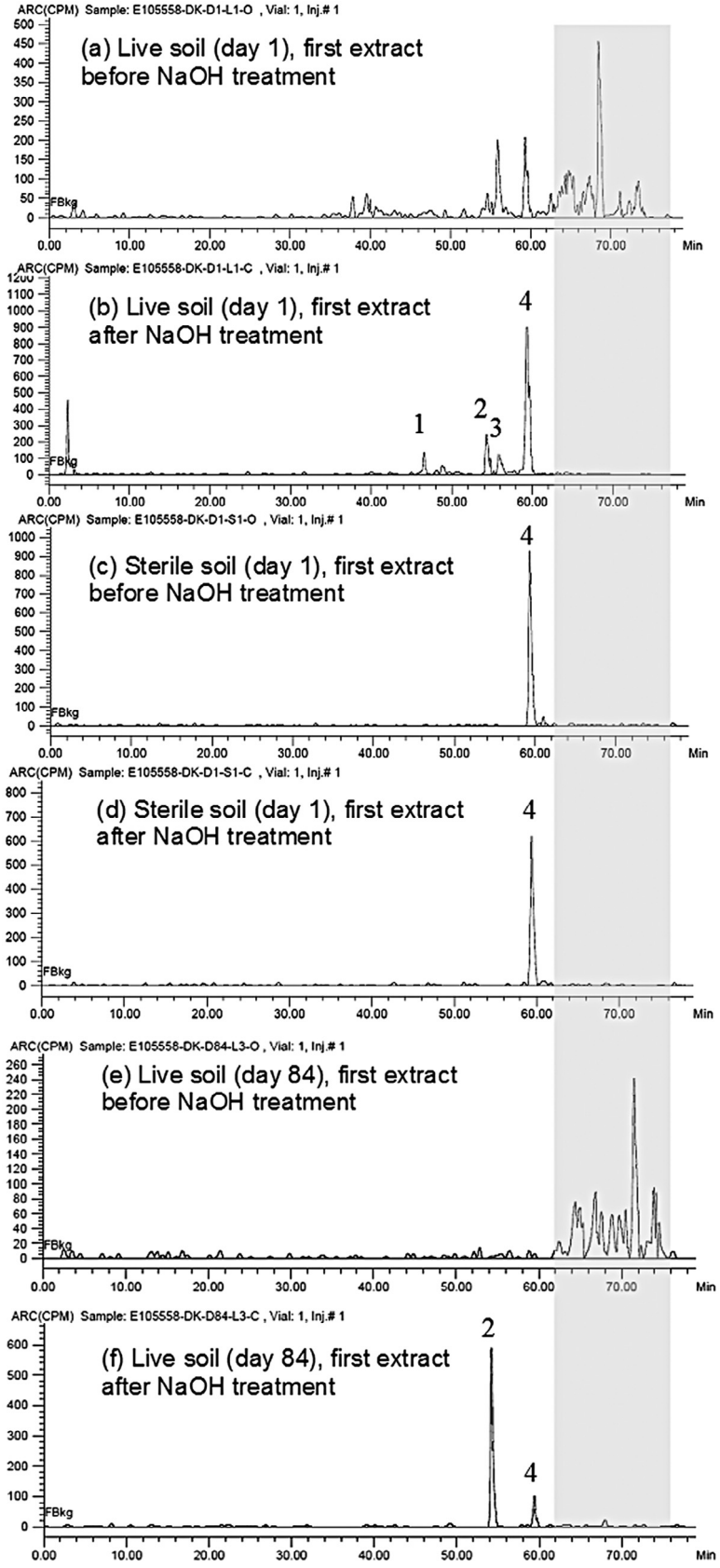

Fig. 1. Chromatograms from accurate radioisotopic counting instruments coupled to liquid chromatography (LC-ARC) of transformation products in [1,2-^{14}C] 6:2 FTOH-dosed live and sterile forest-soil samples (day 1 and day 84), showing alkaline (NaOH) treatment in dispersive solid-phase extraction (DSPE) on elimination of unresolved analyte-matrix conjugates for better recoveries. Peak 1: [1,2-^{14}C] 6:2 FTUCA [$F(CF_2)_5CF{=}{}^{14}CH_2{}^{14}COOH$]; Peak 2: [1,2-^{14}C] 5:3 acid [$F(CF_2)_5CH_2{}^{14}CH_2{}^{14}COOH$]; Peak 3: [^{14}C] 5:2 sFTOH [$F(CF_2)_5CH(OH){}^{14}CH_3$]; and, Peak 4: [1,2-^{14}C] 6:2 FTOH [$F(CF_2)_6{}^{14}CH_2{}^{14}CH_2OH$]. The unresolved peaks in the grey area (retention time: 62–76 min) are ^{14}C-labeled analyte-matrix conjugates. {From [26] with permission, ©Elsevier Ltd.}.

LC-MS/MS operated in the multiple-reaction monitoring (MRM) mode offered both wide linear dynamic range of at least three orders of magnitude and excellent sensitivity at sub-ppb levels. Negative electrospray ionization (ESI) was the most suitable LC-MS interface, and anionic PFASs were obtained as the deprotonated ($[M-H]^-$) ions. Pre-column formation of analyte adducts by adding additives to the LC mobile phases, such as acetate-adducts ($[M+59]^-$) of FTOHs, sFTOHs and FT ketones, could avoid LC-peak broadening and offer sufficient sensitivity for the neutral FT alcohols and ketones (instrument detection limits, IDLs: 0.14–0.22 ng on column) along with anionic PFCAs and PFSAs (IDLs: 4.0–42 pg on column) [27,29,30]. Moreover, LC-atmospheric pressure photoionization (APPI)-MS proved to be effective in direct ionization of FTOHs and FOSAs with excellent IDLs less than 1 pg on column [53].

3.1.2. GC-MS

GC-MS is an effective tool to quantify neutral volatile polyfluoroalkyl precursors, such as FTOHs, FTIs and FT acrylates, in different biotransformation incubation systems [44,54]. DB-WAX, DB-35 and analogous stationary phases were normally used to separate FTOHs and FOSEs [40,41,44,55], whereas DB-5 and DB-624 analytical columns were options for semi-volatile and highly-volatile PFAS precursors, respectively [10,11,37,54].

GC-MS can provide enhanced selectivity and sensitivity to target analytes as well as tolerances to sample-matrix effects. Electron-impact (EI) ionization was suitable for measuring FTOHs, FTIs, 8:2 FTS in organic solvent extracts [37,54,55]. Martin and co-workers also suggested that positive chemical ionization (PCI) was the mode of choice for FTOH routine analysis, whereas negative chemical ionization (NCI) showed sensitivity advantages for sulfonamide-based derivatives, such as N-EtFOSA, N-MeFOSE and N-EtFOSE [55]. The electron-capture detector (ECD) has also been used for GC analysis [41,52]. The element-specific microwave plasma detector was also successfully applied to study 8:2 FTOH biotransformations in rat to avoid the insufficient specificity of ECD in complex biological matrices [52].

3.2. ^{14}C-labeling and detection techniques

Tracking the partitioning of polyfluorinated precursors and formation of novel degradation products in complex test media is quite challenging. Nevertheless, radio-isotopic labeling provides a means of overcoming this by measuring positron emission from radioactive decaying chemicals. Due to low natural abundance and lasting half-life, ^{14}C was selected to label PFAS precursors in order to trace chemical transformations in environmental and biological trials [10,11,26,42].

Liu et al. [26] noted that mass-balance analysis of precursor-biodegradation processes based on solvent extraction and LC-MS/MS quantification methods suffered from matrix effects and formation of bound residues and unknown degradation products in complex sample media. Nevertheless, a simple, accurate liquid scintillation counting (LSC) measurement allowed quantification of novel transformation products and biological conjugates without the need for authentic standards. Accurate radioisotopic counting instruments coupled to LC (LC-ARC) could further promote separation of total radioactive precursor/metabolite mixtures by different physical-chemical properties and also facilitate information sharing with other qualitative equipment, particularly HRMS [10,11,26,29]. Retention times of unknown signals from both LC-ARC and LC-quadrupole-time of flight (Q-TOF) MS were precisely matched, and ^{14}C-labeled 8:2 FTOH transformation products (Fig. 2) were confirmed in activated sludge with 7:3 acid [$F(CF_2)_7{}^{14}CH_2CH_2COOH$] being identified for the first time [10]. Non-extractable soil- or sediment-bound ^{14}C-residues can also be quantified after thermal combustion to $^{14}CO_2$, which was trapped in a liquid scintillation cocktail for radioactivity measurements. Up to 35% of ^{14}C-labeled FTOH precursors and transformation products were irreversibly bound to soils after two-day incubation, and could be recoverable by only high-temperature soil combustion (900°C) [29].

3.3. Mass-spectrometry elucidation of novel transformation products

High resolution power and sufficient sensitivity are both essential to elucidate the molecular structure of novel degradation products at low abundance during polyfluoroalkyl-precursor transformation, so GC-MS or LC-MS emerged as a powerful tool. Use of accurate-mass measurements and multiple-mass fragmentation are two major applicable strategies in identifying unknown metabolites [56]. In accurate-mass measurement, selected suspect-ion monitoring or retrospective screening of the full-scan spectrum are effective in confirming plausible analytes without using authentic standards. For example, typical phase II metabolites (glutathione, glucuronide and sulfate conjugates) of polyfluorinated alcohols, aldehydes and unsaturated telomer acids could easily be observed once deprotonated molecular ions of corresponding conjugates were monitored [19–21]. When searching for plausible unknown metabolites without *a priori* information, exact mass filtering is also one principal step in the so-called "non-target screening" strategy [57]. As the accurate mass of fluorine ($m/z = 18.998$) is very close to its nominal mass, mass-defect filtration (-50 to +10 mDa) of nominal masses could eliminate the majority of irrelevant information in the full-scan MS spectrum [27]. Comparison of differences in full-scan chromatograms of paired live and matrix samples could also help find signals that might represent potential transformation products that existed in live samples only [38]. A limited number of rational molecular compositions by a combination of C, H, F, O, N elements could be further obtained with a mass accuracy of less than 5 ppm [27,39,58].

MS^n is particularly attractive for providing molecular structure information through multiform scanning functions. The elemental composition of cleaved functional groups (e.g., consecutive loss of CO_2+2HF and HF) in given pseudo-molecular ions by collision-induced dissociation (CID) could be accurately measured by HRMS [39]. Besides, precursor-ion scan can be used to search for transformation products structurally related to the parent compound. Once neutral structure segments are lost in MS^n experiments, constant neutral-loss scan could be set to search for plausible metabolites [59] {e.g., a neutral loss of 20 in the MS/MS spectra corresponds to the neutral loss of HF in most polyfluorinated metabolites [19]}. At m/z 306, m/z 272 and m/z 254, there are highly specific ions produced by dissociation of the glutathione moiety, which were used as diagnostic ions in precursor-ion scans for polyfluorinated glutathione conjugates [19]. Moreover, for single-quadrupole MS instruments with no MS^n functions, structural information of novel metabolites could provide confirmation by in-source fragmentation in an MS ionization source with the aid of reference standards [30].

3.4. Computer-aided prediction of biotransformation pathways

Computer-aided pathway prediction relies on quantitative structure-activity (QSAR) models, which link biodegradation potential with molecular descriptors, such as physical-chemical descriptors and connectivity indexes generated from chemical-structure fragments [60]. *In-silico* calculation is a quick, cost-effective assessment to predict chemical-degradation potential, metabolites, and pathways at the screening level.

The University of Minnesota Pathway Prediction System (UM-PPS) is an open-access web interface, which provides information on microbial enzyme-catalyzed reactions. The prediction is based

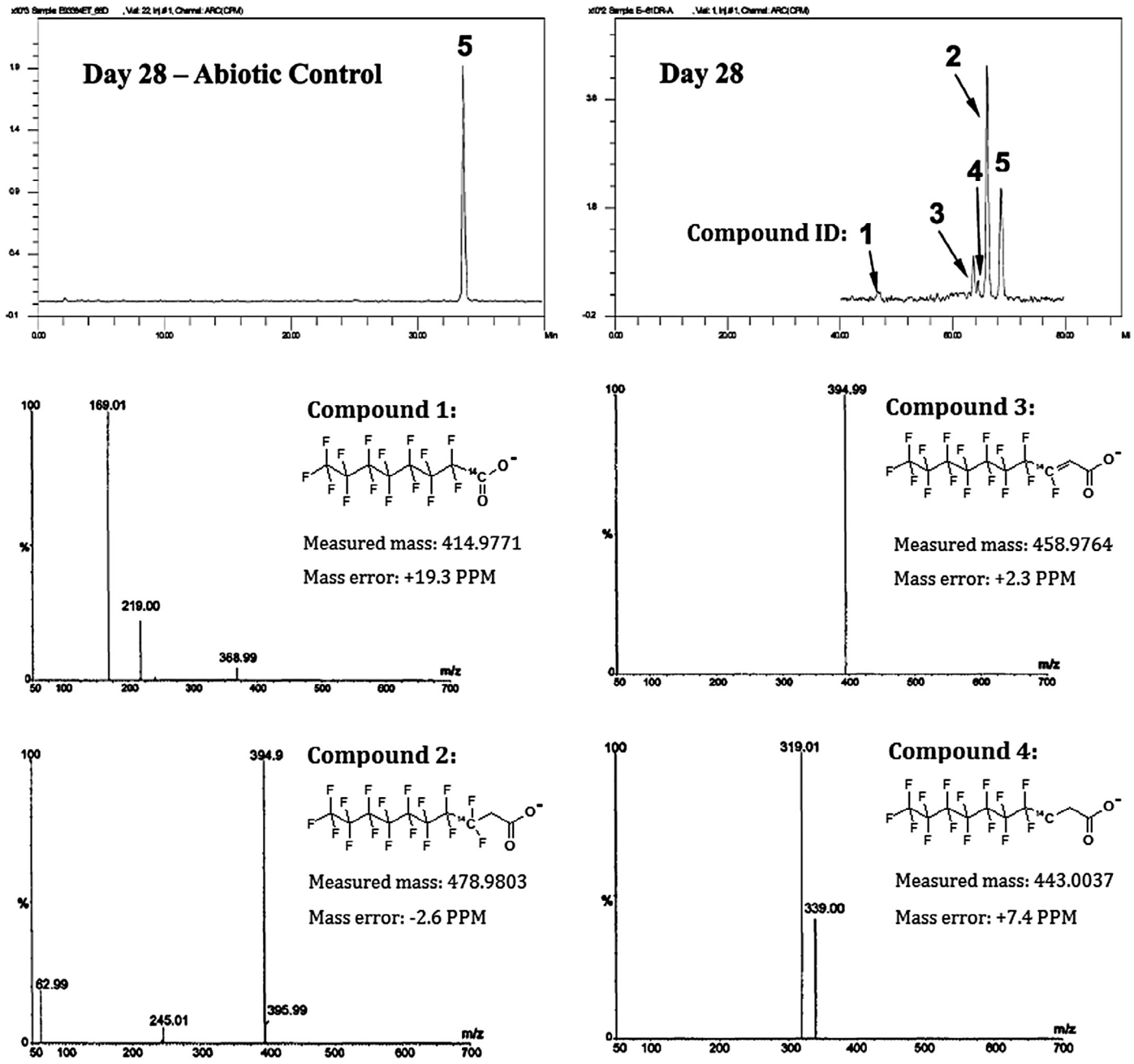

Fig. 2. (A and B) Radiochromatograms from accurate radioisotopic counting instruments coupled to liquid chromatography (LC-ARC) of transformation products in [3-^{14}C] 8:2 FTOH-dosed live and sterile activated sludge samples (day 28). (C–F) LC quadrupole time-of-flight (LC-Q-TOF) product-ion mass spectra of deprotonated molecular ions (Compounds 1–4). Compound 1: [1-^{14}C] PFOA [$F(CF_2)_7{}^{14}COOH$]; Compound 2: [3-^{14}C] 8:2 FTCA [$F(CF_2)_7{}^{14}CF_2CH_2COOH$]; Compound 3: [3-^{14}C] 8:2 FTUCA [$F(CF_2)_7{}^{14}CF{=}CH_2COOH$]; Compound 4: [3-^{14}C] 7:3 acid [$F(CF_2)_7{}^{14}CH_2CH_2COOH$]; and, Compound 5: [3-^{14}C] 8:2 FTOH [$F(CF_2)_7{}^{14}CF_2CH_2CH_2OH$]. {Modified from [10] with permission, ©American Chemistry Society}.

on reorganization of organic functional groups in a coded molecular structure input and predicts transformation according to biotransformation rules from pure culture studies in the UM-BBD database and other scientific literature [61]. Pathways are generated based on a precursor's possible transformation sequence to downstream products with the likelihood being graded as "likely", "neutral" and "unlikely" with no other priority obviation logic provided [61].

Meanwhile, CATALOGIC (formally CATABOL) is proprietary software for simulating catabolism and predicting biodegradation products under biodegradation conditions of a given chemical. Different from UM-PPS, metabolism rules in CATALOGIC are hierarchically ranked by occurrence probabilities judged by expert knowledge and documented metabolic maps [60,62]. Metabolic trees could be generated with quantitative distributions of the precursor and subsequent transformation products.

Fig. 3 gives a detailed comparison between UM-PPS and CATALOGIC software predictions on N-EtFOSE biotransformation pathways. Both models correctly predicted the intermediate, N-ethylperfluorooctane sulfonamido acetic acid and the end-product perfluorooctane sulfonic acid (PFOS), with CATALOGIC predicting more experimentally-confirmed intermediates. However, both models failed to predict novel biotransformation pathways of 6:2 FTOH and 8:2 FTOH that are experimentally confirmed [29,30] due

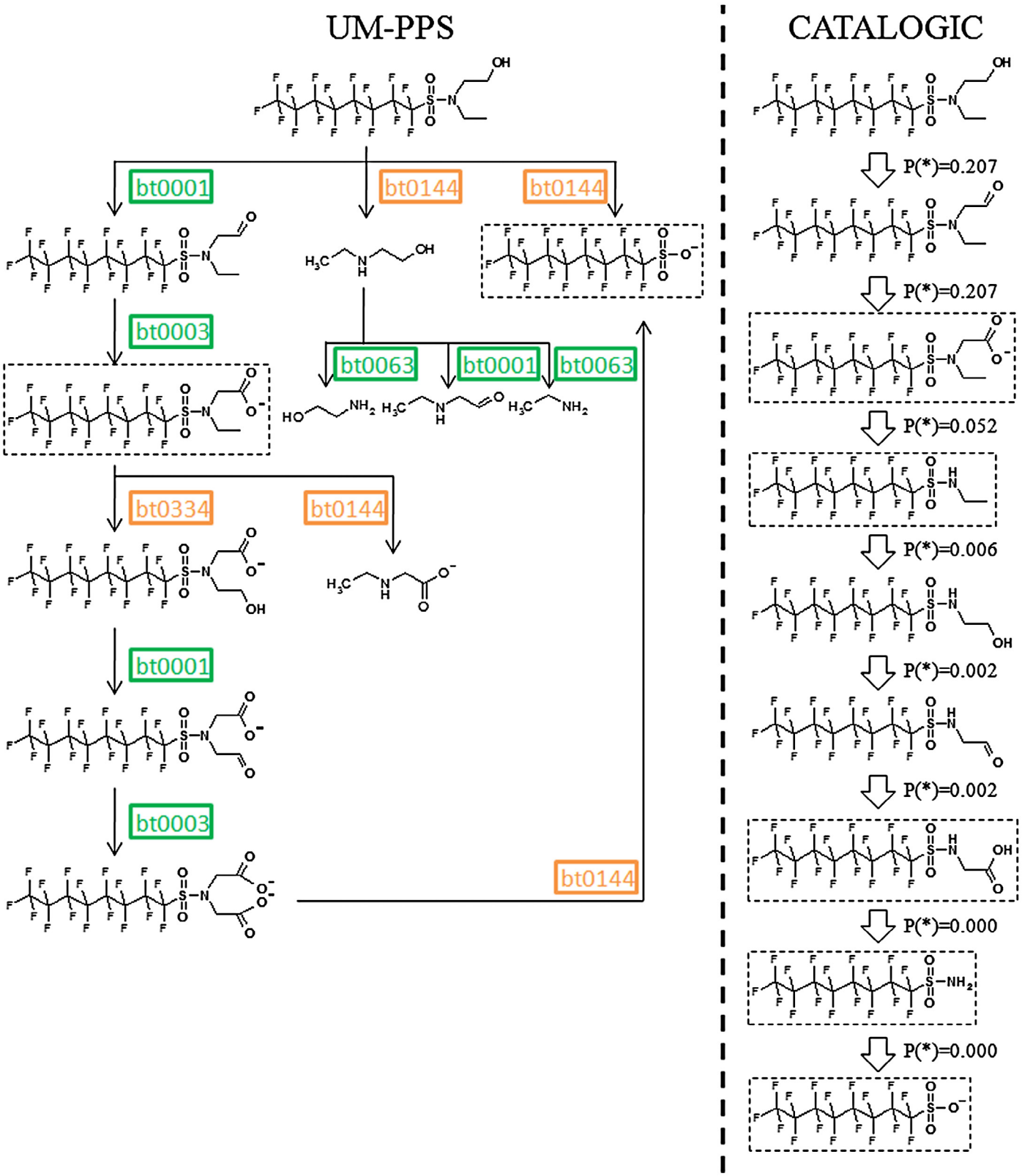

Fig. 3. Comparison of computer-aided predictions of N-EtFOSE biotransformation pathways by UM-PPS framework and CATALOGIC software. The green and orange colors of the solid-line boxes in the UM-PPS framework represent the likelihood of the reactions as "likely" and "neutral", and the numbers inside the solid-line boxes are codes of enzyme reactions in the UM-BBD database. The compounds inside the dashed-line boxes in both UM-PPS and CATALOGIC frameworks are stable transformation products confirmed in laboratory experiments. "*" symbol in CATALOGIC framework represents the probabilities of occurrence of corresponding transformation reactions.

to lack of broad training sets of perfluoroalkyl-transformation products built into the two QSAR models. Inclusion of the latest knowledge on biotransformation reactions and molar yields of transformation products from various polyfluoroalkyl precursors would enhance the accuracy of the *in-silico* predictions.

4. Conclusions and future perspectives

In this review, we summarized up-to-date sample preparation and instrument-analysis methodologies, which were successfully employed in elucidation of major degradation products and biotransformation pathways of biodegradable polyfluorinated chemicals, and might also contribute to future studies on environmental monitoring and ecotoxicology evaluation of emerging PFAS contaminants. Nevertheless, analytical challenges remain in investigating environmental behavior and fate of a broad range of polyfluoroalkyl precursors.

The production volume of polyfluoroalkyl substances with 4 or 6 perfluorinated carbon backbones ("short-chain" precursors) is likely to increase significantly in the next few years. There is an urgent need to expand and to modify current analytical methodologies to focus more on short-chain precursors and to cover more types of environmental matrices and biota species.

Moreover, the application of polyfluoroalkyl alternatives with novel functional groups, such as functionalized perfluoropolyethers (PFPEs) and zwitterionic, cationic and anionic fluoroalkyl aqueous film-forming foams, is also increasing [63,64]. New sample-preparation and instrumental methods are in demand to understand the contributions of these polyfluoroalkyl alternatives to PFCAs and PFSAs detected in the environment and biota.

New analytical technologies could also be adopted to manage vast sets of data besides the state-of-the-art pretreatment and instrumental methods. For example, processing tools (automated data deconvolution, chromatographic peak detection, alignment, feature filtering and scaling) in modern MS analysis could help identify important transformation products that may otherwise be missed by retrospective analysis of HRMS full-scan spectra. Current knowledge on PFAS-biotransformation processes is mainly based on investigating the occurrence and the quantity of relatively stable transformation products. More effective sample-pretreatment techniques to trap and to derivatize possible unstable intermediates may provide new insight into transformation mechanisms among key degradation intermediates and to recognize unknown transformation pathways.

Despite progress in current understanding of biodegradation pathways of polyfluoroalkyl precursors, it is still not well understood what key enzymes and cofactors are involved. Omics studies (e.g., proteomics, transcriptomics, and metabolomics) of polyfluoroalkyl-precursor biotransformation with increased instrumentation sensitivity and throughput may further discern the defluorination mechanisms and other important biotransformation steps.

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