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Critical Review

BIOTRANSFORMATION PATHWAYS OF FLUOROTELOMER-BASED POLYFLUOROALKYL SUBSTANCES: A REVIEW

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Abstract: The study reviews the current state of knowledge regarding the biotransformation of fluorotelomer-based compounds, with a focus on compounds that ultimately degrade to form perfluoroalkyl carboxylates (PFCAs). Most metabolism studies have been performed with either microbial systems or rats and mice, and comparatively few studies have used fish models. Furthermore, biotransformation studies thus far have predominately used the 8:2 fluorotelomer alcohol (FTOH) as the substrate. However, there have been an increasing number of studies investigating 6:2 FTOH biotransformation as a result of industry's transition to shorter-chain fluorotelomer chemistry. Studies with the 8:2 FTOH metabolism universally show the formation of perfluorooctanoate (PFOA) and, to a smaller fraction, perfluorononanoate (PFNA) and lower-chain-length PFCAs. In general, the overall yield of PFOA is low, presumably because of the multiple branches in the biotransformation pathways, including conjugation reactions in animal systems. There have been a few studies of non-FTOH biotransformation, which include polyfluoroalkyl phosphates (PAPs), 8:2 fluorotelomer acrylate (8:2 FTAC), and fluorotelomer carboxylates (FTCAs, FTUCAs). The PAPs compounds and 8:2 FTAC were shown to be direct precursors to FTOHs and thus follow similar degradation pathways. *Environ Toxicol Chem* 2014;33:243–267. © 2013 SETAC

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INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been used in the manufacture of surfactants and polymers since the 1950s [1]. The PFASs are highly stable to chemical reaction and have the unique properties of hydrophobicity and lipophobicity. Thus, many PFASs are primarily used for their stain-repellent properties such as surface treatment for textiles and food contact paper. A detailed description of the sources, manufacture, and uses of PFASs is presented by Buck et al. [1].

In the early 2000s, several studies brought scientific attention to the widespread occurrence of perfluoroalkyl carboxylates (PFCAs) in humans [2] and wildlife [3,4]. Although early research efforts focused on perfluorooctanoate (PFOA), the occurrence of PFCAs of various chain lengths, particularly those with more than 8 carbons, has been extensively reported in humans and wildlife from across the globe [5–8]. The prevalent occurrence of PFCAs was surprising considering that their physical–chemical properties were not consistent with those that typically undergo atmospheric long-range transport. Furthermore, the production of PFCAs themselves was very small compared with the total production of fluorotelomer-based compounds (e.g., fluorotelomer-based polymer and phosphate surfactants) [9]. Therefore, in addition to the direct release of perfluoroalkane sulfonates and PFCAs from industrial emissions and commercial products, the potential contribution of indirect sources from so-called "precursor" compounds has garnered considerable attention. Precursor compounds represent chemicals that can ultimately degrade—through reactions such as atmospheric oxidation, metabolism, and hydrolysis—to form PFCAs. The atmospheric oxidation pathways of PFCA formation have been reviewed by Young and Mabury [10]. Regarding biological transformation, Hagen et al. [11] published the original study investigating the biotransformation of fluorotelomer-based compounds in 1981. Research was stagnant for more than 20 yr until Dinglasan et al. [12] published the biotransformation of 8:2 fluorotelomer alcohol (FTOH) in a mixed microbial system. Since that time there have been an increasing number of studies investigating the metabolism of FTOHs and other fluorotelomer-based compounds. Most studies have used FTOHs as the parent compound, although other fluorotelomer-based compounds have received some attention. In addition, the overwhelming majority of metabolism studies have been performed with microbial systems, rats, or mice, with limited study in fish.

Since the original work by Hagen et al. [11], considerable progress has been made in the state of knowledge regarding fluorotelomer-based chemical biotransformation. In recent years, our understanding rapidly progressed, aided by the advancement in analytical techniques, the use of radiolabeled compounds, and the synthesis of metabolic intermediates. The state of the science has now reached the level where a review of the literature is needed. For example, it is possible to draw general trends with respect to the overall pathways, but knowledge gaps remain.

The objective of the present study is to review and summarize the current state of knowledge regarding biotransformation pathways of fluorotelomer-based compounds that ultimately degrade to PFCAs. The present study is focused on compounds that are biotransformed to yield PFCAs, and thus perfluoroalkane sulfonate sources are not discussed. The review is separated into 3 sections, describing biotransformation in microbial incubations, mammals, and fish. The chemical formulas for compounds mentioned are shown in Table 1. Summaries of the metabolites observed in the individual experiments are presented in Tables 2, 3, and 4 (microbial), Table 5 (rats and mice), and Table 6 (fish), respectively.

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Table 1. Common name, acryonym, and chemical formula of compounds discussed

Common name	Acronym	Chemical formula
Fluorotelomer alcohol	n:2 FTOH	$CF_3(CF_2)_{n-1}CH_2CH_2OH$
Fluorotelomer secondary alcohol	n:2 sFTOH	$CF_3(CF_2)_{n-1}CH(OH)CH_3$
1H,1H,2H,2H,8H,8H-perfluorododecanol	DTFA	$CF_3(CF_2)_3CH_2(CF_2)_5CH_2CH_2OH$
Fluorotelomer ethoxylate	FTEO	$F(CF_2CF_2)_x(CH_2CH_2O)_yH$
Fluorotelomer sulfonate	n:2 FTSA	$CF_3(CF_2)_{n-1}CH_2CH_2SO_3^-$
Fluorotelomer acrylate	n:2 FTAC	$CF_3(CF_2)_{n-1}CH_2CH_2OC(O)CH=CH_2$
Fluorotelomer aldehydes	n:2 FTAL	$CF_3(CF_2)_{n-1}CH_2C(O)H$
	n:3 FTAL	$CF_3(CF_2)_{n-1}CH_2CH_2C(O)H$
	7:3 β-keto aldehyde	$CF_3(CF_2)_6C(O)CH_2C(O)H$
Fluorotelomer unsaturated aldehydes	n:2 FTUAL	$CF_3(CF_2)_{n-2}CF=CHC(O)H$
	n:3 FTUAL	$CF_3(CF_2)_{n-2}CF=CHCHC(O)H$
Fluorotelomer ketone	n:2 Ketone	$CF_3(CF_2)_{n-1}C(O)CH_3$
Fluorotelomer carboxylates	n:2 FTCA	$CF_3(CF_2)_{n-1}CH_2COO^-$
	n:3 FTCA	$CF_3(CF_2)_{n-1}CH_2CH_2COO^-$
Fluorotelomer unsaturated carboxylates	n:2 FTUCA	$CF_3(CF_2)_{n-2}CF=CHCOO^-$
	n:3 FTUCA	$CF_3(CF_2)_{n-1}CH=CHCOO^-$
3-Hydroxy-7:3 saturated fluorotelomer carboxylate	3-OH-7:3 FTCA	$CF_3(CF_2)_6CH(OH)CH_2COO^-$
3-Hydroxy-5:3 saturated fluorotelomer carboxylate	3-OH-5:3 FTCA	$CF_3(CF_2)_4CH(OH)CH_2COO^-$
7:3 Unsaturated amide	7:3 U Amide	$CF_3(CF_2)_6CH=CHC(O)NH_2$
7:3 Taurine amide	7:3 TA	$CF_3(CF_2)_6CH_2CH_2C(O)NHCH_2CHSO_3H$
Perfluoroalkyl carboxylates	PFCA	$CF_3(CF_2)_{n-1}COO^-$
Perfluorobutanoate	PFBA	$CF_3(CF_2)_2COO^-$
Perfluoropentanoate	PFPeA	$CF_3(CF_2)_3COO^-$
Perfluorohexanoate	PFHxA	$CF_3(CF_2)_4COO^-$
Perfluoroheptanoate	PFHpA	$CF_3(CF_2)_5COO^-$
Perfluorooctanoate	PFOA	$CF_3(CF_2)_6COO^-$
Perfluorononanoate	PFNA	$CF_3(CF_2)_7COO^-$
Perfluorodecanoate	PFDA	$CF_3(CF_2)_8COO^-$
Perfluoroundecanoate	PFUnA	$CF_3(CF_2)_9COO^-$
Perfluorododecanoate	PFDoA	$CF_3(CF_2)_{10}COO^-$
Perfluoropropanedioic acid	PFPrDia	$HOOCCF_2COOH$
Perfluorobutanedioic acid	PFBDiA	$HOOC(CF_2)_2COOH$
Perfluoropentanedioic acid	PFPeDiA	$HOOC(CF_2)_3COOH$
Perfluorohexanedioic acid	PFHxDiA	$HOOC(CF_2)_4COOH$
Polyfluorinated carboxylates		
2H,2H,8H,8H-perfluorododecanoate	2H,2H,8H,8H-PFDoA	$CF_3(CF_2)_3CH_2(CF_2)_5CH_2COO^-$
2H,2H,8H,8H-2-perfluorododecanoate	2H,2H,8H,8H-2-PFUDoA	$CF_3(CF_2)_3CH_2(CF_2)_4CF=CHCOO^-$
2H-perfluorooctanoate	2H-PFOA	$CF_3(CF_2)_5CFHCOO^-$
Conjugates		
Fluorotelomer alcohol glucuronide	n:2 FTOH-Gluc	$CF_3(CF_2)_nCH_2CH_2O$-Gluc
Fluorotelomer alcohol sulfate	n:2 FTOH-Sulf	$CF_3(CF_2)_nCH_2CH_2OSO_3^-$
8:2 Unsaturated fluorotelomer carboxylate glutathione conjugate	8:2 FTUCA-GSH	$CF_3(CF_2)_{n-2}C(SG)=CHCOO^-$
8:2 Unsaturated fluorotelomer alcohol glutathione conjugate	8:2 uFTOH-GSH	$CF_3(CF_2)_{n-2}C(SG)=CHCH_2OH$
8:2 Unsaturated fluorotelomer aldehyde glutathione conjugate	8:2 FTUAL-GSH	$CF_3(CF_2)_{n-2}C(SG)=CHCHO$
8:2 Unsaturated fluorotelomer carboxylate cysteine conjugate	8:2 uFTCA-SCys	$CF_3(CF_2)_{n-2}C(SCys)=CHCOO^-$
8:2 Unsaturated fluorotelomer alcohol cysteine conjugate	8:2 uFTOH-SCys	$CF_3(CF_2)_{n-2}C(SCys)=CHCH_2OH$
8:2 Unsaturated fluorotelomer carboxylate cysteinylglycine conjugate	8:2 uFTCA-SCysGly	$CF_3(CF_2)_{n-2}C(SCysGly)=CHCOO^-$
8:2 Unsaturated fluorotelomer alcohol cysteinylglycine conjugate	8:2 uFTOH-SCysGly	$CF_3(CF_2)_{n-2}C(SCysGly)=CHCH_2OH$
8:2 Unsaturated fluorotelomer carboxylate N-acetylcysteine conjugate	8:2 uFTCA-SCysNAcetyl	$CF_3(CF_2)_{n-2}C(SCysNAcetyl)=CHCOO^-$
8:2 Unsaturated fluorotelomer alcohol N-acetylecysteine conjugate	8:2 uFTOH-SCysNAcetyl	$CF_3(CF_2)_{n-2}C(SCysNAcetyl)=CHCH_2OH$
Fluorotelomer phosphates		
Fluorotelomer di-substituted phosphate	n:2 diPAPS	$(CF_3(CF_2)_{n-1}CH_2CH_2O)_2$-P(O)OH
Fluorotelomer mono-substituted phosphate	n:2 monoPAPS	$CF_3(CF_2)_{n-1}CH_2CH_2O$-P(O)$(OH)_2$

Gluc = glucuronic acid ($C_6H_9O_6$); SG = glutathione (S-$C_{10}H_{16}N_3O_6$); SCys = cysteine (S-$C_3H_6NO_2$); SCysGly = cysteinylglycine (S-$C_5H_9N_2O_3$); SCysNAcetyl = N-acetylcysteine (S-$C_5H_8NO_3$).

Reported biotransformation rates are presented in Table 7. No attempt is made to critique the proposed pathways, but fundamental similarities and differences are highlighted.

MICROBIAL

8:2 fluorotelomer alcohol

Dinglasan et al. [12] investigated the biotransformation of 8:2 FTOH in a mixed microbial culture (sediment and groundwater). The parent compound and volatile metabolites were monitored by gas chromagraphy–mass spectrometry (GC-MS) and nonvolatile metabolites monitored by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The study found that 85% of the parent 8:2 FTOH was degraded by day 7. No degradation was observed in the sterile control vessels, indicating that microbial degradation was primarily responsible for the observed degradation. Concurrent with the depletion of 8:2 FTOH was the production of the 8:2 fluorotelomer carboxylate (FTCA) and 8:2 fluorotelomer unsaturated carboxylate (FTUCA). The 8:2 fluorotelomer aldehyde (FTAL) was also identified as a transient intermediate between the 8:2 FTOH and 8:2 FTCA, but levels of this metabolite could not be confidently quantified. The 8:2 FTCA was also a transient metabolite, and depletion of this intermediate was coincident with the increase in 8:2 FTUCA levels. The authors suggested that the 8:2 FTCA degradation could have proceeded via abiotic

Table 2. Summary of metabolites observed in biotransformation studies with microbial systems: 8:2 FTOH and related intermediates, fluorotelomer monomers, and fluorotelomer-based polymers

Model system	Substrate	8:2 FTOH	8:2 FTAL	9:3 FTCA	8:2 FTCA	8:2 FTUCA	7:3 FTCA	7:3 FTUCA	7:2 sFTOH	7:2 Ketone	3-OH-7:3 FTCA	7:3 U Amide	2H-PFOA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	Comments	Reference
Mixed microbial culture, aerobic, closed system	8:2 FTOH[a]		X[b]		X[b]	X[b]										X[a]				[d]	[12]
Activated sludge (domestic), aerobic, closed system	^{14}C 8:2 FTOH[b]				X[b]	X[b]	X[c]									X[a]				[e]	[13]
Mixed bacterial culture, closed	^{14}C 8:2 FTOH[b]		X[b]		X[b]	X[b]	X[b]	X[b]	X[b]			X[c]		X[a]		X[a]				[f]	[14]
Digester sludge, anaerobic	^{14}C 8:2 FTOH[b]				X[b]	X[b]	X[b]									X[a]				[g]	[15]
Soil microsomes, closed bottle	8:2 FTOH[b]				X[b]	X[b]	X[b]	X[b]	X[b]					X[a]	X[a]	X[a]	X[a]			[h]	[16]
Isolated soil bacterial cultures (*Pseduomonas*), open bottle	8:2 FTOH[b]				X[b]	X[b]	X[b]	X[b]						X[a]	X[a]	X[a]	X[a]			[h]	[16]
Isolated soil bacterial cultures (*Pseudomonas*), closed bottle	8:2 FTOH[b]				X[b]	X[b]	X[b]	X[b]	X[b]					X[a]	X[a]	X[a]	X[a]			[h]	[16]
Aerobic soil micocosms, closed bottle & continous air flow	^{14}C 8:2 FTOH[b]		X[b]			X[b]	X[b]	X[b]	X[b]	X[c]	X[c]		X[c]	X[a]		X[a]				[i]	[17]
	8:2 FTUCA[b]						X[b]	X[b]	X[b]							X[a]					
	7:3 FTUCA[b]						X[b]				X[c]										
	7:3 FTCA[b]																				
	7:2 sFTOH[b]															X[a]					
Individual bacterial strains (*Pseudomonas butanovora, Pseudomonas oleovorans*)	8:2 FTOH[a]				X[b]	X[b]	X[b]	X[b]	X[b]	X[a]				X[a]		X[a]				[g]	[18]
Soil microcosm, aerobic conditions, closed	8:2 fluorotelomer stearate	X[b]			X[b]	X[b]	X[b]	X[b]	X[b]					X[a]	X[a]	X[a]				[g]	[22]
Soil microcosm, aerobic conditions, closed	8:2 fluorotelomer stearate, 8:2 fluorotelomer citrate	X[b]			X[b]	X[b]	X[b]		X[b]					X[a]	X[a]	X[a]				[g]	[28]
Soil-water microcosms, closed bottle	8:2 FTCA[b]															X[a]				[g]	[31]
	8:2 FTUCA[b]						X[a]								X[a]	X[a]					
	10:2 FTCA[b]																	X[a]			
	10:2 FTUCA[b]			X[a]													X[a]	X[a]			
Soil microcosm, aerobic conditions	FTAC-based polymer[b]	X[b]			X[b]	X[b]	X[b]		X[b]							X[a]				[j]	[36]
Soil microcosm, aerobic conditions	FTAC-based polymer[b]												X[c]							[k]	[37]
Soil microcosm, aerobic conditions	FT urethane polymer[b]	X[b]			X[b]	X[b]	X[b]		X[b]							X[a]	X[a]	X[a]	X[a]	[l]	[38]

[a]Compound purchased from a commercial source.
[b]Compound synthesized.
[c]Identified using mass spectrometry techniques.
[d]8:2 FTOH monitored in headspace, aqueous concentrations calculated using Henry's Law Constant. Volatile metabolites by gas chromagraphy–mass spectrometry (GC-MS), non-volatiles metabolites by liquid chromatography–tandem mass spectrometry (LC-MS/MS).
[e]^{14}C 8:2 FTOH custom synthesized. Product quantification by LC-ARC and LC-MS/MS, product identification by LC-QTOF.
[f]^{14}C 8:2 FTOH custom synthesized. ^{14}C 8:2 FTOH and ^{14}C metabolites quantified by LC-ARC, product identification by LC-QTOF, GC-TOF. Mixed bacterial culture initially conditioned on 8:2 FTOH. Experiments also conducted with sewage sludge under closed & open conditions, but metabolites not identified.
[g]Metabolite analysis by LC-MS/MS.
[h]Synthesized compounds provided by E.I. du Pont de Nemours and Company. Instrumental analysis performed by LC-MS/MS. PFNA observed near or at detection limits in the soil experiment.
[i]Metabolite analysis by LC-ARC, LC-QTOF and GC-MS. 8:2 FTOH summary from all three soil treatments (Sassafras, Manning, Chalmers) under closed bottle, flow-through conditions. 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, 7:2 sFTOH experiments conducted in Sassafras soil only, closed bottle conditions. Incubation with 7:3 FTCA did not yield any metabolites.
[j]8:2 FTOH analyzed by GC-MS, non-volatile metabolites by LC-MS/MS. FTAc monomer used synthesized from 6:2 FTOH (1%), 8:2 FTOH (55%),10:2 FTOH (29%), 12:2 FTOH (10%), 14:2 FTOH & larger (5%)
[k]FTAC-based polymer was synthesized by a major fluorotelomer polymer manufacturer. Polymer synthesized using various chain-lengths of FTACs; metabolites by LC-MS/MS; FTACs, FTOHs analyzed by GC-MS, non-volatile.
[l]FTOHs used in polymer starting material: 8:2 FTOH (31%), 10:2 FTOH (18%), 12:2 FTOH (9%), 14:2 FTOH & greater (8%). Summary is combined results from analyzed by GC-MS, non-volatile all soil treatments (Alfisol, Inceptisol, Mollisol, Ultisol).
See Table 1 for definition of abbreviations.

Table 3. Summary of metabolites observed in biotransformation studies with microbial systems: 6:2 FTOH and related intermediates

Model system	Substrate	6:2 FTOH	6:2 FTAL	6:2 FTCA	6:2 FTUCA	5:3 FTCA	5:3 FTUCA	5:2 FTCA	5:2 FTUCA	4:3 FTCA	4:3 FTUCA	3:3 FTCA	5:2 sFTOH	5:2 Ketone	5:3 Ketone Aldehyde	3-OH-5:3 FTCA	α-OH-5:3 FTCA	5:3 U Amide	PFBA	PFPeA	PFHxA	PFHpA	Comments	Reference
Digester sludge, anaerobic	6:2 FTOH[a]			X[b]	X[b]	X[b]							X[b]								X[a]		[d]	[15]
Individual bacterial strains (*Pseudomonas butanovora, Pseudomonas oleovorans*)	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[b]						X[b]	X[a]					X[a]		X[a]		[d]	[18]
Mixed bacterial culture, closed bottle	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[c]						X[b]	X[a]	X[c]	X[c]		X[c]	X[a]	X[a]	X[a]		[e]	[21]
	5:2 ketone[a]												X[b]							X[a]	X[a]			
Aerobic soil micocosms, closed bottle	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[c]			X[b]			X[b]	X[a]		X[c]			X[a]	X[a]	X[a]		[e]	[21]
	5:2 ketone[a]												X[b]							X[a]	X[a]			
	5:2 sFTOH[b]													X[a]						X[a]	X[a]			
	5:3 FTCA[b]									X[b]														
Aerobic soil micocosms, continuous headspace air flow	^{14}C 6:2 FTOH[b]			X[b]	X[b]	X[b]	X[c]			X[b]			X[b]	X[a]		X[c]			X[a]	X[a]	X[a]		[f]	[22]
Aerobic sediment microcosms, closed bottle	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[c]			X[b]			X[b]	X[a]					X[a]	X[a]	X[a]		[d]	[23]
Individual bacterial strain (*Mycobacterium vaccae* JOB5)	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[b]						X[b]	X[a]						X[a]	X[a]		[d]	[24]
Individual bacterial strain (*Pseudomonas fluorescens* DSM 8341)	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[b]						X[b]	X[a]					X[a]	X[a]	X[a]		[d]	[24]
Individual bacterial strains (*Pseudomonas butanovora*)	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[b]						X[b]	X[a]						X[a]	X[a]		[d]	[24]
Individual bacterial strains (*Pseudomonas oleovorans*)	6:2 FTOH[a]			X[b]	X[b]	X[b]	X[b]						X[b]	X[a]					X[a]	X[a]	X[a]		[d]	[24]
Activated sludge (domestic), aerobic, closed system	5:3 FTCA[b]							X[b]	X[b]	X[b]	X[b]	X[a]					X[b]		X[a]	X[a]			[e]	[26]
Activated sludge, closed bottle	6:2 FtS[b]				X[b]	X[b]							X[b]	X[a]					X[a]	X[a]	X[a]		[g]	[30]
Raw wastewater/sewage sludge, flow-through conditions	6:2 monoPAP, 6:2 diPAP[b]	X[a]		X[a]	X[a]	X[c]														X[a]	X[a]	X[a]	[h]	[32]

[a]Compound purchased from a commercial source.
[b]Compound synthesized.
[c]Identified using mass spectrometry techniques.
[d]Metabolite analysis by LC-MS/MS.
[e]Metabolites quantified by LC-MS/MS, identified by LQT Orbitrap.
[f]^{14}C 8:2 FTOH custom synthesized by PerkinElmer. Product quantification by LC-ARC, product identification by LC-MS/MS.
[g]6:2 FtS synthesized by DuPont.
[h]Metabolite analysis by GC-MS for 6:2 FTOH, non-volatile metabolites by LC-MS/MS. 6:2 monoPAP and 6:2 diPAP dosed separately, results were similar between treatments. 6:2 monoPAP was formed from 6:2 diPAP dose.
See Table 1 for definition of abbreviations.

hydrolysis or by enzymatic mechanisms. However, it was noted that the abiotic degradation of FTCAs proceeds at a slower rate (half-lives greater than 1 wk) than that observed in the microbial study. Perfluorooctanoate was formed during the experiment, reaching approximately 3% of the total mass by day 81. Perfluorononanoate (PFNA) was not observed, indicating that α-oxidation did not occur in the microbial system. Overall, a poor mass balance was achieved with only approximately 55% of the products accounted for at the end of the experiment. The reduced mass balance may have been the result of the inability to quantify the 8:2 FTAL, the production of other unidentified metabolites, and the potential production of nonextractable metabolites that were covalently bound to biological macromolecules. A biodegradation scheme was developed (Figure 1), building on the experimental results as well as the findings of Hagen et al. [11]. The first biotransformation step was the oxidation of 8:2 FTOH by an alcohol dehydrogenase enzyme to form the 8:2 FTAL, which is subsequently oxidized by an aldehyde dehydrogenase to yield the 8:2 FTCA. In addition, the 8:2 allylic FTOH (an impurity in the 8:2 FTOH commercial product) could form the 8:2 FTUCA via the 8:2 fluorotelomer unsaturated aldehyde (FTUAL). The 8:2 FTCA can enter β-oxidation,

Table 4. Summary of metabolites observed in biotransformation studies with microbial systems: DTFA and fluorotelomer ethyoxlates

Model system	Substrate	2H, 2H, 8H, 8H- PFDoA	2H, 8H, 8H-2-PFUDoA	PFBDiA	PFPeDiA	PFHxDiA	PFBA	PFPeA	PFHxA	PFOA	Comments	Reference
Activated sludge, closed bottle	DTFA[b]	X[b]	X[b]				X[a]	X[a]			[c]	[29]
Activated sludge, flow-through conditions	DTFA[b]	X[b]	X[b]	X[a]	X[a]	X[a]	X[a]	X[a]			[c]	
WWTP effluent, aerobic conditions	Zonyl FSH (mix of fluorotelomer ethoxylates)[a]								X[a]	X[a]	[d]	[34]

[a]Compound purchased from a commerical source.
[b]Compound synthesized.
[c]DTFA (1H, 1H, 2H, 2H, 8H, 8H- perfluorododecanol) synthesized by UNIMATEC. Metabolite analysis by LC-MS.
[d]Metabolite analysis by LC-MS/MS. PFHxA and PFOA formation attributed to residual FTOHs in system. Primary metabolites were fluorotelomer ethoxylate carboxylates.
See Table 1 for definition of abbreviations.

ultimately yielding PFOA. As stated, the 8:2 FTCA could degrade to the 8:2 FTUCA through either abiotic or biotic mechanisms. The formation of PFOA from the 8:2 FTUCA would be slow because of the high thermodynamic cost of oxidizing the FTUCA β-carbon.

Wang et al. [13] examined the biodegradation of ^{14}C-labeled 8:2 FTOH [$CF_3(CF_2)_6{}^{14}CF_2CH_2CH_2OH$] in activated sewage sludge under aerobic, closed conditions for 28 d. The use of the radiolabeled parent compound allowed the researchers to obtain mass balance. At the end of the 28-d experiment, 16% of the parent 8:2 FTOH was unchanged. Furthermore, a significant portion (41%) of the total 8:2 FTOH was adsorbed to the polytetrafluoroethylene septa. The metabolites observed in the active vessels were the 8:2 FTCA, 8:2 FTUCA, PFOA, and the novel metabolite 7:3 FTCA. At the conclusion of the experiment, 27% of the mass balance was 8:2 FTCA, 6.0% was 8:2 FTUCA, and 2.3% was 7:3 FTCA. PFOA accounted for 2.1% of the total mass balance. Several other unidentified metabolites were also observed, but these compounds each contributed less than 1% of the total mass balance. Similar to findings by Dinglasan et al. [12], PFNA was not observed, indicating that α-oxidation of the 8:2 FTCA did not occur. No metabolites were identified in the abiotic control vessels. Based on the experimental results, the authors proposed an 8:2 FTOH biotransformation reaction scheme. The first degradation step was postulated to be the formation of 8:2 FTAL via alcohol dehydrogenase, although this analyte was not observed during the experiment. This is followed by formation of the 8:2 FTCA, which may be mediated by aldehyde dehydrogenase. The 8:2 FTCA can react to yield the 8:2 FTUCA, which can undergo monooxygenase-mediated reactions to yield PFOA. The proton deficiency of the 8:2 FTCA and 8:2 FTUCA effectively prevents these compounds from entering the β-oxidation cycle; thus a novel mechanism for PFOA formation was proposed. The 8:2 FTCA can form the 7:3 FTCA, through an unknown mechanism, which can subsequently act as a substrate for β-oxidation and ultimately form PFOA.

Wang et al. [14] extended their earlier sewage sludge experiments by investigating the biotransformation of ^{14}C-8:2 FTOH in mixed bacterial culture and activated sludge. The mixed bacterial culture collected had previously been exposed to FTOHs and thus was presumably acclimated to degrade telomer-based compounds. Bottles were subject to either closed or continuous air flow conditions for up to 4 mo. No degradation was observed in the sterile controls. Similar to their previous experiment [13], significant 8:2 FTOH sorption to the polytetrafluoroethylene septa in the bottles was observed (5.2%–17.4% of mass balance). Metabolites were identified in the mixed bacterial culture only and included 5 previously reported compounds (8:2 FTAL, 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, and PFOA) as well as 2 novel metabolites (7:2 secondary fluorotelomer alcohol [sFTOH] and 7:3 unsaturated amide). In addition, perfluorohexanoate (PFHxA) was formed in low yields, approximately 1% of the initial 8:2 FTOH concentration, which the authors suggested was evidence for degradation of the fluorinated chain. The 7:2 sFTOH was the most abundant metabolite observed, constituting 14% of the mass balance at day 90. Perfluorooctanoate was formed in comparatively lower yields, accounting for 6% of the mass balance by the conclusion of the experiment. Consistent with their previous study [13], PFNA was not detected in the active vessels, suggesting that α-oxidation of the 8:2 FTCA did not occur. Based on the newly identified metabolites, a novel series of degradation pathways was proposed [14]. The initial degradation steps were consistent with the previous study [13]—that is, the oxidation of the 8:2 FTOH to 8:2 FTAL by alcohol dehydrogenase, followed by oxidation to 8:2 FTCA then conversion to the 8:2 FTUCA by HF elimination. It was postulated that the 8:2 FTUCA represents a key branching point in the microbial degradation of 8:2 FTOH. In the first branch, the 8:2 FTUCA is enzymatically decarboxylated to form the 8:1 FTO, which is defluorinated to yield the 7:2 FTO. The 7:2 FTO could be oxidized through cytochrome P450 reactions to form the 7:2 sFTOH. It was proposed that the 7:2 sFTOH could yield PFOA, although the mechanism is uncertain. The second branch involves defluorination of the 8:2 FTUCA to the 7:3 FTUCA, which can yield the 7:3 unsaturated amide via transaminase. The 7:3 unsaturated amide could form PFOA; however, the mechanism was not explained. The third branch postulates that after formation of the 7:3 FTUCA from the 8:2 FTUCA, the 7:3

Table 5. Summary of metabolites observed in biotransformation studies with rats and mice

Model system	Substrate	8:2 FTAL	8:2 FTUAL	7:3 FTAL	7:3 FTUAL	10:2 FTCA	8:2 FTCA	8:2 FTUCA	7:3 FTCA	7:3 FTUCA	5:3 FTCA	7:2 sFTOH	7:2 Ketone	7:3 β-Keto Aldehyde	7:3 taurine amide	8:2 FTOH-Gluc	4:2 FTOH-Gluc	7:2 sFTOH-Gluc	8:2 FTOH Sulfate	4:2 FTOH Sulfate	8:2 FTUCA-GSH	8:2 FTUAL-GSH	8:2 uFTOH-GSH	8:2 uFTOH-SCys	8:2 uFTUCA-SCys	8:2 uFTOH N-Acetyl Cysteine	8:2 uFTUCA N-Acetyl Cysteine	8:2 uFTOH-SCysGly	8:2 FTUCA-SCysGly	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	Comments	Reference
Male CD rats, gavage dose	8:2 FTOH[a]						X[a]																										X[a]			d	[11]
Sprague-Dawley rats, intraperitoneal injection	8:2 FTOH[a]						X[b]	X[b]	X[c]	X[c]						X[c]			X[c]														X[a]	X[a]		e	[39]
Hepatocytes, Sprague-Dawley rats	8:2 FTOH[a]	X[b]	X[c]				X[b]	X[b]								X[c]			X[c]		X[c]	X[c]											X[a]	X[a]			
	8:2 FTCA[b]							X[b]													X[c]												X[a]	X[a]			
	8:2 FTUCA[b]																																X[a]				
	8:2 FTAL[b]		X[c]				X[b]	X[b]																									X[a]	X[a]			
Male ddY mice, intraperitoneal injection	8:2 FTOH[a]						X[b]																										X[a]	X[a]		f	[40]
Male and female Crl:CD rats, oral gavage dosed	^{14}C 8:2 FTOH[b]						X[b]	X[b]	X[c]	X[c]						X[c]		X[c]			X[c]		X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]			X[a]	X[a]	X[a]	X[a]		g	[20]
Male and female Crl:CD rats, oral gavage dosed	^{14}C 8:2 FTOH, 8:2 FTOH[b]			X[c]	X[c]				X[b]			X[c]	X[c]		X[c]	X[c]		X[c]	X[c]				X[c]		X[c]	X[c]						X[a]	X[a]	X[a]	X[a]	X[a]	h	[42]
Rat hepatocytes	^{14}C 8:2 FTOH[b]	X[b]	X[b]		X[c]		X[b]	X[b]	X[b]	X[b]		X[c]	X[c]	X[c]	X[c]	X[c]			X[c]		X[c]		X[c]								X[a]	X[a]	X[a]	X[a]	X[a]		i	[19]
	8:2 FTCA[b]								X[b]	X[b]		X[c]	X[c]		X[c]																X[a]		X[a]	X[a]				
	8:2 FTUCA[b]								X[b]	X[b]					X[c]																				X[a]				
	7:3 FTCA[b]									X[b]					X[c]																								
	7:3 FTUCA[b]								X[b]						X[c]																								
Rat microsomes, liver	^{14}C 8:2 FTOH[b]		X[b]	X[c]	X[c]		X[b]	X[b]	X[b]	X[b]			X[c]	X[c]																	X[a]	X[a]	X[a]	X[a]	X[a]			
	8:2 FTCA[b]																																						
	8:2 FTUCA[b]																																						
	7:3 FTCA[b]																																						
	7:3 FTUCA[b]																																						
Mouse hepatocytes	^{14}C 8:2 FTOH[b]			X[c]	X[c]		X[b]	X[b]	X[b]	X[b]		X[c]	X[c]	X[c]	X[c]								X[c]								X[a]	X[a]	X[a]	X[a]	X[a]			
	8:2 FTCA[b]								X[b]	X[b]		X[c]	X[c]		X[c]																		X[a]	X[a]				
	8:2 FTUCA[b]								X[b]	X[b]		X[c]	X[c]		X[c]																			X[a]				
	7:3 FTCA[b]									X[b]					X[c]																								
	7:3 FTUCA[b]								X[b]						X[c]																								
Mouse microsomes, liver	^{14}C 8:2 FTOH[b]			X[c]	X[c]		X[b]	X[b]	X[b]				X[c]	X[c]									X[c]								X[a]	X[a]	X[a]	X[a]	X[a]			
	8:2 FTCA[b]																																						
	8:2 FTUCA[b]																																						
	7:3 FTCA[b]																																						
	7:3 FTUCA[b]																																						
Human hepatocytes	^{14}C 8:2 FTOH[b]						X[b]	X[b]	X[b]					X[c]		X[c]							X[c]								X[a]	X[a]	X[a]	X[a]	X[a]			
	8:2 FTCA[b]								X[b]	X[b]		X[c]	X[c]		X[c]																				X[a]				
	8:2 FTUCA[b]								X[b]	X[b]			X[c]		X[c]																				X[a]				
	7:3 FTCA[b]														X[c]																								
	7:3 FTUCA[b]								X[b]						X[c]																								
Human microsomes, liver	^{14}C 8:2 FTOH[b]						X[b]		X[b]																						X[a]	X[a]	X[a]					

(*Continued*)

Table 5. (*Continued*)

Model system	Substrate	8:2 FTAL	8:2 FTUAL	7:3 FTAL	7:3 FTUAL	10:2 FTCA	8:2 FTCA	8:2 FTUCA	7:3 FTCA	7:3 FTUCA	5:3 FTCA	7:2 sFTOH	7:2 Ketone	7:3 β-Keto Aldehyde	7:3 taurine amide	8:2 FTOH-Gluc	4:2 FTOH-Gluc	7:2 sFTOH-Gluc	8:2 FTOH Sulfate	4:2 FTOH Sulfate	8:2 FTUCA-GSH	8:2 FTUAL-GSH	8:2 uFTOH-GSH	8:2 uFTOH-SCys	8:2 uFTUCA-SCys	8:2 uFTOH N-Acetyl Cysteine	8:2 uFTUCA N-Acetyl Cysteine	8:2 uFTOH-SCysGly	8:2 FTUCA-SCysGly	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	Comments	Reference
	8:2 FTCA[b]																																				
	8:2 FTUCA[b]																																				
	7:3 FTCA[b]																																				
	7:3 FTUCA[b]																																				
CD-1 mice, gavage dose	8:2 FTOH[a]																																X[a]	X[a]		[j]	[41]
Male and female Crl:CD rats, inhalation	8:2 FTOH[b]						X[b]	X[b]	X[b]	X[b]																				X[a]	X[a]	X[a]	X[a]				[43]
Male Sprague-Dawley rats, oral gavage	8:2 monoPAPs[b]						X[a]	X[a]	X[a]																							X[a]	X[a]			[k]	[44]
	8:2 diPAPs$_b$						X$_a$	X$_a$	X$_a$																							X$_a$	X$_a$					
Sprague-Dawley rats	4:2, 6:2, 8:2, 10:2 monoPAPs[b]								X[a]		X[a]						X[c]			X[c]										X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	[l]	[45]
	4:2, 6:2, 8:2, 10:2 diPAPs[b]										X[a]						X[c]			X[c]										X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	[m]	

[a]Compound purchased from a commercial source.
[b]Compound synthesized.
[c]Identified using mass spectrometry techniques.
[d]Analysis by GC-microwave plasma detector. 8:2 FTCA supplied by American Hoechst Corporation. PFOA assumed to have been purchased (based on previous papers from the authors). Unknown metabolite also detected.
[e]Metabolites analyzed by LC-MS/MS.
[f]Metabolites analyzed by LC-fluorescence detection after derivatization.
[g]^{14}C 8:2 FTOH synthesized by Perkin-Elmer. Product quantification by LC-ARC, product identification by LC-QTOF. Metabolites detected represent the summary of urine, feces, bile and plasma.
[h]^{14}C 8:2 FTOH synthesized by Perkin-Elmer. Product quantification and identification by LC-ARC and LC-MS/MS. Metabolites detected represent the summary of urine, feces, liver, kidney, fat and plasma in males and females.
[i]^{14}C 8:2 FTOH synthesized by Perkin-Elmer. Product quantification and identification by LC-ARC and LC-MS/MS.
[j]Metabolites analyzed by GC-MS after derivatization.
[k]Metabolites analyzed by LC-MS/MS. Metabolites formed were identical in both doses. 8:2 monoPAPs also detected as a metabolite from 8:2 diPAPS dosing.
[l]Combination of intravenous and gavage dosing. Also very infrequent detections of 4:2 FTCA & FTUCA, 6:2 FTCA & FTUCA, 4:2 FTOH suflate & glucuronide, 6:2 glucuronide, 8:2 sulfate, 10:2 sulfate.
[m]Combination of intravenous and gavage dosing. Also very infrequent detections of 4:2 FTCA & FTUCA, 6:2 FTCA & FTUCA, 8:2 FTUCA, 10:2 FTCA and 10:2 FTOH sulfate.
See Table 1 for definition of abbreviations.

Table 6. Summary of metabolites observed in biotransformation studies with fish

Model System	Substrate	8:2 FTOH	10:2 FTCA	10:2 FTUCA	8:2 FTCA	8:2 FTUCA	7:3 FTCA	7:3 FTUCA	7:2 sFTOH	7:2 Ketone	7:3 β-Keto Aldehyde	7:3 taurine amide	8:2 FTOH-Gluc	8:2 FTUCA-GSH	8:2 uFTOH-GSH	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	Comments	Reference
Rainbow trout, dietary exposure	8:2 FTOH[a]		X[a]	X[a]	X[a]	X[a]													X[a]		X[a]	d	[46]
Rainbow trout, dietary exposure	8:2 FTAC[a]	X[a]			X[a]	X[a]	X[a]	X[c]					X[b]					X[a]	X[a]	X[a]		e	[47]
Rainbow trout, liver S9 fraction	8:2 FTAC[a]	X[a]																				f	[48]
Rainbow trout, intestine S9 fraction	8:2 FTAC[a]	X[a]																				f	[48]
Rainbow trout, dietary exposure	8:2 FTCA[b]					X[a]	X[a]	X[c]										X[a]	X[a]	X[a]		g	[33]
	8:2 FTUCA[b]						X[a]	X[c]						X[c]					X[a]				
	7:3 FTCA[b]							X[c]										X[a]					
Rainbow trout hepatocytes	^{14}C 8:2 FTOH[b]				X[b]	X[b]	X[b]				X[c]		X[c]		X[c]	X[a]	X[a]	X[a]	X[a]	X[a]		h	[19]
	8:2 FTCA[b]						X[b]	X[b]	X[c]	X[c]		X[c]							X[a]				
	8:2 FTUCA[b]						X[b]	X[b]				X[c]							X[a]				
	7:3 FTCA[b]							X[b]				X[c]											
	7:3 FTUCA[b]						X[b]					X[c]											

[a]Compound purchased from a commercial source.
[b]Compound synthesized.
[c]Identified using mass spectrometry techniques.
[d]Metabolite analysis by LC-MS/MS.
[e]8:2 FTOH analyzed by GC-MS, non-volatile metabolites by LC-MS/MS. Results are a combination of blood, liver, kidney, bile and feces.
[f]8:2 FTOH analyzed by GC-MS, non-volatile metabolites not monitored (system not optimized for oxidation metabolism).
[g]Metabolite analysis by LC-MS/MS.
[h]^{14}C 8:2 FTOH synthesized by Perkin-Elmer. Product quantification and identification by LC-ARC and LC-MS/MS.
See Table 1 for definition of abbreviations.

FTUCA is reduced to the 7:3 FTCA, which acts as substrate for β-oxidation, ultimately yielding PFOA. Consistent with their previous mechanism [13], the 8:2 FTCA and 8:2 FTUCA could not proceed through β-oxidation.

Zhang et al. [15] reported the first study of 8:2 FTOH biodegradation under anaerobic conditions. The 6:2 FTOH biodegradation was also investigated, and details are presented in the *6:2 fluorotelomer alcohol* subsection. The study may be relevant for conditions such as landfill leachate and anaerobic wastewater treatment plant (WTTP) sludge. The experiment used anaerobic digester sludge that was collected from a domestic WTTP in Delaware, USA. The incubations were dosed with ^{14}C-8:2 FTOH, and the study continued for 181 d. Similar to the 6:2 FTOH incubations, the 8:2 FTOH also showed a slower degradation half-life (approximately 145 d) compared with previous studies in aerobic soil and sludge [14,16,17]. In addition, the x:3 FTCA (7:3 FTCA) was the major degradation product (27 mol% at 181 d). The 8:2 FTCA was another major degradation product, but this metabolite was transient, peaking at 23 mol% on day 90. The 8:2 FTUCA was a minor metabolite, accounting for 5.1 mol% 181 d. The 7:2 sFTOH, suggested by the authors as the direct precursor to PFOA [17], was not observed during the study. As a result, very low yields of PFOA were observed (0.3 mol%). As noted by Zhang et al. [15], the PFOA yield is much lower than measured from aerobic soil [17] and aerobic activated sludge [14]. Both the 6:2 and 8:2 studies showed that the x:3 FTCAs (5:3 and 7:3 FTCAs) were stable during the anaerobic experiments. Interestingly, 6:2 FTUCA incubations did not yield the expected 5:2 sFTOH, the suspected direct precursor to PFHxA. These results suggest that the low yield of PFCAs, during the anaerobic studies, may be attributable to the very low rate of the n:2 FTCA → x:2 sFTOH step. A detailed biotransformation pathway was presented by Zhang et al. [15].

Liu et al. [16] investigated the aerobic biotransformation of 8:2 FTOH and metabolite profile in a clay loam soil as well as with 2 isolated soil bacterial cultures. In addition, the influence of 3 carrier solvents (ethanol, octanol, 1,4-dioxane), which may act as carbon sources, was investigated. In the soil studies, the fastest 8:2 FTOH biotransformation rate was observed when 1,4-dioxane was the carrier solvent, followed by octanol. Assuming a first-order reaction, the 8:2 FTOH biotransformation rates were 0.28 d^{-1} with 1,4-dioxane, 0.18 d^{-1} with ethanol, and 0.13 d^{-1} for octanol. Some loss of 8:2 FTOH (12%) was observed in the sterile controls. The authors attributed this loss to irreversible sorption, because only trace levels of metabolites were detected. Metabolites formed from the 8:2 FTOH biotransformation included 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, 7:2 sFTOH, PFHxA, perfluoroheptanoate (PFHpA), and PFOA. The metabolite profile was consistent between the 3 carrier solvents. At the end of the experiment (day 7), the dominant metabolites were 8:2 FTUCA (6.1–8.4 mol%, relative to applied 8:2 FTOH) followed by 8:2 FTCA (2.3–2.9 mol%) and 7:2 sFTOH (2.3–3.5 mol%). Formation of PFOA was comparatively low (0.6–0.8%). The mass balance decreased with time, which the authors attributed to irreversibly

Table 7. Biotransformation rates for fluorotelomer-based compounds

Model system	Substrate	Biological half-life	Reference
8:2 FTOH			
Mixed microbial culture, aerobic, closed system	8:2 FTOH	0.2 d/mg biomass protein (initial half-life)	[12]
Soil microsomes, closed bottle	8:2 FTOH	0.13-0.28 d^{-1} (biotransformation rate)	[16]
Aerobic soil microcosms, closed bottle and continuous air flow	^{14}C 8:2 FTOH	<7 days	
Anaerobic digestor sludge	^{14}C 8:2 FTOH	~145 d	[15]
Male and female rats, oral gavage dosed	^{14}C 8:2 FTOH	1-5 h	[20]
Hepatocytes, rat	^{14}C 8:2 FTOH	9.9 min	[19]
Microsomes, rat	^{14}C 8:2 FTOH	14 min	[19]
Cytosol, rat	^{14}C 8:2 FTOH	14.2 min	[19]
Hepatocytes, mouse	^{14}C 8:2 FTOH	12.7 min	[19]
Microsomes, mouse	^{14}C 8:2 FTOH	7.89 min	[19]
Cytosol, mouse	^{14}C 8:2 FTOH	10.6 min	[19]
Hepatocytes, human	^{14}C 8:2 FTOH	35.9 min	[19]
Microsomes, human	^{14}C 8:2 FTOH	33.7 min	[19]
Cytosol, human	^{14}C 8:2 FTOH	13.8 min	[19]
Hepatocytes, trout	^{14}C 8:2 FTOH	103 min	[19]
6:2 FTOH			
Mixed bacterial culture, closed bottle	6:2 FTOH	1.3 d	[21]
Aerobic soil microcosms, closed bottle	6:2 FTOH	1.6 d	[21]
Aerobic soil microcosms, continuous headspace air flow	^{14}C 6:2 FTOH	1.3 d	[22]
Aerobic sediment microcosms, closed bottle	6:2 FTOH	1.8 d	[23]
Anaerobic digestor sludge	6:2 FTOH	~30 d	[15]
Fluorotelomer carboxylates			
Rainbow trout, dietary exposure	7:3 FTCA	5.1 d	[33]
Rainbow trout, dietary exposure	8:2 FTCA	1.2 d	[33]
Rainbow trout, dietary exposure	8:2 FTUCA	0.39 d	[33]
Fluorotelomer phosphates			
Sprague-Dawley rats	4:2 diPAP	1.6-2.0 d (gavage and intravenous dose)	[45]
Sprague-Dawley rats	6:2 diPAP	2.1-3.9 d (gavage and intravenous dose)	[45]
Sprague-Dawley rats	8:2 diPAP	2.4-4.8 (gavage and intravenous dose)	[45]
Sprague-Dawley rats	10:2 diPAP	3.3 d (intravenous dose only)	[45]
Fluorotelomer monomers			
Soil microcosm, aerobic conditions, closed	8:2 Fluorotelomer stearate	10.3 d	[27]
Soil microcosm, aerobic conditions, closed	8:2 Fluorotelomer stearate	5.4-28.4 d	[28]
Fluorotelomer polymers			
Soil microcosm, aerobic conditions	FTAC-based polymer	1200-1700 y	[36]
Soil microcosm, aerobic conditions	FTAC-based polymer	10-17 y (fine grain), 870-1400 y (course grain)	[37]
Soil microcosm, aerobic conditions	FT urethane polymer	102 y	[38]

See Table 1 for definition of abbreviations.

bound metabolites and unmonitored metabolites such as fluorotelomer aldehydes. The 2 soil bacterial strains investigated, *Pseudomonas* species OCY4 and OCW4, were capable of biotransforming 8:2 FTOH without prior exposure or acclimation to 8:2 FTOH. The bacteria did not appear to use 8:2 FTOH as an energy source. The degradation yielded all of the metabolites observed in the soil incubations, with the exception of 7:2 sFTOH in the open system. Similar to the soil incubations, mass balance was not achieved, potentially because of unknown or unmonitored metabolites. The authors postulated that a partial β-oxidation mechanism was responsible for the production of PFOA from 8:2 FTCA or 8:2 FTUCA.

Kim et al. [18] investigated the biotransformation of 4:2, 6:2, and 8:2 FTOH by 2 strains of alkane-degrading microorganisms, *Pseudomonas butanovora* and *Pseudomonas oleovorans*. Short-term experiments (until 24 h for 6:2 and 8:2 FTOH with *P. oleovorans*; until 48 h for 4:2 FTOH with *P. oleovorans*; and until 3 d for 4:2, 6:2, 8:2 FTOH with *P. butanovora*) were conducted and analyzed by GC-MS and GC–electron capture detector (ECD). Long-term experiments were performed for the determination of FTOH biotransformation products using LC-MS/MS. Active and control vessels were sampled at 0 d, 0.5 d, 1 d, 3 d, 7 d, 14 d, and 27 d. It was shown that the 4:2 FTOH was rapidly degraded by the 2 bacterial strains, but a half-life was not reported. Furthermore, no attempt was made to identify metabolites by LC-MS/MS because of the lack of authentic standards. However, GC-ECD analysis revealed the formation of 2 peaks during 4:2 FTOH biotransformation by *P. oleovorans*. Similarly, 4:2 FTOH biotransformation by *P. butanovora* showed the formation of 5 peaks (when incubated without 1-butanol) and 3 peaks (incubation with 1-butanol) by GC-ECD, respectively. Incubation with the 6:2 and 8:2 FTOH showed formation of transformation products that are typically observed in microbial degradation experiments (i.e., FTCAs, FTUCAs, fluorotelomer ketones and secondary alcohols, and PFCAs). For both substrates, the mass balance could not account for approximately 40% to 54% of initial FTOH mass, which the authors attributed to nonextracted bound residues and non-quantified metabolites. In both strains, the FTOHs were quickly transformed to their corresponding n:2 FTCA, n:2 FTUCA, and n:2 ketone, followed by formation of the x:2 sFTOH (where $n = 6$ or 8, $x = n - 1$). These were shown to be major, intermediate metabolites. Furthermore, the PFCAs (PFHxA in the 6:2 FTOH incubations, PFOA in the 8:2 FTOH incubations) were formed early and increased throughout the experiment. Incubations with *P. oleovorans* also showed formation of x:3 FTCA and x:3 FTUCA, but these metabolites were not observed in the *P. butanovora* incubations. These findings suggest that the bacterial strains had differing degradation abilities, specifically with respect to the n:2 FTUCA. The authors proposed an FTOH

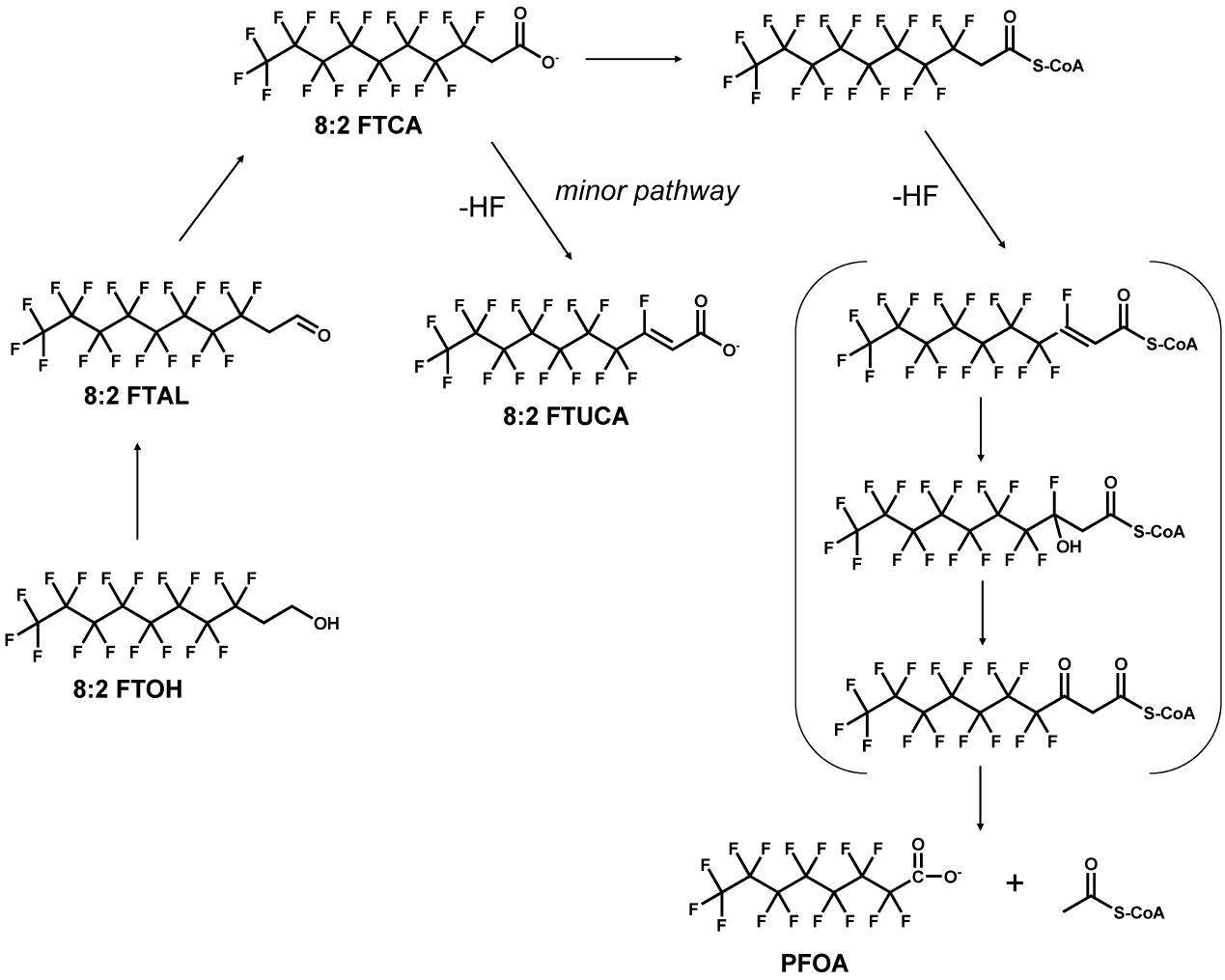

Figure 1. 8:2 Fluorotelomer alcohol (FTOH) biodegradation in mixed microbial system as proposed by Dinglasan et al. [12]. Structures in brackets were not determined in the study. Figure adapted from Dinglasan et al. [12]. See Table 1 for definition of abbreviations.

biotransformation pathway, based on the study findings. The initial steps are the formation of the n:2 FTAL, followed by formation of the n:2 FTCA and then n:2 FTUCA. Degradation of the n:2 FTUCA could proceed by 2 separate pathways. In pathway I, the n:2 FTUCA is transformed to the x:2 ketone, which is converted to the x:2 sFTOH and ultimately PFCAs (PFHxA or PFOA). In pathway II, the n:2 FTUCA is transformed to the x:3 FTUCA followed by formation of either the x:3 FTCA or PFCAs (PFBA or PFHxA).

Wang et al. [17] examined the biodegradation of ^{14}C-8:2 FTOH in aerobic soils under closed conditions and continuous air flow. It was shown that between 10% and 35% of the mass balance was irreversibly bound to the soil and could not be solvent extracted. The 8:2 FTOH was degraded in the soil, and at least 11 metabolites were observed, 7 of which had been previously reported [14]. Three identified metabolites were not previously reported for soil 8:2 FTOH degradation and included the 3-hydroxy-7:2 saturated fluorotelomer carboxylate (3-OH-7:3 FTCA), the 7:2 ketone, and 2H-PFOA. Perfluorooctanoate and the 7:3 FTCA were formed in relatively high yields, averaging 25% and 11% of the mass balance, respectively. These yields are at least 2 orders of magnitude greater than other reported yields for soil [16]. The 7:2 sFTOH, 8:2 FTUCA, and 7:3 FTCA were transient metabolites. Interestingly, the 8:2 FTCA was not observed, which was attributed to the rapid degradation of this compound. The formation of PFNA was not observed; however, PFHxA was observed in the 8:2 FTOH soil incubations. To investigate the 8:2 FTOH biodegradation pathway, several identified or proposed intermediate metabolites were incubated in soil and activated sludge for 90 d, including the 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, and 7:2 sFTOH. Incubations with the 7:2 sFTOH resulted in the formation of PFOA, which was hypothesized to be produced through several monooxygenase-mediated reactions. Therefore, the 7:2 sFTOH was postulated to be the ultimate precursor to PFOA. The 7:2 sFTOH was formed during incubations with the 8:2 FTUCA but not the 7:3 FTUCA. Incubations with the 7:3 FTCA did not yield any metabolites, including PFOA, suggesting that this compound is a terminal metabolite in soil systems. However, 7:3 FTUCA incubations did yield the 7:3 FTCA. Based on the experimental findings and building on previous 8:2 FTOH metabolism studies in microbial systems and mammals performed by this group [13,14,19,20], a series of degradation pathways was proposed (Figure 2). The initial steps of biotransformation are consistent with previous studies (8:2 FTOH > 8:2 FTAL > 8:2 FTCA > 8:2 FTUCA), and the 8:2 FTUCA represents a key branching point. In the first branch, the 8:2 FTUCA can be defluorinated to yield the 7:3 FTUCA, which

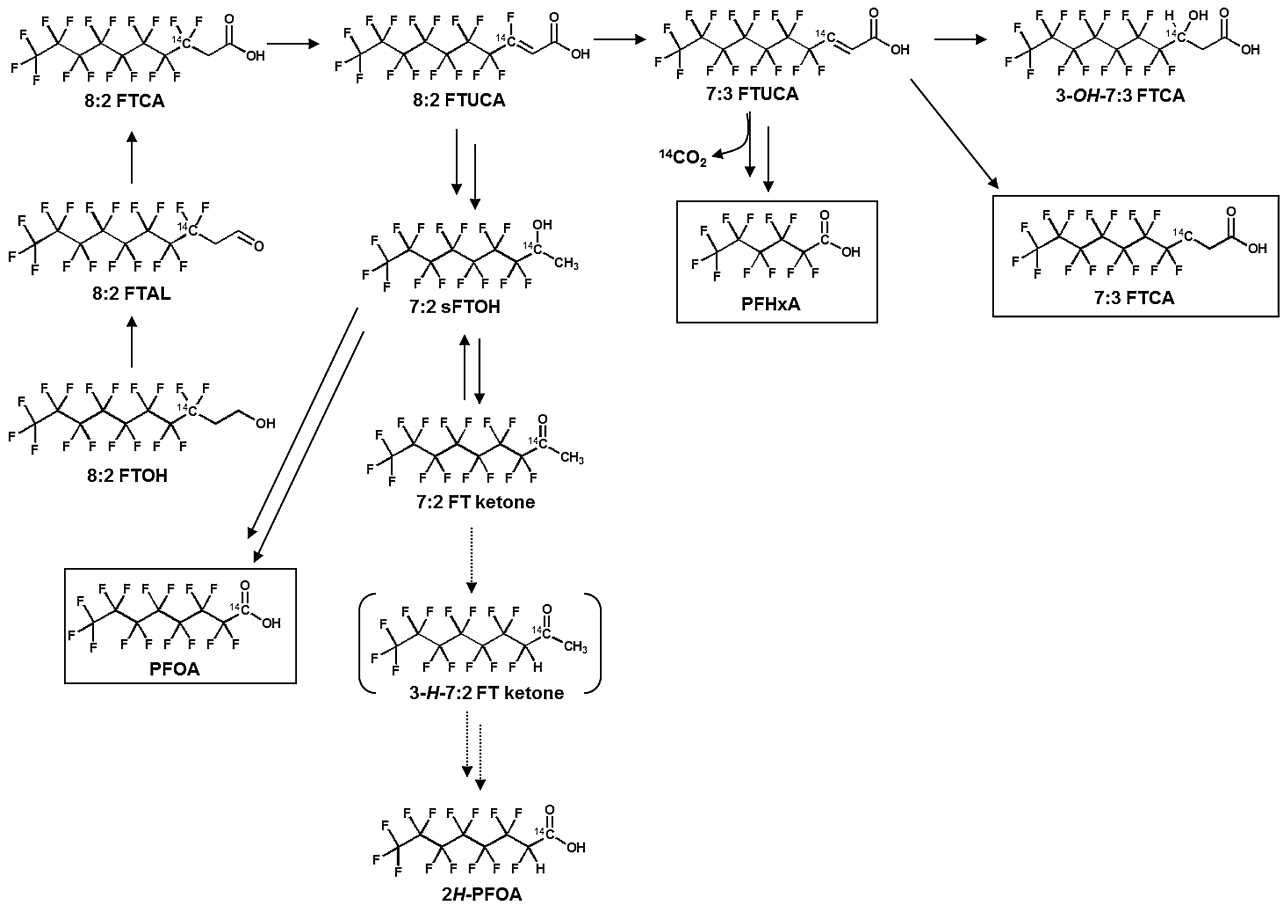

Figure 2. 8:2 Fluorotelomer alcohol (FTOH) biodegradation pathway in soils and activated sludge as proposed by Wang et al. [17]. Solid arrows represent pathways expected to occur based on published literature. Dotted arrows represented hypothesized pathways. Compounds inside boxes are stable metabolites; those in parenthesis are proposed intermediates. Adapted from Wang et al. [17]. See Table 1 for definition of abbreviations.

can either be degraded to the PFHxA, reduced to the 7:3 FTCA, or oxidized to the 3-OH-7:3 FTCA. This is a substantial change from previous proposed pathways from this group that suggested that the 7:3 FTCA would form PFOA through β-oxidation [13,14]. In the second branch, the 8:2 FTUCA forms the 7:2 sFTOH, which, as stated previously, was hypothesized to be the ultimate precursor to PFOA. Alternatively the 7:2 sFTOH can be oxidized to the 7:2 ketone, then defluorinated to the 3-H-7:2 ketone and ultimately the 2H-PFOA. However, a recent study by the same researchers has shown that the 7:2 ketone is actually formed directly from the 8:2 FTUCA, which then forms the 7:2 sFTOH and then PFOA (N. Wang, E.I. du Pont De Nemours & Co. Inc., Wilmington, DE, USA, personal communication).

6:2 fluorotelomer alcohol

Thus far, only 5 studies have investigated the biotransformation of the 6:2 FTOH [15,21–24]. Recently, the global fluorotelomer industry has committed to eliminating PFOA and the precursors that may degrade to PFOA [25]. As a result, there reportedly has been a shift to shorter-chain-length fluorotelomer compounds, such as the 6:2 FTOH [21]. Thus, there is a need to investigate the biotransformation of these shorter-chain-length fluorotelomer compounds and to compare the degradation pathways to those of the 8:2 FTOH.

Liu et al. [21] investigated the biodegradation of the 6:2 FTOH in a mixed bacterial culture (90 d) and an aerobic soil (180 d). The 6:2 FTOH was rapidly degraded in both test systems with measured half-lives of 1.3 d and 1.6 d in the bacterial culture and soil, respectively. In the mixed bacterial culture, the overall mass balance at day 90 was approximately 60%, and the major metabolites were the 6:2 FTUCA (molar yield = 23%) and 5:2 sFTOH (16%). Minor metabolites included the 6:2 FTCA (6%), 5:3 FTCA (6%), and PFHxA (5%), as well as trace quantities of perfluorobutanoate (PFBA) and perfluoropentanoate (PFPeA) (<0.5% each). In the aerobic soil, the overall mass balance was approximately 67% at day 180. In contrast to the bacterial culture, the major metabolites were PFPeA (30%) and the 5:3 FTCA (15%). Minor metabolites included PFHxA (8%), 5:2 sFTOH (7%), PFBA (2%), and trace quantities of the 4:3 FTCA (1%). The 6:2 FTCA, 6:2 FTUCA, and 5:2 ketone were shown to be intermediate metabolites and became undetectable after day 7 (6:2 FTCA, 6:2 FTUCA) and day 60 (5:2 ketone), respectively. Several differences between the 8:2 and 6:2 FTOH biodegradation in soil were noted by Liu et al [21]. Particularly, in the 8:2 FTOH degradation the major PFCA metabolite was PFOA (40% in the Sassafras soil treatment). However, in the 6:2 FTOH degradation, PFHxA, the analogous metabolite, was formed in only 8% yield. In contrast, the major metabolite in the 6:2 FTOH degradation was PFPeA (30%). In addition, to provide further insight into the 6:2 FTOH biodegradation pathway, individual 6:2 FTOH metabolites (5:3 FTCA, 5:2 ketone, 5:2 sFTOH) were incubated with the bacterial culture and aerobic soil. Incubation of the 5:2 ketone in mixed bacterial culture for 95 d yielded the 5:2 sFTOH (78%) and trace quantities of PFHxA (<1%). Incubation of 5:2 ketone in soil for 90 d yielded the 5:2 sFTOH (78%), PFHxA (4%), and PFPeA (18%). Interestingly, when the

5:2 sFTOH was incubated in soil for 60 d, the predominant metabolites were PFHxA (12%) and PFPeA (85%), whereas only trace amounts (<0.5%) of 5:2 ketone were detected. The authors noted that these results suggest the 5:2 ketone > 5:2 sFTOH transformation is essentially irreversible and that the 5:2 sFTOH is the direct precursor to PFHxA and PFPeA in microbial systems. No metabolites were observed when the 5:3 FTCA was incubated (soil only tested). Interestingly, the 7:3 FTCA was also a terminal metabolite in the previous 8:2 FTOH soil biodegradation studies conducted by this group [17]. The 6:2 FTOH biotransformation pathway was proposed based on the study results and previously published 8:2 FTOH biotransformation studies (Figure 3). The initial biodegradation steps are consistent with the 8:2 FTOH; specifically, the 6:2 FTOH is oxidized to the 6:2 FTAL followed by subsequent oxidation to the 6:2 FTCA and then 6:2 FTUCA. The 6:2 FTUCA is then degraded via 2 pathways. It was proposed that the dominant pathway is the 6:2 FTUCA > 5:2 ketone > 5:2 sFTOH > PFHxA and PFPeA. The second proposed 6:2 FTUCA pathway was formation of the 5:3 FTUCA followed by several branching points. These include conversion to the 5:3 FTCA, hydration to form 3-OH-5:3 FTCA (which could undergo β-oxidation to form PFHxA or shorter-chain PFCAs), formation of the PFBA, and formation of the 5:3 unsaturated amide.

Liu et al. [22] followed up their earlier 6:2 FTOH study [21] by examining the aerobic biodegradation of the [1,2,-^{14}C] 6:2 FTOH in a flow-through soil (Sassafras) incubation system for 84 d. The experimental system was similar to that previously used by the group to study 8:2 FTOH biodegradation [17]. A significant portion of the original ^{14}C dose was captured in the airflow during the experiment. Specifically, by 84 d in the active vessels, the ^{14}C captured in the airflow comprised the [1,2,-^{14}C] 6:2 FTOH (14% of total ^{14}C dose), [^{14}C] 5:2 sFTOH (16%), and $^{14}CO_2$ (6%). The $^{14}CO_2$ formed was presumably the result of C1 and C2 carbon mineralization (e.g., 6:2 FTUCA decarboxylation to form 5:2 ketone). In the soil, the half-life of the [1,2,-^{14}C] 6:2 FTOH was approximately 1.3 d in both the active and sterile control treatments. This very short half-life was similar to that observed in the closed system experiment [21]. The intermediate and terminal metabolites identified were similar to those observed in the previous study [21]. As such, no modifications were made to the previously proposed 6:2 FTOH degradation pathway. However, the yields of the terminal metabolites were different between the flow-through and closed incubation experiments. For example, the PFPeA (30% yield, fraction of initial mass dosed) was the major metabolite in the closed experiment [21] but was formed in much smaller amounts in the flow-through experiment (4.2%). Similarly, decreased PFHxA

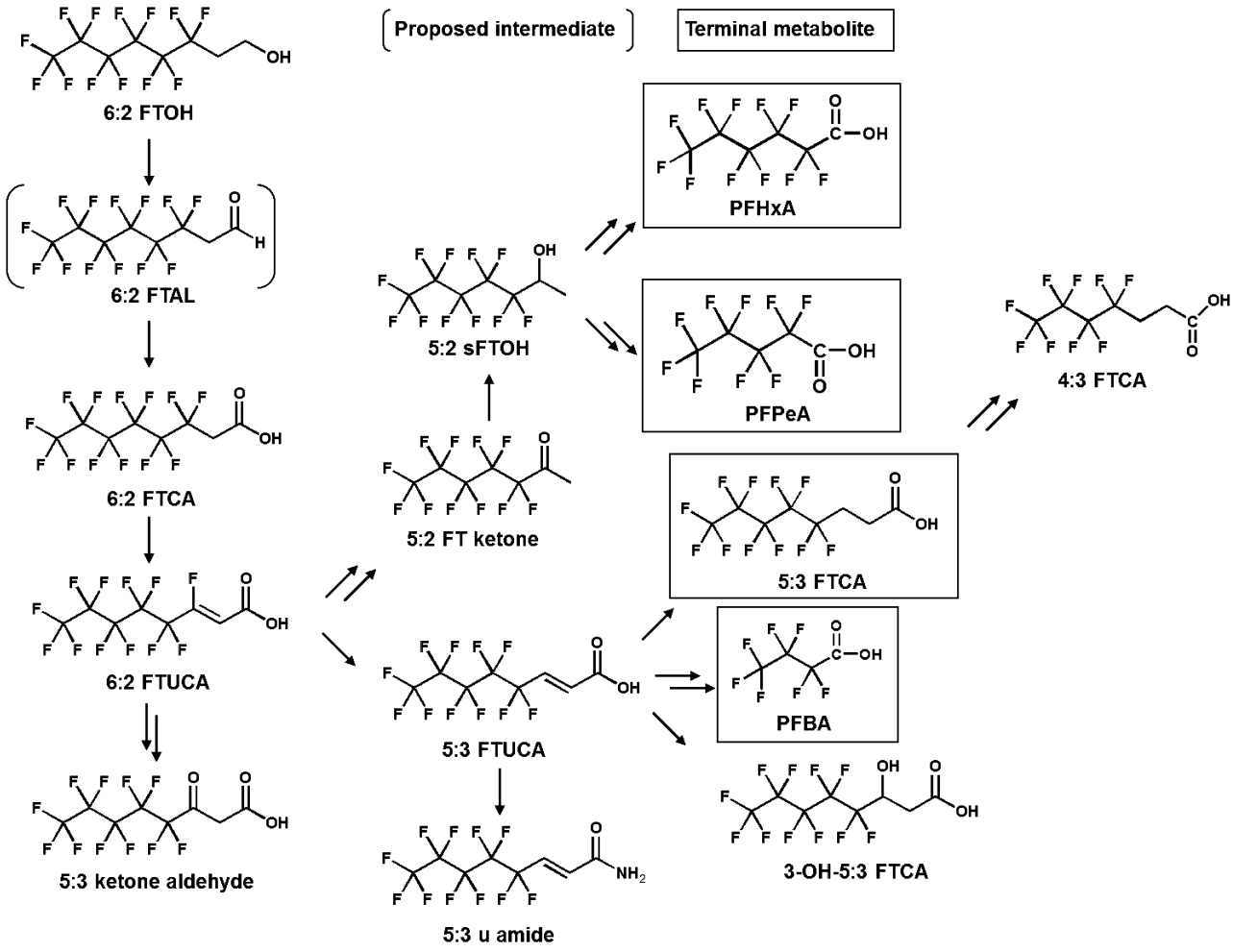

Figure 3. 6:2 Fluorotelomer alcohol (FTOH) aerobic biodegradation pathway as proposed by Liu et al. [21]. Double arrows indicate multiple transformation steps. Structure in brackets indicates proposed intermediate; structures in boxes indicate terminal metabolites. Adapted from Liu et al. [21]. See Table 1 for definition of abbreviations.

yields were observed in the flow-through experiment (4.5%) as compared with the closed system (8.1%). The authors attributed the lower PFPeA and PFHxA yields to the removal of the apparent precursor compound, the 5:2 sFTOH, in the airflow. In contrast, the 5:3 FTCA yields were essentially the same between the flow-through (12%) and closed (15%) experiments, and the 5:2 sFTOH was found not to be a precursor to the 5:3 FTCA. Finally, the sum of the stable products in the soil of the flow-through experiment (23%) was much less than that measured in the closed system (56%). The authors noted that this discrepancy (33%) is similar to the total ^{14}C (36%) measured in the airflow of the flow-through experiment.

The aerobic biotransformation of 6:2 FTOH in river sediments (collected from Brandywine Creek, PA, USA) was investigated by Zhao et al. [23]. The study was unique because it investigated biotransformation in river sediments, which may have different microbial communities, and thus different biotransformation pathways, as compared with soils and WWTP sludges. Similar to previous studies [17,26] by the study co-authors, C18 solid-phase extraction cartridges were inserted into the vessel headspace to monitor volatile compounds as well as to allow for gas exchange. Active and control vessels, and corresponding C18 cartridges, were analyzed on days 0, 2, 7, 14, 28, 56, and 100 by LC-MS/MS. The mass balance was 86 mol% to 98 mol% and 71 mol% to 88 mol% for the sterile control and active treatments, respectively. The 6:2 FTOH half-life was 1.8 d in the active vessels, similar to that shown for aerobic soil and mixed bacterial culture, suggesting rapid biotransformation. The transient, intermediate metabolites were the 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, and 5:2 sFTOH. The 6:2 FTCA and 6:2 FTUCA peaked on d 2 and then rapidly decreased. The 5:2 ketone and 5:2 sFTOH peaked at 7 d and 28 d, respectively. Other metabolites were the 5:3 FTCA, 4:3 FTCA, PFPeA, PFHxA, and PFBA. Most of the 5:3 FTCA was bound to sediment organic constituents, similar to what was shown in soil experiments [21]. The molar yields for the PFCAs were 10.4% (PFPeA), 8.4% (PFHxA), and 1.5% (PFBA). As noted by the authors, the PFCA formation profile was different from that observed in soil 6:2 FTOH biotransformation, which showed a PFPeA yield of 30 mol%. A 6:2 FTOH biotransformation was proposed by the authors, combining the previously reported 6:2 FTOH degradation pathway in soil [21] and the more recent findings from the 5:3 FTCA biotransformation pathway [26]. Similar to the previously proposed pathway [21], the key branching point was at the 6:2 FTUCA, which ultimately can be transformed to either the 4:3 and 5:3 FTCAs or the PFCAs (PFBA, PFPeA, PFHxA). Furthermore, the 5:2 sFTOH was proposed to be the direct precursor to PFPeA and PFHxA.

As mentioned in the *8:2 fluorotelomer alcohol* subsection, Zhang et al. [15] reported the first study of 6:2 FTOH biodegradation under anaerobic conditions. The 8:2 FTOH biodegradation was also investigated, and details are presented in the *8:2 fluorotelomer alcohol* subsection. The 6:2 FTOH biotransformation was investigated in 2 separate studies that were 90 d and 176 d in duration, respectively. In addition, the incubations were dosed with several 6:2 FTOH biotransformation intermediates: 6:2 FTUCA, 5:2 FTUCA, a-OH 5:3 FTCA, and 5:3 FTCA. In both studies, the 6:2 FTOH biotransformation half-life was approximately 30 d, much slower than that observed in aerobic soil, sediment, and activated sludge [22,23]. The major biotransformation product was the 6:2 FTCA (44 mol % and 32 mol% after 90 d and 176 d, respectively). These results contrast previous aerobic biodegradation studies [21,23] that show very low yields of the 6:2 FTCA because of its rapid biotransformation. Another major metabolite was the 5:3 FTCA (23 mol% and 18 mol%). The 5:3 FTCA was apparently stable and, in contrast to aerobic activated sludge studies [26], did not show further biotransformation. The 6:2 FTUCA was a minor formation product (2 mol% and 8 mol%). The 5:3 FTUCA was not measured in the incubations, but the authors attributed this finding to its very rapid biotransformation (half-life < 3 h). The 3-fluoro 5:3 FTCA [$F\text{-}(CF_2)_5CFHCH_2COOH$] was identified as a novel biotransformation product during the 6:2 FTUCA incubation. It was suggested that this metabolite may be converted back to the 6:2 FTUCA or form the 5:3 FTUCA and then the 5:3 FTCA. The 5:2 sFTOH, which was suggested to be the direct precursor to PFHxA, was formed in low yields (2.5 mol% and 0.6 mol%). The authors noted that these yields are approximately 16-fold lower than in aerobic activated sludge. Consequently, only very low yields of PFHxA were measured (0.2 mol% and 0.4 mol%), approximately 28-fold lower than aerobic activated sludge. Several other 6:2 FTOH biotransformation products were not identified in the incubations: PFPeA, PFHpA, 5:2 ketone, 5:2 acid, 5:2 FTUCA, a-OH 5:3 acid, and 3:3 FTCA. As mentioned earlier, both the 6:2 FTOH and 8:2 FTOH studies showed that the x:3 FTCAs (5:3 and 7:3 FTCAs) were stable during the anaerobic experiments. Furthermore, the study results suggested that the low yield of PFCAs during the anaerobic studies may be attributable to the very low rate of the n:2 FTCA $\rightarrow$ x:2 sFTOH step.

Kim et al [24] investigated 6:2 FTOH biotransformation in 3 species of alkane degrading bacteria (*Mycobacterium vaccae* JOB5, *P. oleovorans, P. butanovora*) and 1 fluoroacetate-degrading bacterium (*Pseudomonas fluorescens* DSM 8341) during 28 d experiments. In addition, 5:3 FTCA degradation by *P. oleovorans* and *P. fluorescens* DSM 8341 was examined. Incubations also tested the influence of formate, an external reducing energy source, on 6:2 FTOH degradation. This study was a follow-up of their previous work [18], which investigated 6:2 FTOH biotransformation in *P. oleovorans* and *P. butanovora*. The presence of formate did not affect degradation rates or metabolites profiles by *M. vaccae* JOB5. In contrast to *M. vaccae* JOB5, 6:2 FTOH biotransformation by *P. fluorescens* DSM 8341 was influenced by formate. Specifically, PFBA was only formed in the presence of formate (0.14 mol% at day 28). In addition, it was shown that the 6:2 FTOH biotransformation in *P. oleovorans* was 6-fold greater in the presence of dicyclopropylketone (alkane monooxygenase inducer) or formate. Based on the new findings of the study, the authors proposed a biotransformation pathway that was modified from their earlier study [18]. The initial degradation steps were shared by all 4 bacterial strains: 6:2 FTOH $\rightarrow$ 6:2 FTAL $\rightarrow$ 6:2 FTCA $\rightarrow$ 6:2 FTUCA. Pathway I shows the 6:2 FTUCA $\rightarrow$ 5:2 ketone $\rightarrow$ 5:2 sFTOH. The 5:2 sFTOH forms PFHxA, PFPeA, and PFBA; the composition of PFCAs formed is dependent on bacterial strain and conditions. Pathway II shows 6:2 FTUCA $\rightarrow$ 5:3 FTUCA, followed by formation of either the 5:3 FTCA or PFPeA. However, whether all strains can convert the 5:3 FTUCA to PFPeA is unclear.

8:2 fluorotelomer stearate monoester and 8:2 fluorotelomer citrate triester

Dasu et al. [27] investigated the biodegradation of the 8:2 fluorotelomer stearate monoester in loam soil (collected from the Purdue Agronomy Farm in West Lafayette, IN, USA), using laboratory microcosms. The microcosms were closed but maintained sufficient oxygen concentrations to be aerobic during the 80-d experiment. The 8:2 FTOH stearate was

degraded with a half-life of 10.3 d, decreasing to 22% of the initial applied mass by the end of the experiment. The 8:2 FTOH was rapidly formed, resulting from the hydrolysis of the ester bond, and subsequently degraded with a half-life of approximately 2 d. Coincident with the 8:2 FTOH decline was the formation of the 8:2 FTCA, 8:2 FTUCA, 7:2 sFTOH, 7:3 FTCA, PFHxA, PFHpA, and PFOA. The 7:2 sFTOH was a transient metabolite showing increasing levels until 20 d, followed by decreasing levels until the end of the study. The 7:3 FTCA, PFHxA, PFHpA, and PFOA were considered to be the terminal products because their levels continued to increase throughout the 80-d study, with PFOA comprising the largest fraction (1.7 mol%). No attempt was made to elucidate the degradation pathway.

Dasu et al. [28] followed up their original study [27] by examining the biodegradation of the 8:2 fluorotelomer stearate monoester and 8:2 fluorotelomer citrate triester in forest silt loam soil (collected from West Lafayette, IN, USA) using laboratory microcosms. The microcosms were closed and aerated during the study to ensure aerobic conditions. The 8:2 fluorotelomer stearate was degraded with a half-life of 28.4 d, much slower than observed in the previous study with an agricultural soil [27]. Similar to the previous study, the initial metabolite formed was the 8:2 FTOH, which showed rapid degradation (half-life of approximately 2 d). The 8:2 FTOH reached 9 mol% by 1 d and gradually decreased. Secondary metabolites formed were the 8:2 FTCA, 8:2 FTUCA, 7:2 sFTOH, 7:3 FTCA, 7:2 FTUCA, PFHxA, PFHpA, and PFOA. The 7:2 sFTOH was the major transient metabolite (peaking at 17 mol% at 46 d), and PFOA was the major terminal metabolite (peaking at 4 mol% at 94 d). A higher amount of metabolite formation was shown in the forest soil incubations as compared with the agricultural field incubations. In contrast to the 8:2 fluorotelomer stearate, the 8:2 fluorotelomer citrate was degraded much more slowly, with only 44% depleted by the end of the study (day 218). Formation of 8:2 FTOH and secondary metabolites, identical to those shown in the 8:2 fluorotelomer stearate incubations, was observed. However, determination of the metabolite yield was complicated by the 8:2 FTOH residuals in the 8:2 FTOH citrate triester.

1H, 1H, 2H, 2H, 8H, 8H-perfluorododecanol

Arakaki et al. [29] investigated the biodegradation of the 1H,1H,2H,2H,8H,8H-perfluorododecanol (referred to as "degradable telomer fluoroalcohol" [DTFA]) in activated sludge under closed-bottle and aerated, flow-through conditions for 20 d. In addition, a long-term study (70 d) was performed under aerated conditions. The DTFA is similar to the 10:2 FTOH, with the exception that the CF_2 group at the 8-carbon is replaced with a CH_2 group. In the aerated conditions, volatile compounds were captured on tandem C_{18} traps and 2 NaOH traps and nonvolatile metabolites monitored in the aqueous phase. The activated sludge was collected from a water treatment facility for fluorinated compounds, and thus these microorganisms likely were acclimatized to fluorinated chemicals. In the closed-bottle experiments, the 2*H*,2*H*,8*H*,8*H*-perfluorododecanoate (2*H*,2*H*,8*H*,8*H*-PFDoA) and 2*H*,8*H*,8*H*-2-perfluordodecanoate (2*H*,8*H*,8*H*-2-PFUDoA)—analogous to the 10:2 FTCA and FTUCA, respectively—were shown to steadily increase during the study. Furthermore, trace quantities of the terminal metabolites PFBA and PFPeA were also observed. Metabolite formation was restricted because of the consumption of oxygen during the experiment. In the aerated experiments, the same intermediate and terminal metabolites were formed, with the additional detection of several perfluorinated dicarboxylates: perfluorobutanedioic acid, perfluoropentanedioic acid, and perfluorohexanedioic acid. A DTFA biotransformation pathway was proposed based on the study results and previously reported 8:2 FTOH microbial degradation studies [17,21] (Figure 4). The initial biotransformation steps were consistent with the 8:2 FTOH pathway: DTFA oxidation to the aldehyde analog, via alcohol dehydrogenase, followed by oxidation to the saturated carboxylic acid, via aldehyde dehydrogenase, and finally defluorination to form the unsaturated carboxylic acid, 2*H*,8*H*,8*H*-PFUDoA. These steps are analogous to the formation of 10:2 FTUCA in 10:2 FTOH biodegradation. The 2*H*,8*H*,8*H*-PFUDoA could undergo defluorination at the 8-carbon position, forming a double bond on either side of the internal -CH_2- group. These intermediates could undergo multiple decarboxylation steps, forming carboxylic acids of 8-, 9- and 10-carbons, respectively. Finally, cleavage of the internal double bond would result in the formation of short-chain PFCAs (PFBA, PFPeA) and a series of perfluorinated dicarboxylates: perfluoropropanedioic acid, perfluorobutanedioic acid, perfluoropentanedioic acid, and perfluorohexanedioic acid.

6:2 fluorotelomer sulfonate

Wang et al. [30] investigated the biodegradation of a 6:2 fluorotelomer sulfonate (FTSA) salt [$F(CF_2)_6CH_2CH_2SO_3^-K^+$] in activated sludge over 90 d under aerobic conditions. Sludges from WWTPs in Pennsylvania, Maryland, and Delaware (USA) were tested separately. Interestingly, no biotransformation products were observed in the Delaware incubations, despite the fact that this sludge was capable of degrading the 8:2 FTOH [14]. Overall, the 6:2 FTSA showed minor degradation with the observed transformation products accounting for only 6.3% (molar yield) at day 90 as compared with approximately 55% for the 6:2 FTOH in bacterial culture and soil [21]. The major metabolites observed were the 5:2 sFTOH and 5:2 ketone (combined yield = 3.4% of initial 6:2 FTSA dose) followed by the terminal metabolites, PFPeA (1.5%) and PFHxA (1.1%). The 5:3 FTCA and PFBA were minor metabolites comprising approximately 0.1% of the initial 6:2 FTSA dose. The 6:2 FTUCA was a transient intermediate, peaking between day 7 and day 28 and then declining. The 6:2 FTUCA declines were followed by formation of the 5:2 ketone, 5:2 sFTOH, PFHxA, and PFPeA. The PFHpA was not detected in any of the treatments during the 90 d, indicating that α-oxidation did not occur, consistent with other microbial studies. Furthermore, the 6:2 FTOH and 6:2 FTCA were also not detected during the experiment. Based on the study results and literature on 6:2 FTOH degradation [21], a biotransformation pathway was proposed. It was suggested that the initial step is desulfonation, catalyzed by alkane sulfonate-α-hydroxylase, forming the unstable intermediate, 1-hydroxy 6:2 FTSA [$F(CF_2)_6CH_2CH(OH)SO_3^-$]. The 1-hydroxy 6:2 FTSA is rapidly converted to the 6:2 FTAL, releasing sulfonic acid (HSO_3^-), and bypassing the formation of the 6:2 FTOH. The 6:2 FTAL is rapidly oxidized to the 6:2 FTCA, which is further oxidized to the 6:2 FTUCA, the first measurable intermediate metabolites. The 6:2 FTAL and FTCA were not actually observed in the study, which was attributed to their rapid degradation. The 6:2 FTUCA is further metabolized following a similar pathway to that of the 6:2 FTOH as described by Liu et al. [21,22]. Specifically, the 6:2 FTUCA yields the 5:2 ketone, via decarboxylation and other reactions, which is converted to the 5:2 sFTOH through dehydrogenase. Previous studies from this group [21,22] have shown that the 5:2 sFTOH is directly metabolized to PFHxA and PFPeA. Alternatively, the 6:2 FTUCA may yield the 5:3 FTCA.

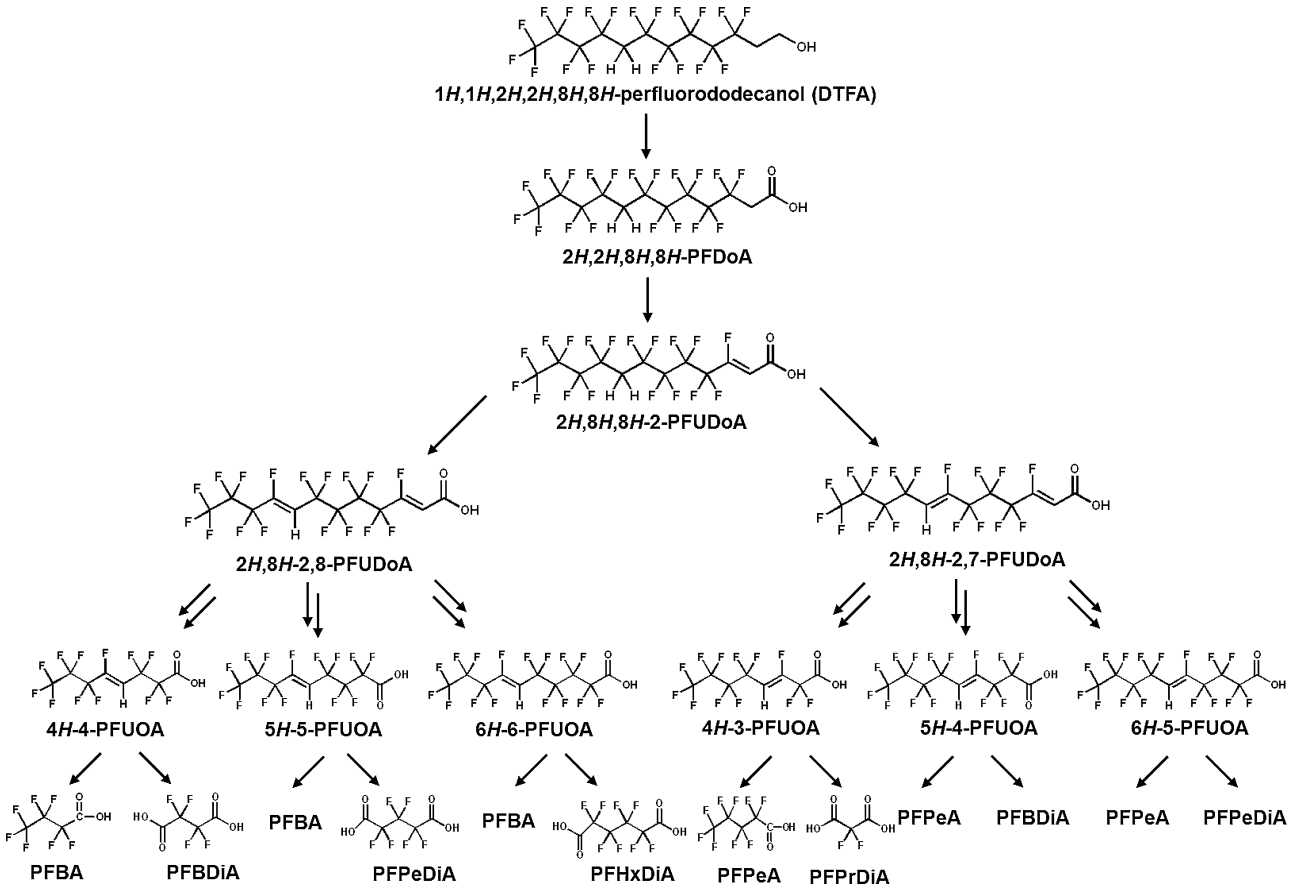

Figure 4. Biotransformation pathway of the 1H,1H,2H,2H,8H,8H-perfluorofluorododecanol (DTFA) as proposed by Arakaki et al. [29]. Adapted from Arakaki et al. [29]. See Table 1 for definition of abbreviations.

Fluorotelomer carboxylates

Myers and Mabury [31] investigated the fate of the 8:2 and 10:2 FTCA and FTUCA in soil–water microcosms. Biotransformation products were monitored, although a degradation pathway was not proposed. In addition to active sediment treatments, sterile sediment and control-water (no sediment) treatments were monitored to assess binding to sediment and abiotic degradation, respectively. In all experiments only the active treatments showed significant formation of metabolites, indicating that biological activity in the sediment was necessary for FTCA and FTUCA degradation. In the active 8:2 FTCA treatments, the parent compound was rapidly depleted and the only metabolite detected was PFOA. However, the 8:2 FTUCA was observed in the sterile sediment and control water microcosms. In the active 8:2 FTUCA treatments, the parent compound was also rapidly degraded and the major metabolite was also PFOA. In addition, the 7:3 FTCA and PFHpA were detected. Analogous trends were shown for the 10:2 FTCA and FTUCA. Specifically, the active 10:2 FTCA treatment showed production of the perfluorodecanoate (PFDA) only, whereas the 10:2 FTUCA treatment showed production of PFDA, the 9:3 FTCA, and trace quantities of PFNA.

Biotransformation of the 5:3 and 7:3 FTCA in domestic WWTP activated sludge (Pennsylvania, USA) was investigated by Wang et al. [26]. A C18 cartridge was inserted into the top of the bottle to collect volatile metabolites and to allow air exchange. When the headspace oxygen decreased to approximately 10%, the vial was purged with fresh air to ensure aerobic conditions. Active and sterile control treatments were sampled, in triplicate, on days 0, 2, 7, 14, 28, 56, and 90. A unique aspect of the study was the elucidation of the 5:3 FTCA biotransformation pathway by separately dosing with α-OH 5:3 acid and the 6:2 FTUCA. At the time of sampling, the C18 cartridges and sludge were extracted with acetonitrile and analyzed by LC-MS/MS. Novel metabolites were identified using a LTQ Orbitrap mass spectrometer. In addition, a more aggressive extraction, using NaOH, was performed to increase the recovery of the 5:3 and 7:3 FTCA. In the 5:3 FTCA incubations, a good mass balance (77–103 mol%) was obtained. No volatile transformation products were measured in the C18 cartridges. At the end of 90 d, the average yield of the biotransformation products was 22 mol%. As noted by the Wang et al. [26], this yield is approximately 10-fold higher than the 5:3 FTCA biotransformation in soil and may be related to the reduced 5:3 FTCA binding in the activated sludge as compared with soil. The most abundant metabolites were the 4:3 FTCA and PFPeA, with molar yields of 14.2 mol% and 5.9% at the end of 90 d. Minor transformation products were the PFBA and 3:3 FTCA, forming approximately 1% each. Formation of PFHxA was not observed. The study also identified the 4 novel biotransformation intermediates: α-OH 5:3 FTCA, 4:3 FTUCA, 5:2 FTCA, and 5:2 FTUCA. Dosing with the α-OH 5:3 FTCA showed the formation of the 4:3 FTCA, PFPeA, PFBA, and 3:3 FTCA. In contrast, the 7:3 FTCA incubations showed very low biotransformation—only 1.7 mol% depleted after 90 d. The only transformation product observed was the PFHpA. In contrast to the 7:3 FTCA, the 5:3 FTCA was rapidly degraded in the

activated sludge. As noted by the authors, this may have been attributable to the 7:3 FTCA binding to sludge organic constituents, rendering the 7:3 FTCA nonbioavailable. The 5:3 FTCA biotransformation pathway was proposed, proceeding through a novel "one-carbon removal" pathway. The initial step is 5:3 FTCA conversion to the 5:3 FTUCA, via a microbial dehydrogenase, followed by hydration across the double bond to form the α-OH 5:3 FTCA. The α-OH 5:3 FTCA is rapidly decarboxylated to the 5:2 FTAL, followed by oxidation to the 5:2 FTCA and then dehydro- and dehalogenation to yield the 5:2 FTUCA. The main fate of the 5:2 FTUCA is defluorination to yield the 4:3 FTUCA followed by reduction to form the 4:3 FTCA. Alternately, the 4:3 FTUCA can undergo further -CF2- loss to form the 3:3 FTCA. In a minor pathway, the 5:2 FTUCA can be decarboxylated to yield the 4:2 ketone, followed by formation of the 4:2 sFTOH and ultimately yielding the PFPeA and PFBA.

Polyfluoroalkyl phosphates

Lee et al. [32] examined the biodegradation of the 6:2 mono- and di-substituted polyfluoroalkyl phosphates (PAPs) in WWTP "mixed liquor" (raw wastewater and sewage sludge). The 6:2 mono- and diPAPs contain 1 and 2 $CF_3(CF_2)_5CH_2CH_2$ substituents attached to a phosphate group, respectively. A biotransformation pathway was proposed based on the study findings and previously published work on FTOHs. The immediate production of 6:2 FTOH was observed in the headspace of both the 6:2 mono- and diPAPS treatments. These findings suggest that the initial degradation step is the microbial hydrolysis of the phosphate ester bonds in the PAPs to yield the FTOHs. In the 6:2 diPAPs treatment, the ester bonds appeared to be cleaved sequentially, as evidenced by the initial formation and subsequent depletion of the monoPAP, followed by production of the FTOH. In addition to the 6:2 mono-PAP and FTOH, a series of acid metabolites were observed in the aqueous phase of the bottles. The 6:2 FTCA was initially formed and consumed, followed by the 6:2 FTUCA and PFHxA. Both the 6:2 FTCA and FTUCA were transient metabolites, peaking during the initial stages of the experiment, whereas the PFHxA was a terminal metabolite. The authors suggested that these metabolites were formed through a β-oxidation pathway, analogous to that of the 8:2 FTCA to PFOA. In addition, the 5:3 FTCA was also formed as an intermediate metabolite, although the 5:3 FTCA levels peaked much later than the 6:2 FTCA and FTUCA. Interestingly, the formation of odd-chain-length PFCAs (PFHpA, PFPeA) was also observed. It was suggested that the PFHpA formed through the α-oxidation of the 6:2 FTCA, which has been shown in mammalian and fish studies, but not previously in microbial systems. The authors attributed this finding to the phosphate-free conditions of the media, which may have selected for a different microbial community. The formation of the PFPeA was suggested to originate from the 5:3 FTCA, analogous to the 7:3 FTCA → PFHpA observed in rainbow trout [33]. In addition, the authors noted that the PFPeA may form through the 6:2 FTUCA → 5:2 ketone → 5:2 sFTOH → PFPeA pathway, as suggested by Liu et al. [21], although the 5:2 ketone and 5:2 sFTOH were not monitored in the PAPs study. In addition, the authors investigated the biodegradation of the 4:2, 6:2, 8:2, and 10:2 monoPAPs (the so-called, "chain length" study). This is one of the few studies to investigate non-8:2 fluorotelomer compound degradation and is important because industrial production has shifted away from 8:2 fluorotelomer chemistry. The study showed that the rate of FTOH production decreased with increasing monoPAP chain length. For example, the 4:2 FTOH production leveled off after day 1, but the 10:2 FTOH production increased throughout the experiment. These trends may have been attributable to the steric hindrance of the longer-chain monoPAPs. In the aqueous phase, the expected terminal PFCA metabolites (PFBA and PFHxA) were observed in the 4:2 and 6:2 monoPAP treatments, respectively. In contrast, the expected terminal PFCAs (PFOA and PFDA) were not observed above the limit of quantification (1.9 ng/mL for PFOA, 0.37 ng/mL for PFDA). Furthermore, the intermediate FTCA and FTUCA metabolites were observed in all treatments, with the exception of the 3:3 FTCA in the 4:2 FTOH dose. These findings suggest that the shorter-chain monoPAPs can be degraded to their terminal PFCA metabolites, whereas the longer-chain monoPAPs are only partially degraded to the corresponding FTCA and FTUCA intermediate metabolites.

Fluorotelomer ethoxylates

Fromel and Knepper [34] reported on the biodegradation of a suite of fluorotelomer ethoxylates (FTEOs) $[F(CF_2CF_2)_x(CH_2CH_2O)_yH$, FTEO] in WWTP effluent under aerobic conditions. The FTEOs were dosed by spiking the Zonyl FSH commercial mixture, which contains FTEOs with perfluorocarbon chain lengths between 4 and 12 and a degree of ethoxylation between 0 and 18; but the experiment focused only on FTEOs with perfluorocarbon chain lengths of 6 and 8 carbons, and a degree of ethyoxylation between 0 and 13 [35]. The FTEOs were rapidly degraded, showing a half-life of approximately 1 d. The only significant metabolites formed were the FTEO carboxylates (FTEOCs). It was proposed that these compounds were by the initial oxidation of the FTEOs to the aldehydes followed by additional oxidation to the FTEOCs. The longer FTEOCs were degraded over the experiment time course, presumably through consecutive removal of the ethoxylate groups via diglycolic acid dehydrogenase. Interestingly, the shorter FTEOCs ($y < 9$) did not show further degradation. The PFHxA and PFOA were also detected in the experimental vessels; however, their formation was attributed to the degradation of residual FTOHs in the commercial mixture.

Fluorotelomer-based polymers

The largest commercial class of polyfluorinated products is reportedly the acrylate-based polymers [36], whereas minor products include methacrylate- and urethane-based polymers. Currently, very few studies have examined the biodegradation potential of fluorotelomer-based polymers. There is considerable variability in the reported PFCA formation rates between studies. As such, the contribution of fluorotelomer-based polymers as an environmental source of PFCAs remains unclear.

Russell et al. [36] monitored the fate of a commercial fluorotelomer acrylate (FTAC)-based polymer in 4 soils over 2 yr under aerobic conditions. The FTAC monomer used in the study was synthesized from a mixture of 6:2 FTOH (1 wt%), 8:2 FTOH (55%), 10:2 FTOH (29%), 12:2 FTOH (10%), and 14:2 FTOH and larger FTOHs (5%). The test polymer comprised the various length FTACs, hydrocarbon acrylates, and $CH_2=CCl_2$ monomers. In addition, the test polymer was shown to contain impurities of various lengths of FTOHs, FTACs, FTOH acetates, and fluorotelomer olefins (FTOs). Headspace samples were analyzed for 8:2 FTOH, and soil samples were analyzed for 8:2 FTOH, 8:2 FTCA, 8:2 FTUCA, C_8-C_{11} PFCAs, 7:2 sFTOH, and 7:3 FTCA. The study did not monitor the test polymer concentrations and thus may underestimate the PFCA contributions for residual precursors. Furthermore, the study did not

monitor for the FTAC, FTOH acetates, and FTO polymer impurities. The initial soil 8:2 FTOH concentration was between 2.5 and 3.3 μmol/kg but declined to below the limit of quantification (0.22 μmol/kg) by day 182. The 8:2 FTCA and 8:2 FTUCA were consistently detected in low concentrations (generally less than 0.04 μmol/kg) throughout the experiment. The 7:2 sFTOH was only detected above the limit of quantification during 1 time point. Perfluorooctanoate soil levels increased initially and reached steady levels (1.8–2.1 μmol/kg) within approximately 180 d. Similar 7:3 FTCA time trends were also observed. Using a molar balance comparison approach, the authors showed that the maximum PFOA soil levels were 24% to 28% of the theoretical maximum from the FTOH residuals in the polymer. This yield is similar to that reported for PFOA production from direct incubation with 8:2 FTOH in soil (25%) and sewage sludge (28%). The authors suggested that the FTOH impurities in the polymer can account for nearly all of the PFOA, and other metabolite concentrations, observed during the experiment. This is also supported by the initial rapid increase in PFOA concentrations followed by minimal increases after 12 mo incubation. However, the study did not monitor levels of other potential PFCA precursors. Using statistical software, the polymer half-life was calculated to be 1200 to 1700 yr. However, the authors noted remarkable differences between the soil types. For example, when considering individual soils, the half-lives ranged between 95 yr (Inceptisol soil) and infinity (Ultisol soil).

Washington et al. [37] also investigated the fate of an FTAC-based polymer in soil microcosms for 546 d. The test polymer was synthesized by a major fluorotelomer polymer manufacturer and, similar to the Russell et al. [36] polymer, was composed of the various length FTACs, hydrocarbon acrylates, and $CH_2=CCl_2$ monomers. Furthermore, the polymer also contained impurities of FTOHs, FTACs, 10:2 FTO, and trace levels of PFCAs and FTCAs. The authors developed an extraction technique to thoroughly "open up" the polymer, apparently resulting in the exhaustive extraction of any residual and degradation compounds that may have been trapped within the polymer matrix. Previous attempts to investigate fluorinated polymer degradation presumably did not adequately extract the polyfluorinated and perfluorinated compounds from the polymer and thus may underestimate degradation. The soil extracts were analyzed at 497 d and 546 d for several FTACs and FTOHs as well as the C_6 through C_{12} PFCAs. The 8:2 FTUCA and 7:3 FTCA also were monitored. Polymer degradation for each PFC group (i.e., C6, C8, C10, C12) was assessed by summing the corresponding FTAC, FTOH, and even-chain-length PFCA (e.g., "C8" = 8:2 FTAC + 8:2 FTOH + PFOA). Exceptions were C12, which comprised the sum of 12:2 FTOH and perfluorododecanoate, and C8, which was evaluated as the sum of the 8:2 FTAC, 8:2 FTOH, 8:2 FTUCA, 7:3 FTCA, PFOA, and PFHxA (in addition to the conditions mentioned previously). As acknowledged by the authors, this simplified degradation scheme ignores several known metabolites and thus presumably underestimates polymer degradation. However, at 497 d and 546 d, several of the PFC groupings had soil concentrations greater than the fresh polymer, demonstrating polymer degradation. The degradation occurred on either the ester linkage or the carbon backbone. The study also identified a potential novel PFOA degradation product, the 2H-PFOA [$CF_3(CF_2)_5CFHC(O)O^-$] in the 546-d extracts. The apparent half-life of the studied polymer was estimated to be approximately 870 yr to 1400 yr, which the authors noted overlaps with that reported by Russell et al. [36] of 1200 yr to 1700 yr. However, the test polymer in the current study had a surface area that was approximately 14-fold lower than typical commercial fluorotelomer-based polymers. Considering that degradation most likely occurred on the polymer surface only, the half-life obtained from the current study overestimated actual more finely graded FTAC-based commercial polymers. Using the study results, the calculated half-life for these finer polymers was approximately 10 yr to 17 yr—much shorter than the previous estimate for an FTAC-based polymer.

Russell et al. [38] investigated the aerobic biodegradation of a fluorotelomer-based urethane polymer in 4 soils (Alfisol, Inceptisol, Mollisol, and Ultisol) over 2 yr. The test polymer was synthesized by reacting FTOHs of various chain lengths with a diisocyanato homopolymer, followed by cross-linking. The FTOH mixture used in the polymer synthesis consisted of 6:2 FTOH (34 wt%), 8:2 FTOH (31%), 10:2 FTOH (18%), 12:2 FTOH (9%), and 14:2 FTOH and larger (8%). In addition, the test polymer was shown to contain impurities of various lengths of FTOHs, fluorotelomer iodides, perfluoroalkyl iodides, FTOs, polyfluorinated ethers, and PFCAs. However, studies conducted by the authors showed that the FTO does not yield PFCAs in aerobic soil. During the experiment, the headspace was monitored for 8:2 FTOH, and the soil was monitored for 8:2 FTOH, 8:2 FTCA, 8:2 FTUCA, PFOA, PFNA, PFDA, and perfluoroundecanoate. In addition, the 7:2 sFTOH and 7:3 FTCA were monitored after 1 yr of study. Polymer degradation could occur through breakage of the covalent urethane bond (releasing FTOHs) or by cleavage of the polymeric backbone. Three of the 4 tested soils showed 8:2 FTOH soil concentration less than the limit of quantification (the Inceptisol soil showed transient levels, peaking approximately midway through the experiment). With the exception of the Alfisol soil, all soils showed formation of the 8:2 FTCA and 8:2 FTUCA. Furthermore, the 7:2 sFTOH was infrequently detected in the Inceptisol and Ultisol soils. In addition, PFOA, PFNA, PFDA, perfluoroundecanoate, and 7:3 FTCA were formed during the experiment, but modeling of the polymer degradation only considered PFOA formation. The PFOA levels measured during the experiment were several orders of magnitude greater than the initial levels of PFOA and potential intermediate precursors. Thus, the authors attributed nearly all of the PFOA formation to originate from the fluorotelomer-based urethane polymer degradation. Using the observed PFOA levels and a mathematical degradation model, the polymer half-life was calculated to be 79 yr to 241 y, with a geometric mean of 102 yr when considering all 4 soils.

RATS AND MICE

The original experiment on FTOH biotransformation in mammals was performed by Hagen et al. [11] in the early 1980s. In that study, Hagen et al. [11] dosed male rats via a single oral dose, and animals were periodically sacrificed up to 20 d post-dosage. Plasma samples were solvent extracted, diazomethane derivatized, and analyzed by GC coupled with a microwave-sustained helium plasma detector. A detailed time course of 8:2 FTOH depletion and metabolite formation was not presented by the authors, nor was the 8:2 FTOH half-life given. However, PFOA, 8:2 FTCA, and an unidentified metabolite were detected 2 h post-treatment. In addition, 8:2 FTUCA was also formed during the experiment. Plasma levels of the 8:2 FTCA and the unidentified metabolite were transient, decreasing with time from an initial peak early in the experiment, ultimately reaching very low levels by 48 h. In contrast, PFOA levels increased throughout the experiment. A detailed mechanism was not

presented; however, it was suggested that β-oxidation was involved.

Approximately 25 y after the initial work by Hagen et al. [11], Martin et al. [39] performed an 8:2 FTOH dosing study using similar experimental conditions. The purpose of the study was to replicate the original findings of Hagen et al. [11] while employing improved analytical techniques. In the Martin et al. [39] study, the dosed animal was euthanized at 6 h post-dosage, and blood, liver, and kidney were analyzed. The 8:2 FTCA, 8:2 FTUCA, PFOA, and PFNA were detected in the tissues, and PFNA was formed at levels approximately 10-fold lower than PFOA. In addition, the conjugated metabolites, the *O*-glucuronide and *O*-sulfate, were identified via comparison with expected mass spectra. The 8:2 FTOH time trends were not reported. In a second experiment, Martin et al. [39] investigated FTOH biotransformation in hepatocytes isolated from male Sprague-Dawley rats. Hepatocytes were incubated with the 4:2, 6:2, 8:2, and 10:2 FTOH, although discussion of study results focused on the 8:2 FTOH experiments. In the 8:2 FTOH incubation, 78% of the parent compound had been depleted by 4 h post-treatment, although poor mass balance was obtained. Formation of the 7:3 FTCA and 7:3 FTUCA was also observed during these incubations. The *O*-glucuronide and *O*-sulfate conjugated metabolites were detected. Three glutathione (GSH)-conjugated metabolites were also detected: GSH-FTUAL, GSH-FTUCA, and a reduced GSH-FTUAL conjugate. The formation of aldehyde metabolites also was investigated by derivatizing the hepatocyte medium with 2,4-dinitrophenylhydrazine at 4 time points (30 min, 1 h, 2 h, and 4 h). The formation of the 8:2 FTAL and 8:2 FTUAL was observed by comparison with an authentic standard product spectrum (8:2 FTAL) and expected spectrum (8:2 FTUAL). The quantified metabolites accounted for only 8.5% of the transformed products: 8:2 FTCA (2.9%), 8:2 FTUCA (4.1%), PFOA (1.4%), and PFNA (<0.2%). These results indicated an overall low PFOA yield from 8:2 FTOH biotransformation. The unaccounted mass balance may be partially explained by the formation of the conjugated metabolites (3 GSH conjugates, 1 glucuronide, and 1 sulfate) and, to a lesser extent, the formation of 7:3 FTCA and 7:3 FTUCA. The contribution of these metabolites to the total mass balance could not be determined because authentic standards were not available. Martin et al. [39] further investigated the FTOH degradation mechanism by incubating the hepatocytes with several of the identified intermediate metabolites. When hepatocytes were incubated with the 8:2 FTAL for 2 h, no 8:2 FTAL or 8:2 FTUAL was detected, but the formation of small quantities of PFOA, PFNA, 8:2 FTCA, and 8:2 FTUCA was observed. However, a low mass balance was obtained, to which the authors suggested the formation of acid metabolites was not the primary fate of the FTAL. Individual incubations with 8:2 FTCA and 8:2 FTUCA showed these compounds biotransformed much more slowly than the 8:2 FTOH, with less than 10% of parent compound biotransformed 2 h post-treatment. In these incubations, a much better mass balance was obtained.

Kudo et al. [40] investigated the biotransformation of 8:2 FTOH in male mice dosed via intraperitoneal injection. Animals were euthanized at periodic intervals up to 72 h posttreatment. The serum and liver were analyzed for suspected 8:2 FTOH metabolites, but the parent compound itself was not monitored. Perfluorooctanoate and 2 unidentified metabolites were observed in the liver within 2 h posttreatment in the serum and liver, implying rapid biotransformation of the 8:2 FTOH. The 8:2 FTCA and the 2 unidentified metabolites peaked around 6 h post-treatment in the liver, implying that these compounds were intermediate metabolites. In contrast, PFOA levels continued to increase throughout the experiment. In a second experiment, Kudo et al. [40] continuously exposed male mice via the diet, and animals were sacrificed at weekly intervals up 28 d. Similar to the intraperitoneal exposure, the 8:2 FTOH was not monitored. Perfluorooctanoate and PFNA levels were shown to increase in a dose- and time-dependent manner. The formation of PFNA was approximately 10-fold lower than that of PFOA. One of the unidentified metabolites in the intraperitoneal exposure ("metabolite a") was low throughout the experiment, but the other metabolite ("metabolite b") was not detected. In both experiments, the authors suggested that the first metabolite formed was the 8:2 FTCA.

Fasano et al. [20] investigated the biotransformation of radiolabeled [3-^{14}C] 8:2 FTOH in male and female rats exposed via a single oral dose. The 8:2 FTOH levels in plasma peaked at 1 h posttreatment and were rapidly eliminated. The half-life was 1 h to 5 h for 8:2 FTOH and was 4 h to 18 h for 8:2 FTCA. Perfluorooctanoate half-lives were much longer, 112 h to 217 h in males and 5.6 h to 17.5 h in females. Most of the ^{14}C activity (>70%) was excreted in the feces, primarily as the parent 8:2 FTOH (34–50%). Bile elimination was also significant, whereas less than 4% of the activity was eliminated in the urine. Bile metabolites were predominately glucuronide and glutathione conjugates. Overall only a minor portion of the parent FTOH biotransformed to PFCAs, with most metabolized to 8:2 FTUCA-GSH and 8:2 FTUAL-GSH conjugates. Interestingly, several PFCAs with chain lengths smaller than PFOA were observed, suggesting the potential for CF_2 degradation. Based on their findings, Fasano et al. [20] proposed a metabolic degradation pathway for 8:2 FTOH in rats. The first step involves the series of oxidations of 8:2 FTOH > 8:2 FTAL > 8:2 FTCA. The 8:2 FTCA can undergo dehydrofluorination to yield 8:2 FTUCA or α-oxidation to yield PFNA. Again, similar to other FTOH biotransformation pathways proposed by this group and others [19,39], the 8:2 FTUCA was an important branching point. One branch involves the reduction and dehydrofluorination of 8:2 FTUCA to form the 7:3 FTUCA, which can be subsequently reduced to yield the 7:3 FTCA. It was postulated that both the 7:3 FTCA and 7:3 FTUCA can undergo β-oxidation to yield PFOA. The second branch involves the formation of the 8:1 FTO from the 8:2 FTUCA. The 8:1 FTO subsequently loses fluorine to yield the 7:2 FTO and ultimately form the 7:2 sFTOH. It was proposed that the 7:2 sFTOH could then react to from PFOA, although the mechanism is unclear. Finally, the FTUAL and FTUCA can form GSH conjugates, and the 8:2 FTOH and 7:2 secondary FTOH can form glucuronide and sulfate conjugate metabolites.

Henderson and Smith [41] investigated 8:2 FTOH biotransformation in time-pregnant mice (gestational day 8) exposed via a single oral gavage dose of 30 mg 8:2 FTOH/kg body weight. The 8:2 FTOH and intermediate metabolites, 8:2 FTCA and 8:2 FTUCA, were not detected in maternal serum or liver 24 h posttreatment (the first sampling point after dosing). However, PFOA and PFNA were detected in the maternal serum and liver 24 h posttreatment. The major metabolite was PFOA, reaching levels approximately 5-fold greater than PFNA. These results are consistent with rapid biotransformation of the 8:2 FTOH observed in previous studies.

Nabb et al. [19] investigated the in vitro metabolism of [3-^{14}C] 8:2 FTOH in rat, mouse, trout, and human hepatocytes and in rat, mouse, and human liver microsomes and cytosol fractions. In addition, incubations were performed with the 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, and 7:3 FTUCA to further

investigate the mechanism of FTOH metabolism. Differences between the species were observed with 8:2 FTOH clearance rates following the rank order of rat > mouse > human ≥ trout. The yield of PFOA was low and represented between 0.02% and 0.47% of the initial 8:2 FTOH dosed, on a molar basis. In general, the major transient metabolites were the 7:3 FTCA and 8:2 FTCA. Several novel metabolites were identified, including the 7:2 ketone, 7:3 β -keto acid, 7:3 FTAL, 7:3 FTUAL, and 7:3 FTCA taurine (–TA) conjugate. A series of biotransformation mechanisms were proposed, building on previous studies as well as the study observations [20,39]. Once again, the initial biotransformation steps are consistent with previous studies [20,39] that comprise the series of oxidations of 8:2 FTOH > 8:2 FTAL > 8:2 FTCA > FTUCA. The 8:2 FTAL can be oxidized to form the 8:2 FTUAL, and the 8:2 FTCA can undergo α-oxidation to yield PFNA and lower odd-chain PFCAs. However, in the mechanism proposed by Nabb et al. [19], the branching points are at both the 8:2 FTUAL and 8:2 FTUCA. It was postulated that the 8:2 FTUAL would form the 7:3 β–hydroxy unsaturated aldehyde followed by the 7:3 β-keto aldehyde, ultimately yielding PFOA. An additional fate of the 8:2 FTUAL is formation of the 7:3 FTUAL, ultimately yielding the 7:3 FTCA and 7:3 FTCA-TA conjugate. Two branches also were proposed for 8:2 FTUCA biotransformation. One branch would ultimately yield the 7:2 sFTOH and 7:2 sFTOH glucuronide conjugate, whereas the second branch would yield the 7:3 FTUCA followed by the 7:3 FTCA and finally the 7:3 FTUCA-TA conjugate.

Fasano et al. [42] followed up their original, single-dose study [20] by investigating 8:2 FTOH kinetics and metabolite formation in male and female rats exposed through daily oral dosing for 45 d at 5 mg/kg/d and 125 mg/kg/d, respectively (kinetic study). Animals were sacrificed at 0 d, 1 d, 8 d, 25 d, and 45 d, and tissue concentrations were monitored by LC-MS/MS. In addition, after the initial 45-d conditioning phase, animals were dosed with a single [3-^{14}C] 8:2 FTOH oral exposure, sacrificed at day 53, and analyzed for mass balance and tissue distribution. Results from the [3-^{14}C] 8:2 FTOH dosing showed that the dominant fate was elimination in the feces (87–93.5% and 74–80.2% of the total radioactivity in the high and low doses, respectively). The 8:2 FTOH was the major analyte in the feces, with much smaller quantities of the 7:2 sFTOH, 7:2 ketone and several unknown metabolites. Urinary elimination (2.5–6.1% and 3.9–9.0% in the high and low doses, respectively) and tissues (4.1–5.7% and 8.4–8.5% in the high and low doses, respectively) constituted minor portions of the radioactivity. The liquid chromatography-accurate radioisotope counting (LC-ARC), LC-MS, and GC-MS analyses of the urine, feces, liver, kidney, fat, and plasma showed the formation of several phase II conjugates, intermediate polyfluorinated metabolites, and terminal PFCAs. However, the metabolite profiles varied by tissue. Perfluorononanoate, PFOA, and PFHpA were measured in the tissues, but PFHxA was only measured in the urine. The observed metabolites were previously identified in past 8:2 FTOH mammalian studies by this group [19,20]. The 45-d 8:2 FTOH dosing study showed that the major metabolite was the 7:3 FTCA, followed by PFOA and 8:2 FTCA. The parent 8:2 FTOH and most of the metabolites showed rapid elimination after 45 d; however, the 7:3 FTCA and PFOA showed much lower rates of elimination. Interestingly, sex differences in the metabolite profiles were observed. Specifically, females generally showed higher concentrations of the 8:2 FTOH parent and intermediate metabolites (e.g., 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, and 7:3 FTUCA), whereas males generally showed higher levels of the terminal metabolites (PFNA, PFOA, and PFHpA). Finally, a 8:2 FTOH biotransformation scheme was proposed that was essentially a combination of the mechanisms previously proposed by Fasano et al. [20] and Nabb et al. [19] (Figure 5). Several notable differences were seen between the newly proposed mechanism and that previously proposed by Fasano et al. [20]. Specifically, in the newly proposed mechanism the ultimate fate of the 7:3 FTCA is conjugation with taurine, whereas in the older mechanism the 7:3 FTCA formed PFOA via β-oxidation. In addition, the older mechanism proposed that the sole fate of the 8:2 FTUAL was conjugation with glutathione, whereas the newer mechanism proposes that 8:2 FTUAL can also form the 7:3 FTUAL and 7:3 β-OH FTUCA. The 7:3 FTUAL and 7:3 β-OH FTUAL can form the 7:3 FTCA and PFOA, respectively. Furthermore, PFOA can be formed from the 7:2 sFTOH.

Himmelstein et al. [43] performed the first 8:2 FTOH biotransformation study, exposed via inhalation, in male and female rats. Two groups of 5 rats (per sex) were exposed by nose-only inhalation for 6 h at 8:2 FTOH concentrations of 3 mg/m^3 or 30 mg/m^3. Blood was sampled at 1 h, 3 h, 6 h, 12 h, and 24 h (sampling points at 1 h, 3 h, and 6 h were during the exposure period). The parent compound and suspected metabolites were monitored by LC-MS/MS. Plasma 8:2 FTOH levels were low, which the authors attributed to rapid clearance. In the 3 mg/m^3 exposure, the 8:2 FTOH was not detected in the blood. In the 30 mg/m^3 treatment, plasma 8:2 FTOH levels ranged from 50 nM to 54 nM and 37 nM to 69 nM in the males and females, respectively. However, the 8:2 FTOH was not observed at the later time points (12 h and 24 h), suggesting rapid elimination/metabolism after the exposure stopped. In both exposures, the formation of PFHpA, PFOA, PFNA, 8:2 FTCA, 7:3 FTCA, 8:2 FTUCA, and 7:3 FTUCA was observed. In addition, the formation of PFHxA was shown in the 30 mg/m^3. At the peak concentrations, the most abundant metabolites were the 7:3 FTCA > PFOA > 8:2 FTCA. Using a 1-compartment kinetic model, the metabolite yield was determined for the 7:3 FTCA (1.6–2.1% in males and females, respectively) and PFOA (1.0% and 0.3%). As noted by the authors, these low yields are consistent with those shown for oral 8:2 FTOH dosing in rats. A biotransformation pathway was not proposed.

D'Eon and Mabury [44] investigated the biotransformation of 8:2 fluorotelomer mono- and diPAPs in male Sprague-Dawley rats. Animals were dosed by oral gavage (200 mg/kg), and blood levels were monitored for the parent compounds and metabolites for 15 d posttreatment. The PAPs could be biotransformed through the cleavage of the phosphate ester linkage via phosphatase enzymes, yielding the 8:2 FTOH, which would be subsequently oxidized to form PFOA. In both the mono- and diPAPs exposure, PFOA was formed and accumulated at low ng/g levels. Also, PFHpA was formed in comparatively lower levels. Furthermore, several transient intermediate metabolites (8:2 FTCA, 8:2 FTUCA, and 7:3 FTCA) were observed. Perfluorononanoate was not detected above blank levels. The formation of these intermediate and terminal metabolites is consistent 8:2 FTOH biotransformation, although the 8:2 FTOH was not monitored in the PAPs experiments. A detailed biotransformation pathway was not proposed.

D'Eon and Mabury [45] followed-up their original study [44] by investigating the biotransformation of the 4:2, 6:2, 8:2, and 10:2 monoPAP and diPAPs in male Sprague-Dawley rats. The animals were dosed with the monoPAP or diPAP mixtures

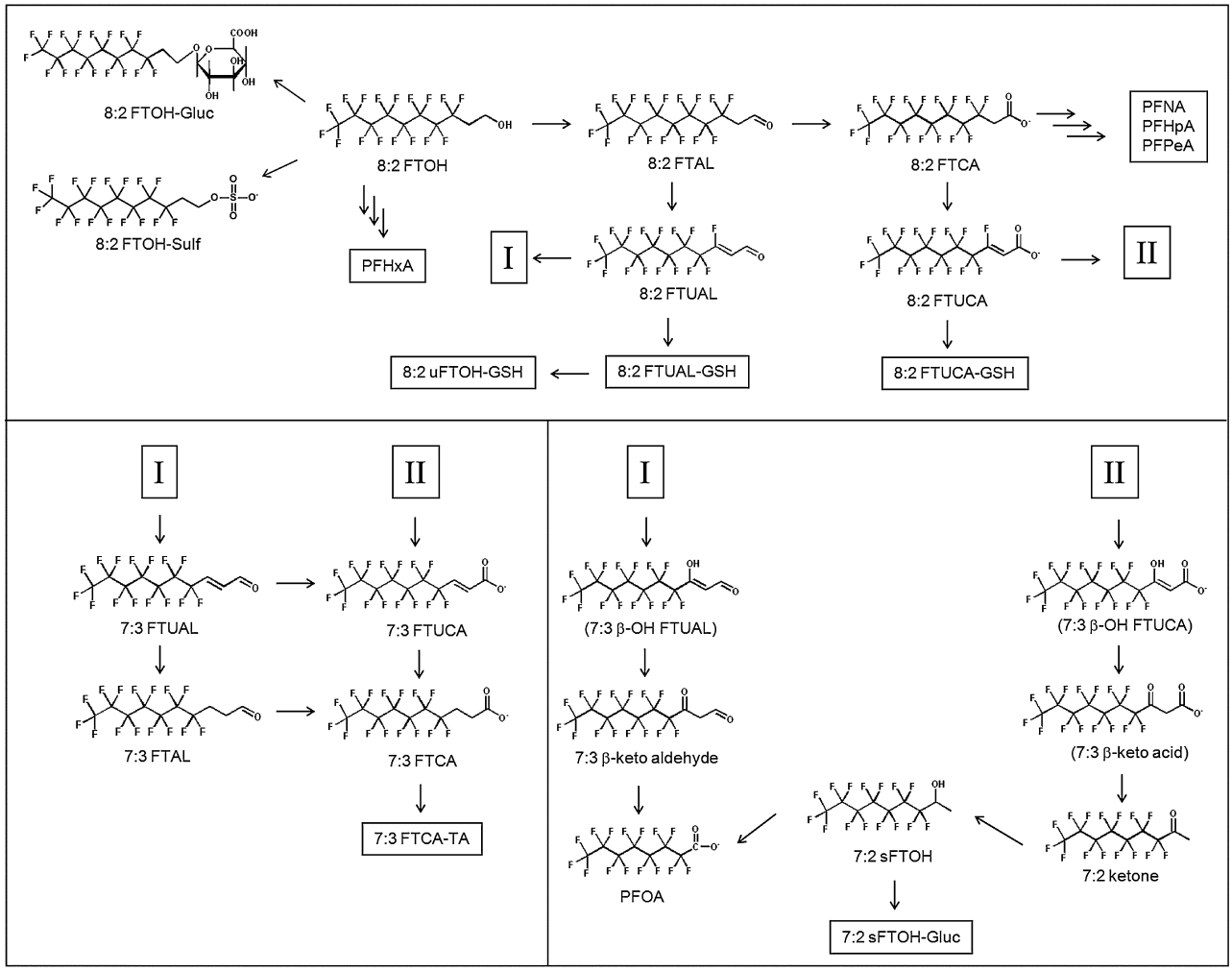

Figure 5. 8:2 Fluorotelomer alcohol (FTOH) biodegradation in rats as proposed by Fasano et al. [42]. Structures labeled in parenthesis are hypothesized intermediates and were not observed in the experiment. Note that glutathione conjugates can undergo further metabolism not presented in this figure. Adapted from Fasano et al. [42]. See Table 1 for definition of abbreviations.

separately and exposed by either oral gavage or intravenous injection. The parent compounds, polyfluorinated metabolic intermediates, selected phase II metabolites, and PFCAs were monitored in the blood, urine, and feces during the 22-d uptake experiment (oral gavage dosed) and 18-d elimination experiment (intravenously dosed). However, in both experiments, urine and feces were collected only during the initial 48 h after dosing. In the uptake experiment, diPAPs were readily absorbed from the gut, with the overall bioavailability decreasing with increasing chain length. In contrast, none of the monoPAP congeners were observed in the blood, suggesting that they were not absorbed from the gut. These findings are contradictory to the previous 8:2 monoPAP study by the authors [44], which showed more than 100 ng/g 8:2 monoPAP in the blood. However, in the monoPAP oral gavage dose, the formation of the 5:3 and 7:3 FTCA, as well as the FTOH-glucuronide and FTOH-sulfate conjugates, were observed up to 2 d after dosing. These trends suggest that the phosphate ester bond was cleaved within the gut, presumably resulting in uptake of the FTOH and further biotransformation. In the diPAPs dosing treatments, the formation of FTCAs, FTUCAs, and conjugated metabolites was only infrequently observed. The authors noted that the overall infrequent detection of polyfluorinated metabolic intermediates is contradictory to previous 8:2 FTOH studies in the rat and may suggest a difference in the metabolic handling of these 2 compound classes. In addition, PFCAs were observed in the blood at typically low ng/g wet weight levels. Interestingly, the 4:2 FTOH-glucuronide was observed in the urine, in apparently very high concentrations, after all dosing treatments.

FISH

Studies on fluorotelomer-compound biotransformation in fish are limited; in fact, the only test species investigated thus far has been rainbow trout. In general, fish have been shown to possess the same metabolic capacity as mammals but at a lower rate. Further work is needed to compare the metabolic pathways between fish and mammals. For brevity, the rainbow trout in vitro work from Nabb et al. [19] is not repeated from the earlier section.

Brandsma et al. [46] investigated 8:2 and 10:2 FTOH biotransformation in rainbow trout. The test compounds were simultaneously dosed via the diet during the 30-d uptake phase, and clean food was fed during the 30-d depuration phase. The study only monitored formation of the C_6-C_{14} PFCAs and the 8:2 and 10:2 FTCAs and FTUCAs. The FTCAs and FTUCAs were detected immediately after dosing (first time point = 1 d), and levels were essentially steady throughout the uptake phase.

These results are consistent with the very rapid metabolism observed in the 8:2 FTAC rainbow trout experiment by Butt et al. [47]. Levels of the 10:2 FTUCA and FTUCA were higher than the 8:2 analogs, presumably because of the longer depuration half-lives of the 10:2 compounds. Furthermore, FTCA levels were generally greater than their corresponding FTUCAs. These results are consistent with the much shorter biological half-life of FTUCAs in fish [33]. Trace quantities of PFOA and PFDA were also detected, confirming the formation of PFCAs from FTOHs in rainbow trout. PFNA was not detected, presumably because of the relatively low formation yields.

The biotransformation of the 8:2 FTAC in rainbow trout was investigated by Butt et al. [47]. Fish were exposed via the diet, and the parent 8:2 FTAC and suspected metabolites were monitored in liver, blood, kidney, bile, and feces during the 5-d uptake and 8-d elimination phases. Very low levels of the 8:2 FTAC were detected in the internal tissues and feces, and the authors attributed these trends to the suspected rapid biotransformation in the gut or liver. This finding was supported through additional work by Butt et al. [48], which showed very rapid 8:2 FTAC in vitro biotransformation in subcellular fractions (S9) isolated from the stomach and liver. Several intermediate and terminal metabolites were observed in the liver, kidney, and blood, and these metabolites generally followed similar time trends between the tissues. Specifically, the 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, and PFOA were rapidly formed, with detectable levels measured within 1 h to 4 h of initial dosing. In addition, PFHpA, PFNA, and the 7:3 FTUCA were formed in very low levels. In general, the rank order of metabolite yield was 8:2 FTCA > 7:3 FTCA > 8:2 FTUCA > PFOA > PFNA. The 8:2 FTCA and 8:2 FTUCA were rapidly removed from the tissues during the elimination phase, with half-lives ranging from 1.1 d to 1.2 d and from 1.0 d to 1.5 d for the 8:2 FTCA and 8:2 FTUCA, respectively. In contrast, the 7:3 FTCA and PFOA showed increasing levels during the initial 72 h to 96 h of the elimination phase, indicating that these metabolites were continuing to be formed from precursors (i.e., 8:2 FTCA and 8:2 FTUCA) still present in the body. The intermediate and terminal metabolites were also measured in the bile at levels within 2-fold of those in the liver. In addition, the 8:2 FTOH glucuronide conjugate was measured in the bile. This indicates that the bile may be a significant elimination route for the poly- and perfluoroalkyl carboxylates in fish. Interestingly, the levels of all measured metabolites were up to 270-fold higher than the parent 8:2 FTAC. This represented a rare situation in which the metabolites were more biologically persistent than the parent compound. The 8:2 FTAC biotransformation pathway was proposed based on the study results, the results of the in vitro stomach and liver S9 experiments [48], and additional work from the authors that examined the in vivo biotransformation of the 8:2 FTCA, 8:2 FTUCA, and 7:3 FTCA in rainbow trout [33]. The in vitro study confirmed that the initial biotransformation step was the formation of the 8:2 FTOH [48]. The results suggest that this may occur in the gut before uptake or within the internal tissues, with apparently equal efficiency.

Butt et al. [33] investigated the biotransformation of the 8:2 FTCA, 8:2 FTUCA, and 7:3 FTCA, respectively, in rainbow trout exposed via the diet. The study objective was to empirically investigate the FTOH biotransformation pathway by using 3 key 8:2 FTOH metabolic intermediates as the substrates. The parent compounds and suspected intermediate and terminal metabolites were monitored in the blood and liver during the 7-d uptake and 10-d elimination phases. The study showed that PFOA was formed from the 8:2 FTCA and 8:2 FTUCA but not from the 7:3 FTCA. In contrast, the 7:3 FTCA was shown to form the 7:3 FTUCA as well as PFHpA, a novel metabolite. Exposure to the 8:2 FTCA resulted in the rapid formation of the 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, PFHpA, PFOA, and PFNA. The 8:2 FTUCA exposure resulted in the formation of the 7:3 FTCA, 7:3 FTUCA, PFOA, and the 8:2 FTUCA glutathione conjugate. Small quantities of the 8:2 FTCA were also accumulated in the tissues, but the authors suggested that this was the result of trace 8:2 FTCA impurities in the food and not 8:2 FTUCA biotransformation. Remarkable differences were found between the half-lives of the parent compounds. Specifically, the half-lives for the 7:3 FTCA were 5.1 d and 10.3 d for blood and liver, respectively. The 8:2 FTCA elimination half-lives were 1.2 d and 1.3 d for blood and liver, respectively. Finally, the 8:2 FTUCA elimination half-life was 0.39 d in the blood, whereas the liver elimination half-life could not be calculated because of nondetectable levels immediately after the beginning of depuration. The difference between elimination half-lives was attributed to differences in metabolism rate. The 8:2 FTOH biotransformation pathway was proposed by combining the study results with previously reported pathways from the literature (Figure 6). The initial step of metabolism is the oxidation to the 8:2 FTAL, and subsequent oxidation to the 8:2 FTCA, or direct formation of the glucuronide and sulfate conjugates. The authors suggested that the biotransformation of 8:2 FTCA proceeds via a beta-like oxidation pathway, specifically 8:2 FTCA > 8:2 FTUCA > 7:3 β-keto acid > 7:2 ketone > PFOA. Furthermore, it was suggested that the 7:3 β-keto acid could enter the beta-oxidation cycle and form PFOA directly. The 8:2 FTCA could also undergo α-oxidation to yield PFNA. In addition, as shown by the study, the 7:3 FTCA can form the PFHpA, presumably proceeding through the 7:3 FTUCA. The 8:2 FTAL could also be oxidized to the 8:2 FTUAL, which could undergo conjugation with glutathione or further biotransformation to 7:3 β-keto aldehyde > 7:3 β-keto acid with eventual formation of PFOA or the 7:2 sFTOH. In contrast to previously reported pathways [17,21], the 7:2 sFTOH would not form PFOA.

CHALLENGES, FUTURE DIRECTIONS, AND CONCLUSIONS

Investigating the biotransformation of fluorotelomer-based compounds is challenging, and much has been learned since the original study by Hagen et al. [11]. For example, the 8:2 FTOH is a volatile compound and has a high Henry's law constant [49]. During in vitro studies with microsomes and hepatocytes, Nabb et al. [19] found that the 8:2 FTOH volatilized from the aqueous fraction (which contains the co-factors and cellular constituents responsible for the biotransformation) and migrated into the headspace. Loss of the parent compound was minimized by using closed conditions, but this was shown to negatively impact the FTOH biotransformation, and thus the experimental vessels were periodically flushed with clean air. Obviously, the flushed air must be collected (i.e., passed through an solid-phase extraction cartridge) to account for any volatile compounds present in the headspace. Furthermore, 8:2 FTOH biotransformation studies with sludge and soil (e.g., Wang et al. [13]) showed that a significant portion of the 8:2 FTOH had volatilized from the solid/aqueous matrix and deposited onto the polytetrafluoroethylene (PTFE) septa of the experimental vessels. Thus, to properly account for the mass balance, it was necessary to also extract the vessel septa. This is also relevant for volatile intermediate metabolites such as the secondary FTOHs. Presumably, such considerations are relevant for other volatile

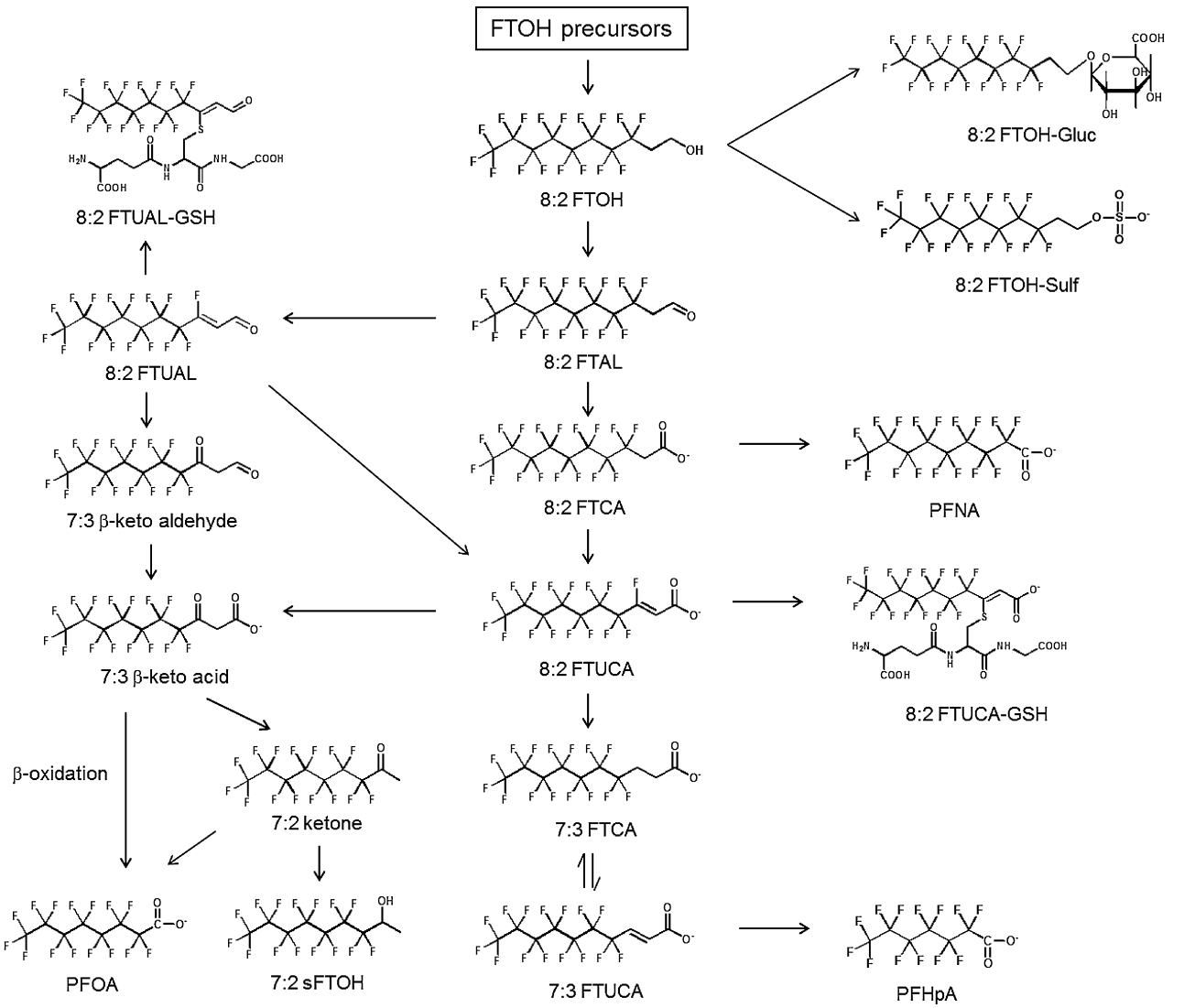

Figure 6. 8:2 Fluorotelomer alcohol (FTOH) biotransformation pathway in rainbow trout as proposed by Butt et al. [33]. "FTOH Precursors" refers to fluorotelomer-based compounds that degrade to the 8:2 FTOH. Adapted from Butt et al. [33]. See Table 1 for definition of abbreviations.

fluorotelomer compounds such as the FTACs. Similarly, quantification of the FTALs is challenging because of their high reactivity. Thus, some researchers [12,19] have used 2,4-dinitrophenylhydrazine (DNPH) to extract and stabilize FTALs for instrumental analysis.

An additional experimental challenge is the quantification of metabolites that are covalently bound to organic compounds. Dinglasan et al. [12] first postulated that the lack of mass balance in their experiments could be attributable to the formation of covalently bound metabolites that were not extracted using their experimental procedures. Furthermore, Wang et al. [26] showed that a significant fraction of the x:3 FTCAs were bound to the organic constituents in the experimental system. These metabolites could only be extracted using an aggressive extraction procedure (i.e., NaOH). Also, Rand and Mabury [50] showed that the FTUALs form covalent bonds with proteins in rat liver microsomes and bovine blood plasma. The formation of nonextractable metabolites could also explain why the mass balance typically decreases during the time course of the experiment (e.g., Liu et al. [16]).

There has been considerable evolution of the proposed biotransformation mechanisms since the original work by Hagen et al. [11]. In particular, with the observation of novel metabolites, the proposed mechanisms have become increasingly complex. For example, the original microbial 8:2 FTOH biodegradation mechanism comprised only 5 metabolites (8:2 FTAL, 8:2 FTCA, 8:2 FTUCA, 8:2 FTCA-S-CoA, and PFOA), but the most recent pathway by Wang et al. [17] comprised 12 metabolites and multiple branches. Much of this development is the result of improved analytical capabilities (i.e., mass spectrometry), synthesis of novel metabolites, and the use of ^{14}C-labeled compounds.

Numerous studies have shown that 8:2 FTOH metabolism results in the formation of PFOA and, to a smaller fraction, PFNA and lower-chain-length PFCAs. However, uncertainty and sometimes contradictory mechanisms still exist in the literature. It is clear that the overall yield of PFOA is low, presumably because conjugation reactions with the 8:2 FTOH occur as well as the apparent branching in the overall biotransformation mechanism. It is difficult to propose an overall biotransformation pathway since this research field is still relatively new and progressing rapidly. Furthermore, many of the very recent advances have been related to microbial biotransformation, and whether these findings apply to animal

models is unknown. However, some comments and synthesis can be resolved. There is universal agreement that the initial steps in the 8:2 FTOH degradation are formation of the 8:2 FTAL → 8:2 FTCA → 8:2 FTUCA. Analogous pathways have been shown for 6:2 FTOH biotransformation. In animal models, PFNA is formed by α-oxidation of the 8:2 FTCA. More recent studies have proposed that the key branching point in the pathway is at 8:2 FTUCA (or 6:2 FTUCA) in both microbial systems [14,18,21,22,26,32] and animal models [19,33,39,42]. In animal models, branching may also occur at the 8:2 FTUAL [19,33,39,42]. Nearly all of the proposed pathways show that the 8:2 FTUCA (or 6:2 FTUCA) yields PFOA (or PFHxA). The exception is Nabb et al. [19], which showed PFOA forming from the 8:2 FTUAL and not the 8:2 FTUCA. The ultimate precursor to PFOA remains unclear, but recent research has shown progress. Early studies proposed that the 7:3 FTCA could enter β-oxidation and yield PFOA [13,20], but this was disproven in subsequent studies [17,19,33]. In addition, earlier studies suggested that the 8:2 FTUCA could enter the β-oxidation cycle to yield PFOA [12,39]. However, the biotransformation is much more complex and appears to include the formation of fluorotelomer ketones and sFTOHs [14,17–23,42]. In microbial systems, direct incubation with the 7:2 sFTOH showed the formation of PFOA [17], suggesting that the 7:2 sFTOH is the ultimate precursor to PFOA. Furthermore, incubation with 5:2 sFTOH resulted in formation of PFHxA and PFPeA [21]. However, additional studies are needed to confirm these findings, particularly in animal models.

Several researchers have attempted to interrogate the biotransformation mechanism through dosing experimental systems with previously identified intermediate metabolites [17,19,21,26,33,39], greatly improving our knowledge of the biotransformation pathways. For example, as noted, the 7:3 FTCA was initially proposed to be a direct precursor to PFOA [13,20], proceeding through the β-oxidation pathway. However, additional work by Wang et al. [17] did not show PFOA formation. Studies with mammalian cells [19] and fish [33] also did not show PFOA formation from the 7:3 FTCA. Furthermore, the sFTOHs were first identified as an FTOH metabolite by Wang et al. [14], and additional work by Wang et al. [17] showed that incubation with the 7:2 sFTOH yielded PFOA. These results led the authors to propose that the 7:2 sFTOH was the ultimate precursor to PFOA.

Some notable differences exist between microbial systems and animal models. For example, the animal models are capable of phase II metabolism, which results in the formation of conjugated metabolites such as the glucuronide, sulfate, and glutathione conjugates. Furthermore, the FTCAs appear to be more stable in animal models, whereas the FTCAs are relatively labile in microbial systems. In contrast, the FTUCAs appear to be readily degraded in animal models. Finally, in animal models, PFNA is formed through the α-oxidation of 8:2 FTCA, but this is generally not observed in microbial systems.

Metabolite yield and profile has been shown to vary between experimental systems (i.e., between soil and sediments, between different soil types), which is presumably attributable to the differences in biotransformation capabilities (i.e., enzymes) between microbial communities. For example, during 8:2 fluorotelomer stearate biodegradation, Dasu et al. [27,28] showed remarkable differences in the degradation half-life between agricultural and forest soils. Differences in the degradation rate could have been attributable to the type of microbial communities present in the soils or total amount of bacteria. With respect to differences in metabolite profile, during 6:2 FTOH biotransformation in river sediments, the 6:2 FTCA and 6:2 FTUCA were shown to be major intermediate metabolites [23], but these metabolites were not observed in soil incubations because of their rapid degradation [17]. Also, Kim et al. [18] showed that 2 bacterial strains formed different metabolites during FTOH biodegradation, specifically with respect to x:3 FTCA and x:3 FTUCA formation. These results indicate that extrapolating metabolite yields across different matrixes (e.g., between sediment and soil) or among matrixes of different origin (e.g., between different soil types) is not possible.

Most fluorotelomer-compound metabolism studies have been performed with either microbial systems or rats and mice. To date, there has been very limited study of FTOH-based compounds in fish, with few exceptions [19,33,46–48]. These include in vitro studies performed by Nabb et al. [19] and Butt et al. [48], as well as the in vivo studies by Brandsma et al. [46] and Butt et al. [33,47]. In addition, no research has been conducted with avian species. Furthermore, only 1 study has investigated inhalation exposure [43]. Volatile precursors are widely detected in the atmosphere and thus could represent important PFCA sources via inhalation.

Biotransformation studies thus far have predominately used the 8:2 FTOH as the parent compound. As a result of industry's transition to shorter-chain fluorotelomer chemistry, an increasing number of studies have investigated 6:2 FTOH biotransformation [15,18,21–23]. Research is very limited, but some studies have shown that metabolite yield can vary between compounds of different chain lengths (e.g., 6:2 and 8:2 FTOH). For example, Liu et al. [21] showed that, for soil incubations, the major metabolite of 8:2 FTOH degradation was PFOA. For 6:2 FTOH degradation, however, the analogous metabolite (PFHxA) was a minor metabolite, and the major metabolite was PFPeA. Differences between the metabolite profiles may be attributable to the covalent bonding of some of the metabolic intermediates to organic matter [21,23]. For example, the x:3 FTCAs have been identified as key intermediates in fluorotelomer compound biotransformation (7:3 FTCA formed from 8:2 FTOH, 5:3 FTCA formed from 6:2 FTOH). During 5:3 and 7:3 FTCA biotransformation in WWTP sludge, Wang et al. [26] showed the 5:3 FTCA was slowly biotransformed (22 mol% after 90 d) but the 7:3 FTCA was essentially not biodegraded (only 1.7 mol% loss after 90 d). The differences may have been attributable to increased binding of the 7:3 FTCA to sludge organic matter, rendering the 7:3 FTCA nonbioavailable. Additional studies are needed, particularly with non-microbial model systems.

In addition, a few studies have examined fluorotelomer-based polymer degradation [36–38]. These studies were not explicitly designed to investigate biotransformation pathways but rather to quantify polymer degradation half-lives. The large discrepancy in the degradation half-lives between the studies warrants further investigation. This is particularly relevant considering the largest commercial category of polyfluorinated compounds is reportedly the fluorotelomer acrylate–based polymers [36]. Finally, there have been a few studies of other fluorotelomer-based compound biotransformation (i.e., not FTOHs). These include PAPs compounds [44,45], 8:2 FTAC [47,48], FTCAs and FTUCAs [31,33], and the 6:2 FTSA [30]. The PAPs compounds and 8:2 FTAC were shown to be direct precursors to FTOHs and thus follow similar degradation pathways. Thus, additional studies are needed on the biotransformation of compounds that are potential FTOH precursors, such as the FTOs and fluorotelomer iodides.

REFERENCES

1. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, De Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SPJ. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classifications and origins. *Integr Environ Asess Manag* 7:513–541.
2. Hansen KJ, Clemen LA, Ellefson ME, Johnson HO. 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ Sci Technol* 35:766–770.
3. Giesy JP, Kannan K. 2001. Distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 35:1339–1342.
4. Martin JW, Smithwick MM, Braune BM, Hoekstra PF, Muir DCG, Mabury SA. 2004. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ Sci Technol* 38:373–380.
5. Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DCG. 2006. Biological monitoring of polyfluoroalkyl substances: A review. *Environ Sci Technol* 40:3463–3473.
6. Houde M, De Silva AO, Muir DCG, Letcher RJ. 2011. Monitoring of perfluorinated compounds in aquatic biota: An updated review of PFCs in aquatic biota. *Environ Sci Technol* 45:7962–7973.
7. Sturm R, Ahrens L. 2010. Trends of polyfluoroalkyl compounds in marine biota and in humans. *Environ Chem* 7:457–484.
8. Butt CM, Berger U, Bossi R, Tomy GT. 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci Total Environ* 408:2936–2965.
9. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. 2006. Sources, fate and transport of perfluorocarboxylates. *Environ Sci Technol* 40: 32–44.
10. Young CJ, Mabury SA. 2010. Atmospheric perfluorinated acid precursors: Chemistry, occurrence, and impacts. *Rev Environ Contam Toxicol* 208:1–109.
11. Hagen DF, Belisle J, Johnson JD, Venkateswarlu P. 1981. Characterization of fluorinated metabolites by a gas chromatographic-helium microwave plasma detector: The biotransformation of 1H,1H,2H,2H-perfluorodecanol to perfluorooctanoate. *Anal Biochem* 118:336–343.
12. Dinglasan MJA, Ye Y, Edwards EA, Mabury SA. 2004. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ Sci Technol* 38:2857–2864.
13. Wang N, Szostek B, Folsom PW, Sulecki LM, Capka V, Buck RC, Berti WR, Gannon JT. 2005. Aerobic biotransformation of C-14-labeled 8-2 telomer B alcohol by activated sludge from a domestic sewage treatment plant. *Environ Sci Technol* 39:531–538.
14. Wang N, Szostek B, Buck RC, Folsom PW, Sulecki LM, Capka V, Berti WR, Gannon JT. 2005. Fluorotelomer alcohol biodegradation: Direct evidence that perfluorinated carbon chains breakdown. *Environ Sci Technol* 39:7516–7528.
15. Zhang S, Szostek B, McCausland PK, Wolstenholme BW, Lu XX, Wang N, Buck RC. 2013. 6:2 and 8:2 Fluorotelomer alcohol anaerobic biotransformation in digester sludge from a WWTP under methanogenic conditions. *Environ Sci Technol* 47:4227–4235.
16. Liu J, Lee LS, Nies LF, Nakatsu CH, Turco RF. 2007. Biotransformation of 8:2 fluorotelorner alcohol in soil and by soil bacteria isolates. *Environ Sci Technol* 41:8024–8030.
17. Wang N, Szostek B, Buck RC, Folsom PW, Sulecki LM, Gannon JT. 2009. 8-2 Fluorotelomer alcohol aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. *Chemosphere* 75:1089–1096.
18. Kim MH, Wang N, McDonald T, Chu KH. 2012. Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane-degrading Pseudomonas strains. *Biotechnol Bioeng* 109:3041–3048.
19. Nabb DL, Szostek B, Himmelstein MW, Mawn MP, Gargas ML, Sweeney LM, Stadler JC, Buck RC, Fasano WJ. 2007. In vitro metabolism of 8-2 fluorotelomer alcohol: Interspecies comparisons and metabolic pathway refinement. *Toxicol Sci* 100:333–344.
20. Fasano WJ, Carpenter SC, Gannon SA, Snow TA, Stadler JC, Kennedy GL, Buck RC, Korzeniowski SH, Hinderliter PM, Kemper RA. 2006. Absorption, distribution, metabolism, and elimination of 8-2 fluorotelomer alcohol in the rat. *Toxicol Sci* 91:341–355.
21. Liu JX, Wang N, Szostek B, Buck RC, Panciroli PK, Folsom PW, Sulecki LM, Bellin CA. 2010. 6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. *Chemosphere* 78:437–444.
22. Liu JX, Wang N, Buck RC, Wolstenholme BW, Folsom PW, Sulecki LM, Bellin CA. 2010. Aerobic biodegradation of C-14 6:2 fluorotelomer alcohol in a flow-through soil incubation system. *Chemosphere* 80:716–723.
23. Zhao LJ, Folsom PW, Wolstenholme BW, Sun HW, Wang N, Buck RC. 2013. 6:2 Fluorotelomer alcohol biotransformation in an aerobic river sediment system. *Chemosphere* 90:203–209.
24. Kim MH, Wang N, Chu KH. 2013. 6:2 fluorotelomer alcohol (6:2 FTOH) biodegradation by multiple microbial species under different physiological conditions. *Appl Microbiol Biotechnol* dx.doi.org/10.1007/s00253-013-5131-3
25. US Environmental Protection Agency. 2006. 2010/2015 PFOA Stewardship Program. EPA HQ/2003/0012/1071. [cited 2013 July 13]. Available from: http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship.htm.
26. Wang N, Buck RC, Szostek B, Sulecki LM, Wolstenholme BW. 2012. 5:3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel "one-carbon removal pathways." *Chemosphere* 87:527–534.
27. Dasu K, Liu JX, Lee LS. 2012. Aerobic soil biodegradation of 8:2 fluorotelomer stearate monoester. *Environ Sci Technol* 46:3831–3836.
28. Dasu K, Lee LS, Turco RF, Nies LF. 2013. Aerobic biodegradation of 8:2 fluorotelomer stearate monoester and 8:2 fluorotelomer citrate triester in forest soil. *Chemosphere* 91:399–405.
29. Arakaki A, Ishii Y, Tokuhisa T, Murata S, Sato K, Sonoi T, Tatsu H, Matsunaga T. 2010. Microbial biodegradation of a novel fluorotelomer alcohol, 1H,1H,2H,2H,8H,8H-perfluorododecanol, yields short fluorinated acids. *Appl Microbiol Biotechnol* 88:1193–1203.
30. Wang N, Liu JX, Buck RC, Korzeniowski SH, Wolstenholme BW, Folsom PW, Sulecki LM. 2011. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* 82:853–858.
31. Myers AL, Mabury SA. 2010. Fate of fluorotelomer acids in a soil-water microcosm. *Environ Toxicol Chem* 29:1689–1695.
32. Lee H, D'Eon J, Mabury SA. 2010. Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. *Environ Sci Technol* 44:3305–3310.
33. Butt CM, Muir DCG, Mabury SA. 2010. Elucidating the pathways of poly- and perfluorinated acid formation in rainbow trout. *Environ Sci Technol* 44:4973–4980.
34. Fromel T, Knepper TP. 2010. Fluorotelomer ethoxylates: Sources of highly fluorinated environmental contaminants—Part I: Biotransformation. *Chemosphere* 80:1387–1392.
35. DuPont Chemicals and Flouroproducts. 2011. Material safety data sheet: Zonyl® FSH. Ver 2.0. Ref 130000000346. Wilmington (DE). [cited 2013 July 13]. Available from: http://msds.dupont.com/msds/pdfs/EN/PEN_09004a3580542b04.pdf.
36. Russell MH, Berti WR, Szostek B, Buck RC. 2008. Investigation of the biodegradation potential of a fluoroacrylate polymer product in aerobic soils. *Environ Sci Technol* 42:800–807.
37. Washington JW, Ellington JJ, Jenkins TM, Evans JJ, Yoo H, Hafner SC. 2009. Degradability of an acrylate-linked, fluorotelomer polymer in soil. *Environ Sci Technol* 43:6617–6623.
38. Russell MH, Berti WR, Szostek B, Wang N, Buck RC. 2010. Evaluation of PFO formation from the biodegradation of a fluorotelomer-based urethane polymer product in aerobic soils. *Polym Degrad Stabil* 95:79–85.
39. Martin JW, Mabury SA, O'Brien PJ. 2005. Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes. *Chem-Biol Interact* 155:165–180.
40. Kudo N, Iwase Y, Okayachi H, Yamakawa Y, Kawashima Y. 2005. Induction of hepatic peroxisome proliferation by 8-2 telomer alcohol feeding in mice: Formation of perfluorooctanoic acid in the liver. *Toxicol Sci* 86:231–238.
41. Henderson WM, Smith MA. 2007. Perfluorooctanoic acid and perfluorononanoic acid in fetal and neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol. *Toxicol Sci* 95:452–461.
42. Fasano WJ, Sweeney LM, Mawn MP, Nabb DL, Szostek B, Buck RC, Gargas ML. 2009. Kinetics of 8-2 fluorotelomer alcohol and its metabolites, and liver glutathione status following daily oral dosing for 45 days in male and female rats. *Chem-Biol Interact* 180:281–295.
43. Himmelstein MW, Serex TL, Buck RC, Weinberg JT, Mawn MP, Russell MH. 2012. 8:2 fluorotelomer alcohol: A one-day nose-only inhalation toxicokinetic study in the Sprague-Dawley rat with application to risk assessment. *Toxicology* 291:122–132.
44. D'Eon JC, Mabury SA. 2007. Production of perfluorinated carboxylic acids (PFCAs) from the biotransformation of polyfluoroalkyl phosphate surfactants (PAPS): Exploring routes of human contamination. *Environ Sci Technol* 41:4799–4805.
45. D'Eon JC, Mabury SA. 2011. Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): Evaluating uptake, elimination, and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat. *Environ Health Perspect* 119:344–350.
46. Brandsma SH, Smithwick M, Solomon K, Small J, de Boer J, Muir DCG. 2011. Dietary exposure of rainbow trout to 8:2 and 10:2 fluorotelomer alcohols and perfluorooctanesulfonamide: Uptake, transformation and elimination. *Chemosphere* 82:253–258.

47. Butt CM, Muir DCG, Mabury SA. 2010. Biotransformation of the 8:2 fluorotelomer acrylate in rainbow trout. 1. In vivo dietary exposure. *Environ Toxicol Chem* 29:2726–2735.
48. Butt CM, Muir DCG, Mabury SA. 2010. Biotransformation of the 8:2 fluorotelomer acrylate in rainbow trout. 2. In vitro incubations with liver and stomach S9 fractions. *Environ Toxicol Chem* 29:2736–2741.
49. Lei YD, Wania F, Mathers D, Mabury SA. 2004. Determination of vapor pressures, octanol-air, and water-air partition coefficients for polyfluorinated sulfonamide, sulfonamidoethanols, and telomer alcohols. *J Chem Eng Data* 49:1013–1022.
50. Rand AA, Mabury SA. 2013. Covalent binding of fluorotelomer unsaturated aldehydes (FTUALs) and carboxylic acids (FTUCAs) to proteins. *Environ Sci Technol* 47:1655–1663.