6:2 Fluorotelomer iodide *in vitro* metabolism by rat liver microsomes: Comparison with [1,2-¹⁴C] 6:2 fluorotelomer alcohol

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HIGHLIGHTS

- 6:2 FTI metabolism in rat liver microsomes was explored for the first time.
- 5:3 Acid was the primary stable metabolite in the microsomes dosed with 6:2 FTI.
- 6:2 FTI metabolism in the microsomes also produced PFBA, PFPeA, and PFHxA.
- 6:2 FTOH in the microsomes produced more 5:3 acid, PFBA, PFPeA, and PFHxA.
- More PFHpA was produced from 6:2 FTI metabolism than observed from 6:2 FTOH metabolism.

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ABSTRACT

6:2 Fluorotelomer iodide [6:2 FTI, F(CF₂)₆CH₂CH₂I] is the industrial raw material used to manufacture 6:2 fluorotelomer alcohol [6:2 FTOH, F(CF₂)₆CH₂CH₂OH] and 6:2 FTOH-based products. During its manufacture and industrial use, workers may be exposed to via oral, dermal or inhalation of 6:2 FTI. Therefore it is useful to understand how 6:2 FTI may be metabolized and into what transformation products. 6:2 FTI *in vitro* rat liver microsomal metabolism was explored for the first time to compare its biotransformation potential with that of [1,2-¹⁴C] 6:2 FTOH [F(CF₂)₆¹⁴CH₂CH₂OH]. 6:2 FTI and 6:2 FTOH metabolite yields were determined in closed-bottle systems using Sprague Dawley and Wistar Han rat microsomes after incubation at 37 °C for up to 6 h with NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)-addition and NADPH-regenerating systems, respectively. 5:3 acid [F(CF₂)₅CH₂CH₂COOH] was the most abundant metabolite for 6:2 FTI (3.3–6.3 mol%) and 6:2 FTOH (9–12 mol%). Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), and perfluorohexanoic acid (PFHxA) in sum accounted for 1.3–2.2 mol% from 6:2 FTI and 2.7–4.4 mol% from 6:2 FTOH biotransformation. Perfluoroheptanoic acid (PFHpA) accounted for 0.14–0.36 mol% from 6:2 FTI but only 0.01–0.06 mol% from 6:2 FTOH biotransformation. These results suggest that mammalian systems exposed to 6:2 FTI or 6:2 FTOH would form 5:3 acid, PFBA, PFPeA, PFHxA as the primary stable metabolites, whereas more PFHpA would be expected from 6:2 FTI biotransformation.

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1. Introduction

Long-chain PFCAs (≥8 carbon chain length) such as PFOA (perfluorooctanoic acid) and perfluoroalkane sulfonic acids (≥6 carbon

chain length) such as PFOS (perfluorooctanesulfonic acid) and their potential precursors are being phased out (U.S. EPA, 2013) due to their presence in the global environment and biota including humans, persistence and toxicological properties (Houde et al., 2011; Lau, 2012). 6:2 FTI is the industrial raw material used to manufacture 6:2 FTOH and 6:2 FTOH-based products (Kissa, 2001), which are being used to replace the long-chain 8:2 FTOH [F(CF₂)₈CH₂CH₂OH]-based products (Ritter, 2010). 8:2 FTOH can degrade to form PFOA in the environment via microbial biotransformation (Wang et al., 2005, 2009; Liu et al., 2007; Dasu et al., 2013). Further, 8:2 FTOH metabolism in mammalian species including humans formed low levels of PFOA and 7:3 acid

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[F(CF₂)₇CH₂CH₂COOH], and to a less extent PFNA (perfluorononanoic acid) (Martin et al., 2005; Fasano et al., 2006; Nabb et al., 2007; Nilsson et al., 2013). Historically, 5000–6000 t per year equivalent of perfluoroalkyl iodide was manufactured globally between 2000 and 2002 (Prevedouros et al., 2006). The alternatives, which cannot break down to long-chain PFCAs, have been approved by regulators and are based on short-chain 6:2 FTI as a key raw material.

6:2 FTI is more volatile with an estimated vapor pressure of 386 Pa compared to 129 Pa for 6:2 FTOH (U.S. EPA, 2011). 6:2 FTI can be emitted to the air and other environmental matrices during its manufacture and industrial use (Ruan et al., 2010a,b). For instance, 6:2 FTI levels reported at a manufacturer site and the local vicinity in China ranged from 1.4 to 368 ng m⁻³ air in the atmosphere and 17 ng kg⁻¹ in surface soil (Ruan et al., 2010a). At point sources such as manufacturing sites, inhalation may be a potential worker exposure route to 6:2 FTI, which would be further metabolized by cellular enzymes. No published information is available on 6:2 FTI metabolism in mammalian species including humans. Therefore, it is not clear whether 6:2 FTI can be biotransformed similarly to 6:2 FTOH and what would be the primary transformation products once 6:2 FTI entered mammalian cellular systems. Very limited information is available in the published scientific literature about 6:2 FTI potential toxicity. An *in vitro* study of MCF-7 cancer cell culture dosed with 6:2 FTI showed that 6:2 FTI did not elicit estrogenic activities while other perfluoroalkyl iodides did (Wang et al., 2012a).

6:2 FTOH can be rapidly biotransformed by microbes in soil, sediment, activated sludge, and bacterial cultures to form perfluorocarboxylic acids (PFCAs) including PFBA, PFPeA, and PFHxA as well as x:3 acids [F(CF₂)_xCH₂CH₂COOH, x = 3, 4, 5] (Liu et al., 2010a; Kim et al., 2012; Zhao et al., 2013a,b). No conjugated metabolites via phase II metabolism (e.g., sulfation, glucosylation, and glutathione-mediated reactions) were observed in these studies. 6:2 FTOH is a structural analog and an initial biotransformation product of 6:2 FTI whose terminal iodide was substituted with a hydroxyl group by a microbial enzyme in soil (Ruan et al., 2013). In addition, 6:2 FTI biotransformation in soil also yielded PFHpA (16 mol%) via a pathway still yet to be elucidated (Ruan et al., 2013). Low levels of PFHpA (<1.5 mol%) were also observed along with PFHxA and 5:3 acid in activated sludge dosed with 6:2 fluorotelomer phosphates (Lee et al., 2010). Similarly, PFPeA, PFHxA, and 5:3 acid were observed during microbial biotransformation of 6:2 FTOH-based ethoxylates and 6:2 fluorotelomer sulfonic acid [6:2 FTSA, F(CF₂)₆CH₂CH₂SO₃H] (Frömel and Knepper, 2010; Wang et al., 2011). Biomonitoring studies found low levels of short-chain PFCAs including PFBA and PFHpA in biota (Houde et al., 2011).

6:2 FTOH *in vitro* metabolism in rat, mouse and human hepatocytes has been reported (Gannon et al., 2010). Major metabolic pathways for 6:2 FTOH in hepatocytes from all three species included either the formation of glutathione (major route), glucuronic acid, or sulfate conjugates of the parent. Another important metabolic pathway resulted in the formation of 5:3 acid, PFPeA, and PFHxA via various transient intermediates. Similarly, *in vivo* studies have reported the profile of 6:2 FTOH and its metabolites in male and female rat plasma following 90 d of oral exposure and 1, 5, and 28 d of inhalation exposure (Gannon et al., 2012; Himelstein et al., 2012). 75–90 mol% of the dose was eliminated within 24 h and metabolite profiles for both dosing regimens were similar. The observed metabolites in both *in vitro* and *in vivo* studies include PFBA, PFHxA, PFHpA, 6:2 FTCA [F(CF₂)₆CH₂COOH], 6:2 FTUCA [F(CF₂)₅CF = CHCOOH], and 5:3 acid. 5:3 acid was the metabolite observed in plasma at the highest concentration (~1–2 mol%).

The objectives of this study were to investigate 6:2 FTI and 6:2 FTOH metabolism, quantify metabolites, and establish biotransformation pathways using *in vitro* rat liver microsomes as a model

system. This allowed for quantitative comparison of yields of primary and stable metabolites from 8:2 FTOH metabolism using similar rodent systems. Information from this study may help elucidate the similarities of 6:2 FTI biotransformation pathways in mammalian species compared to those of 6:2 FTOH and 8:2 FTOH. The study outcomes can also provide insight to assess potential risk from occupational exposure to 6:2 FTI and associated primary and stable metabolites and to understand the sources and distribution of PFCAs in biota including humans.

2. Material and methods

2.1. Chemicals

The chemical names, acronyms and molecular structures of the poly- and perfluoroalkyl target analytes described in this article are shown in Table S1. [1,2-¹⁴C] 6:2 FTOH (radiochemical purity: 99%) was custom synthesized by Perkin Elmer (Boston, MA) and has a specific activity of 29.9 mCi mmol⁻¹. The ¹⁴C-labeled 6:2 FTOH contains 64.7% of unlabeled 6:2 FTOH based on LC/MS/MS analysis (Liu et al., 2010a) and a 23.6 µCi mL⁻¹ stock solution in acetonitrile was prepared and stored at -20 °C. 6:2 FTI (98% purity) was obtained from Alfa Aesar (WardHill, MA). Other fluorinated metabolite standards used for quantitative analysis were the same as previously described with 96% or better purity (Ruan et al., 2013; Zhang et al., 2013). These standards include: 6:2 FTOH, 6:2 FTCA, 6:2 FTUCA, 5:2 ketone [F(CF₂)₅C(O)CH₃], 5:2 sFTOH [F(CF₂)₅CH(OH)CH₃], PFBA, PFPeA, PFHxA, PFHpA, 5:3 acid, 5:3 Uacid [F(CF₂)₅CH = CHCOOH], 4:3 acid [F(CF₂)₄CH₂CH₂COOH], and α-OH 5:3 acid [F(CF₂)₅CH₂CH(OH)COOH]. [1,1,2,2-D; 3-¹³C] 6:2 FTOH [F(CF₂)₅¹³CF₂CD₂CD₂OH] (DuPont, Wilmington, DE) and [1,2-¹³C] PFHxA [F(CF₂)₄¹³CF₂COOH] (Wellington Laboratories, Ontario, Canada) were used as internal standards in LC/MS/MS analysis. Pooled liver microsomes of male Sprague Dawley (SD) and Wistar Han (WH) rats, respectively, glucose-6-phosphate, glucose-6-phosphate dehydrogenase were purchased from BD Inc. (Franklin Lakes, NJ) and Tris-HCl buffer solution (pH = 7.4) was from Sigma-Aldrich (St Louis, MO). Acetonitrile used for LC/MS/MS analysis were LC/MS grade and all other solvents were HPLC grade, and all other chemicals were reagent grade or higher. De-ionized water (18 MΩ) was generated by a Barnstead E-Pure system.

2.2. 6:2 FTI metabolism in rat microsomal incubation system

Pooled liver microsomes of male SD and WH rats, respectively, were incubated in 10 mL serum bottles containing 2 mL reaction media at 37 °C under dark. Liver microsomes from male rats were able to metabolize 8:2 FTOH to form various metabolites (Nabb et al., 2007). In order to investigate potential effects of different reducing-energy systems on microsomal metabolism kinetics, both NADPH re-generating and direct NADPH-addition systems were used separately as the reducing-energy sources. The use of liver microsomes from multiple types (e.g., SD and WH) of rats under different reducing-energy systems allowed us to determine the potential of maximal metabolite yields that otherwise may have been missed with single type under only one reducing-energy system.

For the NADPH re-generating system, 40 µL of 71.4 mg mL⁻¹ MgCl₂ aqueous solution, 20 µL of 2.92 mg mL⁻¹ ethylene diamine tetraacetic acid (EDTA) solution, 40 µL of 39.5 mg mL⁻¹ NADP solution and 40 µL of 282 mg mL⁻¹ glucose-6-phosphate solution were added into 1.83 mL of 0.1 M Tris-HCl buffer (pH 7.4). One milligram (50 µL) of live liver microsomes, 3 units of glucose-6-phosphate dehydrogenase and 20 µL of 0.5 mg mL⁻¹ 6:2 FTI in acetonitrile were then dosed into the suspension, respectively, to initiate the reaction. Afterward, the bottles were sealed immediately with grey

butyl stoppers and aluminum-lined caps. Acetonitrile pre-rinsed C₁₈ cartridges (600 mg, Alltech, Deerfield, IL) coupled with syringe needles were inserted through the butyl stoppers into the headspace of the sample bottles to capture the parent 6:2 FTI and volatile metabolites. Then, the vessels were transferred into an orbital shaker with 250 rpm shaking at 37 °C for up to 6 h. Acetonitrile at 1% (20 µL/2.0 mL, v/v) in the incubation systems was supposed to have no apparent effects on multiple P450-mediated metabolic activities in rat liver microsomes (Li et al., 2010). For the NADPH-addition system, 100 µL of 25.0 mg mL⁻¹ NADPH solution was added into 1.83 mL 0.1 M Tris–HCl buffer before 1 mg of live microsomes and 20 µL of 0.5 mg mL⁻¹ 6:2 FTI solution were dosed.

Heat-inactivated negative control groups with NADPH addition were also prepared by replacing the live microsomes with equal amounts of heat-inactivated ones, which were placed in 100 °C water bath for 10 min before use, while other conditions remained the same as in the live incubation systems. Both 6:2 FTI and 6:2 FTOH have high vapor pressures (e.g. 386 Pa and 129 Pa, respectively) and portions of them are expected to be partitioned to the headspace during incubation, unavailable for microsomal metabolism. Thus, two types of experiment treatments were prepared: (1) live or heat-inactivated microsomes in NADPH-addition system; and (2) live or heat-inactivated microsomes in NADPH-regenerating system. Each of the treatments consisted of triplicate serum bottles per time point. Standard deviation was calculated from the triplicate samples and plotted in figures to account for experimental error or variations.

2.3. [1,2-¹⁴C] 6:2 FTOH metabolism in rat microsomal incubation system

6:2 FTOH is an analog of 6:2 FTI. To compare 6:2 FTI metabolism in rat microsomes with that of 6:2 FTOH, similar experiments were conducted by adding 20 µL of [1,2-¹⁴C] 6:2 FTOH ethanol stock solution (0.47 µCi or 5.7 µg) into the rat microsomal incubation system with identical experimental conditions.

2.4. Sampling and sample preparation procedures

At each sampling time (Time 0, 120 min, 360 min), the headspace of each live and heat-inactivated bottle was purged with approximately 1.5 L of ambient air through a C₁₈ cartridge to capture volatile parent and metabolites. Each C₁₈ cartridge was then eluted with 5 mL acetonitrile and the eluent was stored at below –10 °C before analysis. After headspace purging, the septum from each bottle was removed and immersed into another serum bottle containing 5 mL of acetonitrile for extraction. Afterward, 6:2 FTI or [1,2-¹⁴C] 6:2 FTOH metabolism was quenched by adding 3 mL acetonitrile into the vial and re-sealed with a fresh septum. The quenched-reaction media in the re-sealed bottles were then transferred to a 50 °C incubator and kept shaking at ~200 rpm overnight to extract the parent, 6:2 FTI or [1,2-¹⁴C] 6:2 FTOH, and metabolites. The extracts were then centrifuged at approximately 3000 rpm for 15 min to collect the supernatant, which was filtered through 0.45 µm-pore nylon filters and stored below –10 °C. Finally, the C₁₈ cartridge eluent, septum and reaction media extracts were transferred into LC vials for LC/MS/MS analysis. For [1,2-¹⁴C] 6:2 FTOH, these samples were also analyzed for radioactivity with a LS 5000 TD liquid scintillation counter (Beckman, Fullerton, CA) to determine total ¹⁴C recovery from the experimental systems. For 6:2 FTI experiments, all extracts were also analyzed by GC/MS to quantify 6:2 FTI concentrations.

2.5. Instrumental analysis

The LC/MS/MS quantification method for the target metabolites was similar to a previously described method (Zhang et al., 2013) with some modifications. In brief, the LC/MS/MS system consisted of a Waters (Milford, MA) Quattro Micro triple quadrupole mass spectrometry coupled with a Waters 2795 HPLC module. Electrospray ionization was operated in negative mode and multiple reaction monitoring (MRM) was used. The HPLC separation column was an Agilent (Santa Clara, CA) Zorbax® RX-C8 (150 mm × 2.1 mm, 5 µm particle size, pore size 80 Å, not end-capped with 5.5% carbon loading). The mobile phase consisted of 0.15% acetic acid in de-ionized water and 0.15% acetic acid in acetonitrile in a gradient manner with a flow rate of 0.4 mL min⁻¹. Before LC/MS/MS analysis, all samples were spiked with 50 µL internal standard solution containing 10 ng [1,2-¹³C] PFHxA and 250 µg [1,1,2,2-D;3-¹³C] 6:2 FTOH. GC/MS quantification of 6:2 FTI was conducted in an Agilent 6890 GC/5973 MS system in electron impact ionization positive mode (Ruan et al., 2013). A DB-5 MS column (5% phenyl, 95% dimethylarylene siloxane, 0.25 mm i.d., 30 m long, 1 µm film thickness, J&W Scientific, Folsom, CA) was chosen for analyte separation. Selective ion monitoring (SIM) was applied to increase the sensitivity in quantitative data acquisition. As no isotope-labeled internal standards were available for 6:2 FTI, external calibration standard curves were used for the quantification analysis. Detailed information on the instrumental methods and the detection limit for each of the analytes are provided in the [Supplementary material \(Tables S2 and S3\)](#).

3. Result and discussion

3.1. Observed metabolites from rat microsomal biotransformation

To our knowledge, this is the first report on 6:2 FTI *in vitro* biotransformation in mammalian species. 6:2 FTI was readily transformed in both SD and WH rat microsomes to at least 15 Phase I metabolites. No metabolism was observed in the negative control groups. Potential Phase II conjugates were not monitored as the main purpose of this work was to quantify primary stable metabolites.

5:3 Acid, 4:3 Acid and PFCAs (PFBA, PFPeA, PFHxA and PFHpA) were found in the live incubation systems as stable transformation products. Transient metabolites were also detected, including 6:2 FTOH, 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, and 5:3 Uacid. Other transient metabolites {α-OH 5:3 acid, 5:2 acid [F(CF₂)₅CH₂-COOH], and 5:2 Uacid [F(CF₂)₄CF = CHCOOH]} from 5:3 acid one-carbon removal pathways reported by Wang et al. (2012b) were only detected in SD rat microsomes with NADPH-regenerating system at 0.3 mol% together. The non-detection of above transient metabolites in WH rats may be attributed to intra-species variation in metabolizing 5:3 acid between SD and WH rats. Nevertheless, the presence of 5:3 acid and 4:3 acid, as well as other transient metabolites indicates the importance of the 5:3 acid pathway in 6:2 FTI metabolism in SD rat liver microsomal systems. Most of the corresponding transient and stable metabolites have also been reported in a 6:2 FTI aerobic soil study (Ruan et al., 2013), suggesting similarities in mammalian and microbial biotransformation pathways. Moreover, 6:2 FTCA detected in this work is consistent with the formation of 8:2 FTCA in *in vitro* mammalian metabolism of 8:2 FTOH (Nabb et al., 2007). However, 6:2 FTCA and 8:2 FTCA were not observed in soil spiked with 6:2 FTOH and 8:2 FTOH, respectively, due to their rapid microbial conversion to 5:3 acid and 7:3 acid (Wang et al., 2009; Liu et al., 2010a,b).

The [1,2-¹⁴C] 6:2 FTOH metabolism study presented here in rat liver microsomal systems showed a metabolite profile similar to

those for 6:2 FTI, prior 6:2 FTOH hepatocyte and *in vivo* metabolism studies (Himmelstein et al., 2012; Gannon et al., 2010, 2012) as well as 8:2 FTOH metabolism studies (Martin et al., 2005; Nabb et al., 2007). Six stable transformation products (5:3 acid, 4:3 acid, PFBA, PFPeA, PFHxA and PFHpA) and five transient metabolites (6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, 5:3 Uacid) were observed in 6:2 FTOH-dosed rat liver microsomes. Other transient metabolites (α -OH 5:3 acid, 5:2 Uacid, and 5:2 Uacid) were only detected sporadically among the different experimental systems at low levels (<0.15 mol% together). 5:3 acid was also detected in serum samples of ski-wax workers exposed to 6:2 FTOH via inhalation (Nilsson et al., 2013).

3.2. Mass balance in the incubation systems

The overall molar mass balance is defined as the quantifiable parent and metabolites recovered from reaction media extracts and the headspace captured by C18 cartridges and septa. For the heat-inactivated SD rat microsomal control group in NADPH-addition system, 84–122 mol% of initially dosed 6:2 FTI were recovered (Table 1). For the heat-inactivated WH rat microsomal control group in NADPH-addition system, 120–126 mol% of initially dosed 6:2 FTI were recovered (Table 1). For the live SD microsomal incubation systems, 59–122 mol% of dosed 6:2 FTI and metabolites were recovered with the exception in NADPH-regenerating system at 2 h with only 43 mol% recovery (Table 1). This lower mass balance may be due to losses of vapor-phase 6:2 FTI not captured in the C18 cartridges, reflecting the challenge of working with 6:2 FTI since it is more volatile than 6:2 FTOH. For the live WH microsomal incubation systems, 103–123 mol% of dosed 6:2 FTI and metabolites were recovered (Table 1). Overall, the mass balance in both the live and heat-inactivated groups is satisfactory with one exception noted above.

Most of the 6:2 FTI in the incubation system partitioned to the headspace after dosing into the aqueous media. In the heat-inactivated control groups, the molar percentage of 6:2 FTI in the headspace increased from 72.0 mol% at 2 h to 99 mol% after 6-h incubation. Similarly, 6:2 FTI in the headspace of the dosed live samples increased from 69 mol% at 2 h to 99% after 6-h incubation.

6:2 FTI partitioning to the headspace explains the low metabolite yields in the live incubation systems in comparison with 6:2 FTOH. The 6:2 FTI in the headspace apparently did not partition back to the incubation system and was not available for biotransformation.

[1,2- 14 C] 6:2 FTOH mass balance in the dosed systems is provided in Table 2. For the heat-inactivated control groups in NADPH-addition system, 96–99 mol% of initially dosed 14 C was recovered from the SD rat microsomal system and 91–97 mol% from the WH rat microsomal system. For the live incubation systems, 91–121 mol% of dosed 14 C were recovered from the SD microsomal system and 103–115 mol% from the WH microsomal system.

3.3. Metabolite yields from 6:2 FTI biotransformation

5:3 Acid was the most abundant stable metabolite observed in all experimental systems (e.g., SD and WH rat liver microsomes in NADH-addition and NADH-regenerating systems) from 6:2 FTI biotransformation (Fig. 1). For SD microsomes (Fig. 1A), the NADPH-regenerating system produced more 5:3 acid (6.3 mol%) than in NADPH-addition system (3.3 mol%) after 6-h of incubation. For WH microsomes (Fig. 1B), 5:3 acid molar yields are essentially the same in NADPH-addition and NADPH-regenerating systems (5.2 mol% versus 5.1 mol%). On an average basis, 5:3 acid accounted for 5 mol% of initially dosed 6:2 FTI in all experimental systems, 3–4 times lower than observed in soil, sediment, and activated sludge dosed with 6:2 FTOH (Liu et al., 2010a; Zhao et al., 2013a,b). 4:3 acid ranged 0.02–0.29 mol% in all experimental systems after 6-h incubation, corresponding to a maximum conversion ratio of 0.058 (0.29 mol% divided by 5 mol%) from 5:3 Acid. PFBA, PFPeA, and PFHxA together ranged 1.3–2.2 mol% and PFHpA ranged 0.14–0.36 mol% of initially dosed 6:2 FTI in all experimental systems after 6-h incubation. 5:3 acid molar yields in all experimental systems are 2.3–2.6 times higher than the sum of the PFCAs discussed above whereas 7:3 acid is about 22% higher than the sum of PFOA and PFNA in SD rat microsomes in NADPH-addition system dosed with 8:2 FTOH (Table 3 and reference therein).

All other transient metabolites including 6:2 FTOH, 6:2 FTCA, 6:2 FTUCA, 5:3 Uacid, 5:2 ketone, and 5:2 sFTOH together

Table 1

Total recovery of 6:2 FTI and quantifiable metabolites (transformation products) from microsomal reaction media and volatile phase captured by C18 cartridges and septa as mol% (average $\pm$ standard deviation for $n = 3$) of initially dosed 6:2 FTI [$F(CF_2)_6CH_2CH_2I$] at 5 $\mu g mL^{-1}$ reaction media.

Sample	Sprague Dawley rat		Wistar Han rat	
	NADPH addition	NADPH regeneration	NADPH addition	NADPH regeneration
<i>120-min incubation</i>				
Live reactions	59 $\pm$ 8	43 $\pm$ 6	123 $\pm$ 2	103 $\pm$ 20
Heat-inactivated	84 $\pm$ 6	–	120 $\pm$ 9	–
<i>360-min incubation</i>				
Live reactions	79 $\pm$ 15	59 $\pm$ 3	116 $\pm$ 5	115 $\pm$ 19
Heat-inactivated	122 $\pm$ 8	–	126 $\pm$ 5	–

Table 2

Total recovery of ^{14}C radioactivity from microsomal reaction media and volatile phase captured by C18 cartridges and septa as % (average $\pm$ standard deviation for $n = 3$) of initially dosed [1,2- ^{14}C] 6:2 FTOH [$F(CF_2)_6^{14}CH_2CH_2OH$] at 0.47 μCi or 5.7 $\mu g mL^{-1}$ reaction media.

Sample	Sprague Dawley rat		Wistar Han rat	
	NADPH addition	NADPH regeneration	NADPH addition	NADPH regeneration
<i>120-min incubation</i>				
Live reactions	91 $\pm$ 2	110 $\pm$ 2	104 $\pm$ 7	103 $\pm$ 18
Heat-inactivated	96 $\pm$ 8	–	97 $\pm$ 4	–
<i>360-min incubation</i>				
Live reactions	96 $\pm$ 5	121 $\pm$ 5	111 $\pm$ 10	115 $\pm$ 9
Heat-inactivated	99 $\pm$ 13	–	91 $\pm$ 8	–

Fig. 1. Molar yields of 6:2 FTI biotransformation products in rat liver microsomal incubation systems dosed with 5 μg 6:2 FTI mL^{-1} reaction media. Data are average from triplicate samples and error bars represent standard deviation. (A) Sprague-Dawley rat; (B) Wistar Han rat.

accounted for 2.8–3.2 mol% in SD microsomal systems and 3.7–3.8 mol% in WH microsomal systems dosed with 6:2 FTI after 6-h incubation. No significant discernible patterns were observed for these transient metabolites among the different experiment systems. For SD microsomes, the total yields of stable and transient metabolites averaged 7.9 and 12 mol% in NADPH-addition and NADPH-regenerating systems, respectively (Fig. 1A). For WH

Fig. 2. Molar yields of [1,2- ^{14}C] 6:2 FTOH biotransformation products in rat liver microsomal incubation systems dosed with 0.47 μCi or 5.7 μg [1,2- ^{14}C] 6:2 FTOH mL^{-1} reaction media. Data are average from triplicate samples and error bars represent standard deviation. (A) Sprague-Dawley rat; (B) Wistar Han rat.

microsomes, the total yields of stable and transient metabolites averaged 11 and 10 mol% in NADPH-addition and NADPH-regenerating systems, respectively (Fig. 1B). These yields on average (10 mol%) are about twice as higher than the SD rat microsomes in NADPH-regenerating system dosed with 8:2 FTOH (Nabb et al., 2007) and similar to those in rainbow trout liver microsomes in

Table 3
Comparison of biotransformation yields (mol%) of stable metabolites in rat microsomal incubation systems dosed with 8:2 FTOH [F(CF₂)₈CH₂CH₂OH], [1,2- ^{14}C] 6:2 FTOH, and 6:2 FTI.

	8:2 FTOH (Nabb et al., 2007)	[1,2- ^{14}C] 6:2 FTOH (this study ^a)	6:2 FTI (this study ^a)
Loss of 1 C (one -CH ₂ - group)	0.04 (PFNA)	0.02–0.06 (PFHpA)	0.36 (PFHpA)
Loss of 2 C (two -CH ₂ - groups)	1.9 (PFOA)	4.0 (PFHxA)	0.77 (PFHxA)
Loss of 3 C (one -CF ₂ - group, two -CH ₂ - groups)	<0.01 (PFHpA)	0.16 (PFPeA)	0.76 (PFPeA)
Loss of 4 C (two -CF ₂ - groups, two -CH ₂ - groups)	0.13 (PFHxA)	0.21 (PFBA)	0.66 (PFBA)
x:3 acids	2.5 (7:3 acid)	12 (5:3 acid)	6.3 (5:3 acid)
		0.68 (4:3 acid)	0.29 (4:3 acid)
Sum of stable metabolites	4.6	17	9.1

^a The metabolite yields of [1,2- ^{14}C] 6:2 FTOH and 6:2 FTI biotransformation are results of the dosed live groups in NADPH re-generating system at 360 min incubation time, similar to the system used by Nabb et al. (2007).

NADPH-addition system dosed with a PFOS precursor, *N*-ethyl perfluorooctanesulfonamide [$C_8F_{17}SO_2NH(C_2H_5)$] (Tomy et al., 2004).

3.4. Metabolite yields from $[1,2-^{14}C]$ 6:2 FTOH biotransformation

Similar to 6:2 FTI biotransformation, 5:3 acid was also the most abundant stable metabolite observed in all experimental systems among the quantifiable metabolites from $[1,2-^{14}C]$ 6:2 FTOH biotransformation as summarized in Fig. 2. For SD microsomes (Fig. 2A), NADPH-addition system generated 9.7 mol% 5:3 acid while NADPH-regenerating system produced 12 mol% after 6-h of incubation. For WH microsomes (Fig. 2B), 5:3 acid molar yields are similar in NADPH-addition and NADPH-regenerating systems (10 mol% versus 9.1 mol%). On an average basis, 5:3 acid accounts for 10 mol% of initially dosed 6:2 FTOH in all experimental systems, two times higher than the yields from 6:2 FTI biotransformation. This suggests that 6:2 FTOH biotransformation is faster than 6:2 FTI, most of which partitioned to the headspace and was not available for biotransformation. 4:3 acid was only formed in NADPH-regenerating systems and accounts for 0.69 mol% and 0.32 mol% in SD and WH microsomes, respectively.

PFHxA was the primary PFCA observed from 6:2 FTOH *in vivo* and *in vitro* biotransformation in mammalian systems. For SD microsomes, PFHxA accounted for 2.3 mol% in NADPH-addition system after 6-h incubation and 4.0 mol% in NADPH-regenerating system. For WH microsomes, PFHxA accounted for 2.2 and 2.7 mol% in the two systems, respectively, after 6-h incubation. On an average basis, PFHxA accounted for 2.8 mol% of initially dosed 6:2 FTOH, 5.6-fold higher than the average sum of PFBA and PFPeA in all experimental systems. The distinctly higher PFHxA yield than PFBA and PFPeA was not observed in 6:2 FTI biotransformation in all the rat microsomal systems, indicating a more prominent PFHxA biotransformation pathway from 6:2

FTOH. This observation is consistent with 8:2 FTOH biotransformation in SD microsomes (Nabb et al., 2007) in which PFOA level is much higher than the sum of shorter-chain PFHpA and PFHxA (Table 3). PFHpA was barely detected in the NADPH-addition system of SD microsomes (0.01 mol%) and WH microsomes (0.02 mol%) after 6-h incubation with $[1,2-^{14}C]$ 6:2 FTOH. All other transient metabolites including 6:2 FTCA, 6:2 FTUCA, 5:3 Uacid, 5:2 ketone, and 5:2 sFTOH together accounted for 16–19 mol% in SD microsomal systems and 21–27 mol% in WH microsomal systems dosed with 6:2 FTI after 6-h incubation.

For SD microsomes, the total yields of stable and transient metabolites averaged 32 and 34 mol% in NADPH-addition and NADPH-regenerating systems, respectively (Fig. 2A). For WH microsomes, the total yields of stable and transient metabolites averaged 40 and 24 mol% in NADPH-addition and NADPH-regenerating systems, respectively (Fig. 1B). These yields on average (33 mol%) are at least 6 times higher than 8:2 FTOH biotransformation in SD microsomes (Nabb et al., 2007). This faster 6:2 FTOH transformation may be due to the shorter perfluoroalkyl chain length compared with 8:2 FTOH analog.

5:3 Uacid level is much higher in NADPH-addition system than in NADPH-regenerating system after 6-h incubation with $[1,2-^{14}C]$ 6:2 FTOH. For SD microsomes, 5:3 Uacid accounted for 9.4 mol% in NADPH-addition system versus 1.1 mol% in NADPH-regenerating system. For WH microsomes, 5:3 Uacid accounted for 16 mol% in NADPH-addition system versus 1.0 mol% in NADPH-regenerating system. The greater amounts of 5:3 Uacid indicate that the reducing energy source, NADPH, was nearly depleted in NADPH-addition system, since converting 5:3 Uacid to 5:3 acid needs reducing energy (Zhang et al., 2013). On the other hand, NADPH-regenerating system is more effective at regenerating NADPH from NADP via glucose oxidase electron-and-proton shuttle system, providing sufficient NADPH to reduce 5:3 Uacid to 5:3 acid.

Fig. 3. Proposed 6:2 FTI biotransformation pathways in rat liver microsomes. The double arrows indicate multiple transformation steps. The red arrows indicate pathway to short-chain perfluorocarboxylic acids (i.e., PFBA, PFPeA, and PFHxA), dashed blue arrows represent possible pathway to PFHpA, and green arrows show pathway to 5:3 acid and 4:3 acid. The metabolites in solid rectangular boxes are observed stable metabolites and the metabolite inside a dashed bracket is a putative transient metabolite, 6:2 FTUI, which is not analyzed and verified in this study due to lack of authentic standard. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. 6:2 FTI biotransformation pathways

6:2 FTI biotransformation pathways in rat liver microsomes are proposed in Fig. 3 based on observed transformation products in this study as well as prior knowledge of 6:2 FTI and 6:2 FTOH biotransformation pathways in other environmental matrices (Ruan et al., 2013; Liu et al., 2010a; Zhao et al., 2013a,b). 6:2 FTI generally followed similar biotransformation pathways observed for 6:2 FTOH to form the stable metabolites 5:3 acid, 4:3 acid, PFBA, PFPeA, and PFHxA. 6:2 FTI was first converted to 6:2 FTOH via nucleophilic substitution of the terminal iodide with a hydroxyl group, also occurred in aerobic soil dosed with 6:2 FTI (Ruan et al., 2013). 6:2 FTOH could then be oxidized to 6:2 FTAL [(CF₂)₆-CH₂CHO] as suggested in 6:2 FTOH biotransformation in soil (Liu et al., 2010a) or via similar reaction by converting 8:2 FTOH to 8:2 FTAL [(CF₂)₈-CH₂CHO] in mammalian systems (Martin et al., 2005; Nabb et al., 2007). 6:2 FTAL was not quantified in this study because authentic standard is not currently available. 6:2 FTAL may be further transformed to 6:2 FTCA and 6:2 FTUCA via an oxidation reaction and HF elimination, respectively (Nabb et al., 2007; Liu et al., 2010a).

6:2 FTUCA is the branching point leading to PFPeA and PFHxA via decarboxylation and other steps to form 5:2 ketone, which was converted to 5:2 sFTOH and subsequently to PFPeA and PFHxA via multiple enzymatic steps still yet to be elucidated (Liu et al., 2010a). 6:2 FTUCA was defluorinated to form 5:3 Uacid using NADPH as a co-factor to provide reducing energy via similar one-step reaction reported in soil and activated sludge (Liu et al., 2010a; Wang et al., 2012b). 5:3 Uacid could be converted to PFBA via similar multiple enzymatic steps as reported in activated sludge (Wang et al., 2012b). 5:3 Uacid could also be reduced to 5:3 acid and this step is facile in anaerobic digester sludge dosed with 6:2 FTOH under methanogenic conditions with rich reducing energy sources (Zhang et al., 2013). 5:3 acid biotransformation pathway to 4:3 acid has been elucidated, consisting of 6 enzymatic steps (Wang et al., 2012b).

There may be a unique biotransformation pathway available for 6:2 FTI to be converted to PFHpA via a putative intermediate, 6:2 FTUI [F(CF₂)₆CH₂CH₂I], as suggested but not verified with authentic standard by Ruan et al. (2013). 6:2 FTUI was not quantified in this study due to lack of authentic standard. This hypothetical pathway is plausible to explain the formation of PFHpA from 6:2 FTI via 6:2 FTUI but not from 6:2 FTOH pathways, since PFHpA levels are very low (0.01–0.02 mol% in NADPH addition system and 0.02–0.06 mol% in NADPH re-generating system) in rat liver microsomes dosed with [1,2-¹⁴C] 6:2 FTOH and cannot account for the 0.14–0.36 mol% of PFHpA generated from 6:2 FTI. Future work needs to investigate PFHpA molar yields from 6:2 FTCA and 6:2 FTUI, respectively, in rat microsomal systems and *in vivo* rodent systems to estimate the relative contribution of 6:2 FTUI and 6:2 FTCA to PFHpA in mammalian systems.

4. Conclusion

6:2 FTI and 6:2 FTOH biotransformation in rat microsomes produced similar stable metabolites (i.e., 5:3 acid, 4:3 acid, PFBA, PFPeA, PFHxA, and PFHpA), although the metabolite yields are lower for 6:2 FTI with the exception of PFHpA, which is much higher than from 6:2 FTOH biotransformation. 6:2 FTI was initially converted to 6:2 FTOH and then followed 6:2 FTOH biotransformation pathways to form all the stable metabolites except for PFHpA. Most of the PFHpA observed in rat microsomes dosed with 6:2 FTI is likely from an independent pathway possibly via a putative and unconfirmed transient metabolite, 6:2 FTUI. This suggests that 6:2 FTI may be a more important potential PFHpA precursor than

6:2 FTOH at least in the rat live microsomal systems. This PFHpA pathway, with detailed enzymatic steps yet to be elucidated, bypassed 6:2 FTOH biotransformation pathways. The lower metabolite yields from 6:2 FTI are most likely related to its higher vapor pressure, which led to more 6:2 FTI partitioning into the headspace during the incubation. 5:3 acid was observed as the predominant stable metabolite for both 6:2 FTI and 6:2 FTOH compared with PFCAs, whereas the yields of PFCAs are generally higher or similar to 5:3 acid in aerobic microbes incubated with 6:2 FTOH or 6:2 FTI, illustrating the differences between mammalian and microbial biotransformation processes. Further *in vivo* 6:2 FTI biotransformation studies in mammalian species are needed to investigate the rates of stable metabolite formation to help assess potential 6:2 FTI exposure risk.

Conflict of Interest

Four coauthors are employees of E.I. duPont de Nemours & Company, Inc., a manufacturer of 6:2 FTI.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2014.02.068>.

References

- Dasu, K., Lee, L.S., Turco, R.F., Nies, L.F., 2013. Aerobic biodegradation of 8:2 fluorotelomer stearate monoester and 8:2 fluorotelomer citrate triester in forest soil. *Chemosphere* 91 (3), 399–405.
- Fasano, W.J., Carpenter, S.C., Gannon, S.A., Snow, T.A., Stadler, J.C., Kennedy, G.L., Buck, R.C., Korzenowski, S.H., Hinderliter, P.M., Kemper, R.A., 2006. Absorption, distribution, metabolism and elimination of 8–2 fluorotelomer alcohol in the rat. *Toxicol. Sci.* 91, 341–355.
- Frömel, T., Knepper, T.P., 2010. Fluorotelomer ethoxylates: sources of highly fluorinated environmental contaminants part I: biotransformation. *Chemosphere* 80 (11), 1387–1392.
- Gannon, S., Nabb, D.L., Snow, T.A., Mawn, M.P., Serex, T., Buck, R.C., 2010. In vitro metabolism of 6–2 fluorotelomer alcohol in rat, mouse and human hepatocytes. *Toxicol.—Off. J. Soc. Toxicol.* 114 (1), 97.
- Gannon, S.A., Mawn, M.P., Munley, S.W., Serex, T.L., Buck, R.C., 2012. Toxicokinetic evaluation of 6:2 fluorotelomer alcohol and metabolites in rats following 90-days of oral exposure. *Toxicol.—Off. J. Soc. Toxicol.* 126 (1), 402.
- Himmelstein, M., Shen, Z., Ng, S.P., DeLorme, M.P., Buck, R.C., Serex, T., 2012. Plasma metabolites and dosimetry of 6:2 fluorotelomer alcohol in rats following 1, 5 and 28-day inhalation exposures. *Toxicol.—Off. J. Soc. Toxicol.* 126 (1), 406.
- Houde, M., De Silva, A.O., Muir, D.C.G., Letcher, R.J., 2011. Monitoring of perfluorinated compounds in aquatic biota: an updated review on PFCs in aquatic biota. *Environ. Sci. Technol.* 45, 7962–7973.
- Kim, M.H., Wang, N., McDonald, T., Chu, K.H., 2012. Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane degrading *Pseudomonas* strains. *Biotechnol. Bioeng.* 109, 3041–3048.
- Kissa, E., 2001. *Fluorinated Surfactants and Repellants*, second ed. Marcel Dekker, New York, USA.
- Lee, H., Deon, J., Mabury, S.A., 2010. Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. *Environ. Sci. Technol.* 44, 3305–3310.
- Li, D., Han, Y., Meng, X., Sun, X., Yu, Q., Li, Y., Wan, L., Huo, Y., Guo, C., 2010. Effect of regular organic solvent on cytochrome P450-mediated metabolic activities in rat liver microsomes. *Drug Metab. Dispos.* 38, 1922–1925.
- Lau, C., 2012. Perfluorinated compounds. *Mol. Clin. Environ. Tox. Experientia Supplementum* 101 (101), 47–86.
- Liu, J., Lee, L.S., Nies, L.F., Nakatsu, C.H., Turco, R.F., 2007. Biotransformation of 8:2 fluorotelomer alcohol in soil and by soil bacterial isolates. *Environ. Sci. Technol.* 41, 8024–8030.

- Liu, J.X., Wang, N., Szostek, B., Buck, R.C., Panciroli, P.K., Folsom, P.W., Sulecki, L.M., Bellin, C.A., 2010a. 6–2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. *Chemosphere* 78, 437–444.
- Liu, J.X., Wang, N., Buck, R.C., Wolstenholme, B.W., Folsom, P.W., Sulecki, L.M., Bellin, C.A., 2010b. Aerobic biodegradation of [^{14}C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system. *Chemosphere* 80, 716–723.
- Martin, J.W., Mabury, S.A., O'Brien, P.J., 2005. Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes. *Chem.-Biol. Int.* 155, 165–180.
- Nabb, D.L., Szostek, B., Himmelstein, M.W., Mawn, M.P., Gargas, M.L., Sweeney, L.M., Stadler, J.C., Buck, R.C., Fasano, W.J., 2007. In vitro metabolism of 8–2 fluorotelomer alcohol: Interspecies comparisons and metabolic pathway refinement. *Toxicol. Sci.* 100, 333–344.
- Nilsson, H., Kärman, A., Rotander, A., van Bavel, B., Lindström, G., Westberg, H., 2013. Biotransformation of fluorotelomer compound to perfluorocarboxylates in humans. *Environ. Int.* 51, 8–12.
- Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* 40, 32–44.
- Ritter, S.K., 2010. Fluorochemicals go short. *Chem. Eng. News* 88, 12–17.
- Ruan, T., Wang, Y.W., Wang, T., Zhang, Q.H., Ding, L., Liu, J.Y., Wang, C., Qu, G.B., Jiang, G.B., 2010a. Presence and partitioning behavior of polyfluorinated iodine alkanes in environmental matrices around a fluorochemical manufacturing plant: Another possible source for perfluorinated carboxylic acids? *Environ. Sci. Technol.* 44, 5755–5761.
- Ruan, T., Wang, Y.W., Zhang, Q.H., Ding, L., Wang, P., Qu, G.B., Wang, C., Wang, T., Jiang, G.B., 2010b. Trace determination of airborne polyfluorinated iodine alkanes using multisorbent thermal desorption/gas chromatography/high resolution mass spectrometry. *J. Chromatogr. A* 1217, 4439–4447.
- Ruan, T., Szostek, B., Folsom, P.W., Wolstenholme, B.W., Liu, R., Liu, J., Jiang, G., Wang, N., Buck, R.C., 2013. Aerobic soil biotransformation of 6:2 fluorotelomer iodide. *Environ. Sci. Technol.* 47, 11504–11511.
- Tomy, G.T., Tittlemier, S.A., Palace, V.P., Budakowski, W.R., Braekvelt, E., Brinkworth, L., Friesen, K., 2004. Biotransformation of N-ethyl perfluorooctanesulfonamide by rainbow trout (*Onchorhynchus mykiss*) liver microsomes. *Environ. Sci. Technol.* 38, 758–762.
- U.S. EPA, 2011. Exposure Assessment Tools and Models, Estimation Program Interface (EPI) Suite, V 4.1; U.S. Environmental Protection Agency, Exposure Assessment Branch, Washington, D.C.
- U.S. EPA, 2013. 2010/2015 PFOA Stewardship Program. <<http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship.htm>> (accessed 03.08.13).
- Wang, C., Wang, T., Liu, W., Ruan, T., Zhou, Q.H., Liu, J.Y., Zhang, A.Q., Zhao, B., Jiang, G.B., 2012a. The in vitro estrogenic activities of polyfluorinated iodine alkanes. *Environ. Health Perspect.* 120, 119–125.
- Wang, N., Szostek, B., Folsom, P.W., Sulecki, L.M., Capka, V., Buck, R.C., Berti, W.R., Gannon, J.T., 2005. Aerobic biotransformation of ^{14}C -labeled 8–2 Telomer B alcohol by activated sludge from a domestic sewage treatment plant. *Environ. Sci. Technol.* 39, 531–538.
- Wang, N., Szostek, B., Buck, R.C., Folsom, P.W., Sulecki, L.M., Gannon, J.T., 2009. 8–2 Fluorotelomer alcohol aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. *Chemosphere* 75, 1089–1096.
- Wang, N., Liu, J., Buck, R.C., Korzeniowski, S.H., Wolstenholme, B.W., Folsom, P.W., Sulecki, L.M., 2011. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* 82, 853–858.
- Wang, N., Buck, R.C., Szostek, B., Sulecki, L.M., Wolstenholme, B.W., 2012b. 5:3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel “one-carbon removal pathways”. *Chemosphere* 87, 527–534.
- Zhao, L., Folsom, P.W., Wolstenholme, B.W., Sun, H., Wang, N., Buck, R.C., 2013a. 6:2 Fluorotelomer alcohol biotransformation in an aerobic river sediment system. *Chemosphere* 90, 203–209.
- Zhao, L., McCausland, P.K., Folsom, P.W., Wolstenholme, B.W., Sun, H., Wang, N., Buck, R.C., 2013b. 6:2 Fluorotelomer alcohol aerobic biotransformation in activated sludge from two domestic wastewater treatment plants. *Chemosphere* 92, 464–470.
- Zhang, S., Szostek, B., McCausland, P.K., Wolstenholme, B.W., Lu, X., Wang, N., Buck, R.C., 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.