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Short review

# Is there a human health risk associated with indirect exposure to perfluoroalkyl carboxylates (PFCAs)?

Amy A. Rand[a,*], Scott A. Mabury[b]

[a] Departments of Entomology and Nematology and UC Davis Comprehensive Cancer Center, University of California Davis, Davis, CA, United States
[b] Department of Chemistry, University of Toronto, Toronto, Ontario, Canada

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ABSTRACT

The production and widespread use of poly- and perfluoroalkyl substances (PFAS) has led to their presence in the environment, wildlife, and humans. Particularly, the perfluoroalkyl carboxylates (PFCAs) are pervasive throughout the world and have been found at ng/mL concentrations in human blood. PFCAs, especially those having longer carbon chain lengths (≥C6), are associated with developmental and hormonal effects, immunotoxicity, and promote tumor growth in rodents through their role as PPARα agonists. Humans are directly exposed to PFCAs primarily through contaminated food, drinking water, and house dust. However, indirect exposure to PFCAs through the biotransformation of fluorotelomer-based substances may also be a significant, yet relatively underappreciated pathway. We are exposed to fluorotelomer-based substances through use of consumer products, ingestion of food, or from inhalation of dust particles, but the risk of this exposure has been largely uncharacterized. Here, we summarize the work that has been done to characterize toxicity of the classes of fluorotelomer-based substances shown to biotransform to PFCAs: the polyfluoroalkyl phosphate esters (PAPs), fluorotelomer alcohols (FTOHs), fluorotelomer iodides (FTIs), and fluorotelomer acrylate monomers (FTAcs). These fluorotelomer-based substances biotranform to yield PFCAs, yet also form bioactive intermediate metabolites, which have been observed to be more toxic than their corresponding PFCAs. We address what is known regarding the toxicity of the fluorotelomer-based substances and their metabolites, with focus on covalent binding to biological nucleophiles, such as glutathione, proteins, and DNA, as a possible mechanism of toxicity that may influence the risk of indirect exposure to PFCAs.

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## 1. Introduction

Poly- and perfluoroalkyl substances (PFAS) are environmental pollutants that have received attention due to their environmental persistence, global occurrence, and presence in human blood. One class of PFAS, the perfluoroalkyl carboxylates (PFCAs), has received particular regulatory and scientific focus to determine and mitigate risk of exposure, especially with respect to perfluorooctanoate (PFOA). PFOA is persistent, having a serum half-life of 3.8 years in humans (Olsen et al., 2007), and has been associated in laboratory animal toxicology studies with tumor development through its role as a PPARα agonist (Lau, 2015; Lau et al., 2007), developmental (Fenton et al., 2007; Lau et al., 2006; White et al., 2007; Wolf et al., 2007) and hormonal effects, (Biegel et al., 1995; Liu et al., 1996; White et al., 2011) and immunotoxicity (DeWitt et al., 2009, 2008; Yang et al., 2002a,b, 2000). Epidemiological studies conducted primarily on occupationally exposed workers have focused on mortality and cancer incidence, although generally no consistent association between serum fluorochemical levels and adverse health effects have been observed (Alexander, 2001; Gilliland and Mandel, 1993; Lau et al., 2007). To alleviate the exposure risk to PFOA, industrial and government actions have been set in place to reduce PFCA residuals from products, eliminate processing methods using perfluorooctyl-based chemistries, and change these methods to hexyl-based chemistries (3M, 2000; HBM Commission, 2016; Parsons et al., 2008). After these actions to reduce PFOA emissions, PFOA concentrations in humans have generally decreased, although at a slower rate than predicted (Calafat et al., 2007; Haug et al., 2009; Olsen et al., 2008, 2007). The slow declination rate has been suggested by D'eon and Mabury to correspond not only to continuous exposure to PFCAs from direct sources, but also from exposure to PFCA precursor materials, which subsequently transform to yield PFOA and other PFCAs (D'eon and Mabury, 2011a). As outlined in Table 1, these precursors are the

* Corresponding author.
*E-mail address:* arand@ucdavis.edu (A.A. Rand).

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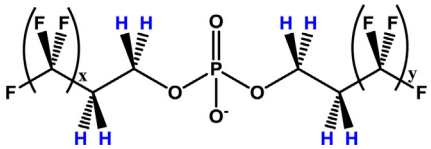
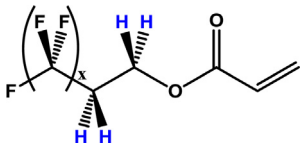
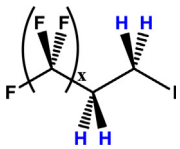
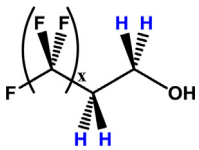
**Table 1**
Structures, acronyms, and nomenclature of the fluorotelomer-based substances examined in this work.
Acronyms correspond to the following substances: polyfluoroalkyl phosphate diester (diPAP), fluorotelomer acrylate monomer (FTAc), fluorotelomer iodide (FTI), and fluorotelomer alcohol (FTOH).

| Fluorotelomer-based substances: Perfluorocarboxylate (PFCA) precursors | | |
|---|---|---|
| Structure | | Acronym |
|  | x = 4,6,8 or 10<br>y = x or x + 2 | If y = x, x:2 diPAP<br>If y = x + 2, x:2/y:2 diPAP |
|  | x = 6,8 | x:2 FTAc |
|  | x = 6,8,10 | x:2 FTI |
|  | x = 6,8,10 | x:2 FTOH |

commercial fluorinated surfactants, including the polyfluoroalkyl phosphates (PAPs), and the following industrial fluorotelomer building blocks: fluorotelomer alcohols (FTOHs), fluorotelomer iodides (FTIs), and fluorotelomer acrylates (FTAcs). The surfactants, particularly the polyfluoroalkyl phosphate mono- and diesters (mono- and diPAPs), can biotransform to yield PFCAs (D'eon and Mabury, 2011a, 2007). Likewise, the FTAc, industrially used to produce fluorinated polymers, can transform in fish and soil to yield PFCAs (Butt et al., 2010a, 2010b; Rankin et al., 2014). The FTIs, used to produce either the FTAc or fluorinated surfactants, may also biotransform to PFCAs (Ruan et al., 2014). All of these materials transform to PFCAs through a common metabolic pathway, by first metabolizing to yield FTOHs (Butt et al., 2014). We can be exposed to FTOHs not only through the metabolism of FTOH precursors, but also from indoor air, dust, and use of commercial products (Dinglasan-Panlilio and Mabury, 2006; Dreyer et al., 2009; Haug et al., 2011; Kim et al., 2012; Shoeib et al., 2011).

Assessing risk of PFCAs is complicated because of their many exposure sources. We are not only exposed via direct sources, such as intake of PFCAs primarily through diet, drinking water, indoor air, and household dust, but also through indirect sources, where PFCAs form through transformation of fluorotelomer-based materials, including the PAPs, FTAcs, FTIs, and FTOHs (D'eon and Mabury, 2011b; Vestergren and Cousins, 2009). Direct exposure to PFCAs has been proposed to be the dominant pathway, however D'eon and Mabury reported that indirect exposure may be more significant than previously understood (D'eon and Mabury, 2011b). Compared to what is known about the direct PFCA exposure risk, there has not been as much focus on the risk of indirect exposure to PFCAs. DiPAPs have been reported in low ppb levels in blood samples in a few countries (D'eon et al., 2009; Lee and Mabury, 2011; Loi et al., 2013; Yeung et al., 2013), indicating an on-going source of exposure to these commercial surfactants and, given that their half-lives range between 2 and 4 days (D'eon and Mabury, 2011b), an on-going indirect exposure source to PFCAs. DiPAPs have also been globally observed in dust, food, and sewage sludge, suggesting multiple routes of exposure (De Silva et al., 2012; D'eon et al., 2009; Gebbink et al., 2015, 2013; Liu et al., 2013; Trier et al., 2011).

Upon exposure, fluorotelomer-based materials can be transformed to form PFCAs, the general mechanism of which is shown in Fig. 1. There are several intermediate metabolites that form through this process, including the fluorotelomer carboxylates (FTCAs), fluorotelomer unsaturated carboyxlates (FTUCAs), fluorotelomer aldehydes (FTALs) and fluorotelomer unsaturated aldehydes (FTUALs) (Butt et al., 2014; Fasano et al., 2006; Martin et al., 2005; Nabb et al., 2007 Fasano et al., 2006; Martin et al., 2005; Nabb et al., 2007). These intermediates are short-lived, yet they may have inherent toxicity upon formation. The FTCAs and FTUCAs have been observed to be up to 10,000 times more toxic than PFCAs to aquatic organisms (Phillips et al., 2007; Mitchell et al., 2011 Mitchell et al., 2011). FTUALs have been shown to covalently bind to proteins, which may lead to toxicity through altered function (Rand and Mabury, 2012a, 2013). However, the studies that illustrate intermediate metabolite toxicity are few, and it is therefore challenging to compare their toxicity to the PFCAs.

Compared to what is known about the direct exposure to PFCAs and their toxicity, it is surprising that relatively little is known about the consequences of indirect exposure to PFCAs, given that indirect exposure may be a significant contribution to the PFCA

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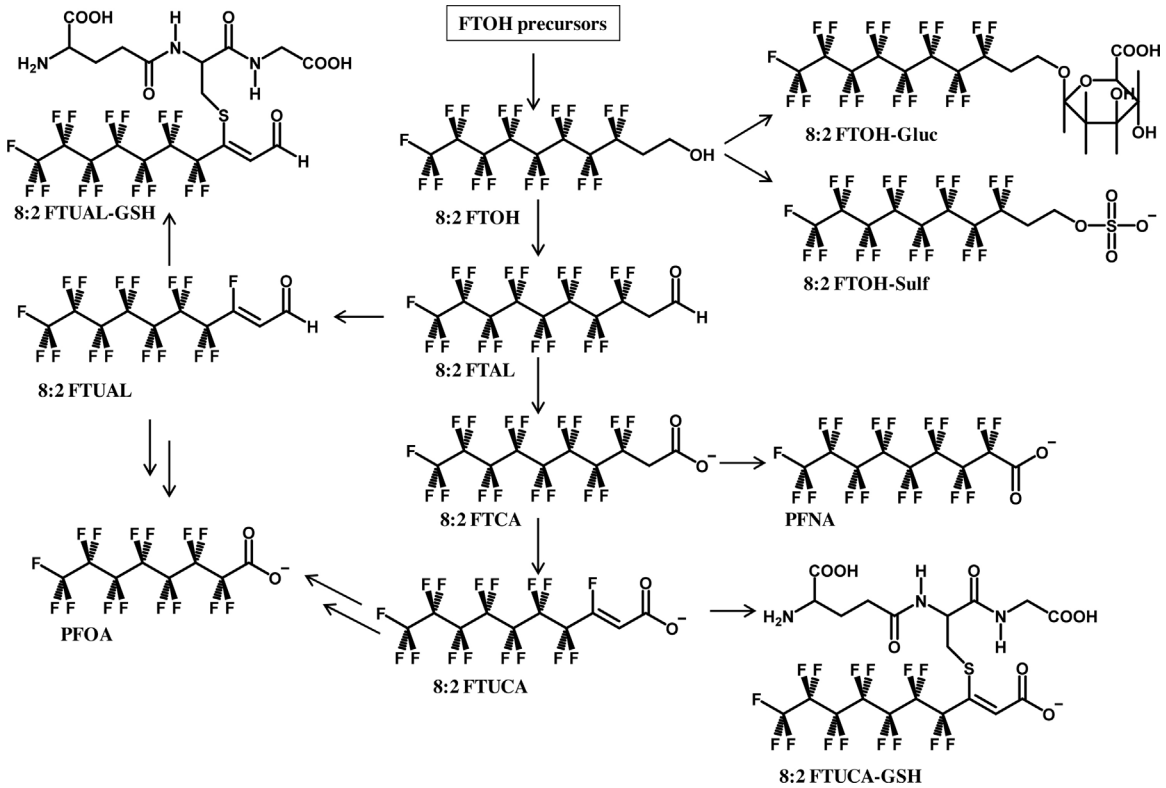


**Fig. 1.** General biotransformation pathway of the 8:2 FTOH, where "FTOH precursors" refers to fluorotelomer-based substances (8:2 PAPs, 8:2 FTI, and 8:2 FTAc) that transform to 8:2 FTOH. Adapted from Butt et al. (2014).

load observed within humans. Here, we highlight what is known about the toxicity corresponding to the commercial PAP surfactants, the industrial fluorotelomer building blocks, FTAcs, FTIs, and FTOHs, and their intermediate metabolites, to further understand the overall risk of indirect exposure to PFCAs. For reference, common names and acronyms of all compounds discussed can be found in Table 2.

## 2. Toxicity of commercial PAP surfactants

Despite our known exposure to diPAPs, there have been limited published toxicity studies for these compounds. D'eon and Mabury orally dosed a mixture of 4:2–10:2 diPAPs in rats each at 50 mg/kg, and did not observe any overt clinical signs of toxicity (D'eon and Mabury, 2011b), although specific mechanisms were not the focus of this investigation. One study demonstrated that PAPs (8:2 monoPAP, diPAP, triPAP, and 10:2 diPAP) affect steroid hormone synthesis *in vitro* by lowering androgen and increasing estrogen levels (Rosenmai et al., 2013). In this study, all four PAPs caused decreased levels of progesterone and 17α-OH-progesterone, and increased estrone levels, while the 8:2 mono- and diPAP inhibited androgen levels (Rosenmai et al., 2013). These observed effects were also similar to other fluorochemicals including PFOA and 8:2 FTOH, however whether the PAPs affected these targets *in vivo* at real exposure levels remains uncertain (Rosenmai et al., 2013; Taxvig et al., 2014).

The toxicity directly associated with exposure to these compounds may be influenced by their ability to hydrolyze through phosphatases to form FTOHs, thus having a short lifetime and reduced potential for the parent compound to elicit a toxic response of its own. The pharmacokinetic parameters for diPAPs are listed in Table 3. In rats, 8:2 diPAP has a bioavailability of 5% and a short half-life (2.4 ± 0.2 days) (D'eon and Mabury, 2011b). Likewise, the bioavailability and half-life for 6:2 diPAP is 74% and 3.9 ± 0.7 days, respectively (D'eon and Mabury, 2011b). Detection of these FTOH precursors in human blood may indicate that they are present in this form for long enough time to have some biological consequence, but may also proceed through a mechanism similar to FTOHs upon their *in vivo* transformation.

**Table 2**
Common Names and acronyms for the compounds discussed.

| Common name | Acronym |
|---|---|
| Poly-/Perfluoroalkyl substances | PFAS |
| Polyfluoroalkyl phosphate diester | x:2 diPAP |
| Polyfluoroalkyl phosphate monoester | x:2 monoPAP |
| Fluorotelomer iodide | x:2 FTI |
| Fluorotelomer acrylate | x:2 FTAc |
| Fluorotelomer alcohol | x:2 FTOH |
| Fluorotelomer aldehyde | x:2 FTAL |
| Fluorotelomer unsaturated aldehyde | x:2 FTUAL |
| Fluorotelomer carboxylates | x:2 FTCA |
| | x:3 FTCA |
| Fluorotelomer unsaturated carboxylates | x:2 FTUCA |
| | x:3 FTUCA |
| Perfluoroalkyl carboxylate | PFCA |
| Perfluorohepanoate | PFHpA |
| Perfluorooctanoate | PFOA |
| Perfluorosulfonate | PFSA |
| Fluorotelomer alcohol glucuronide | x:2 FTOH-Gluc |
| Fluorotelomer alcohol sulfate | x:2 FTOH-Sulf |
| Fluorotelomer unsaturated carboxylate glutathione conjugate | x:2 FTUCA-GSH |
| Fluorotelomer unsaturated aldehyde glutathione conjugate | x:2 FTUAL-GSH |
| N-acetyl-para-aminophenol | APAP |
| N-acetyl-*p*-benzoquinoneimine | NAPQI |
| Naphthalene | NA |
| Diethyl maleate | DEM |

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**Table 3**
Reported pharmacokinetic parameters for diPAPs and FTOH determined from analyte concentrations in rat plasma (Fasano et al., 2006) and whole blood (D'eon and Mabury). Dietary 8:2 FTAc uptake and elimination was examined in rainbow trout, though depurated and metabolized too quickly for pharmacokinetic measurements (Butt et al., 2010a,b).

| Acronym | Sex | Bioavailability (%) | Dose Form | Dose (mg/kg) | Biotransformation (%) | Half-life (h) | $T_{max}$ (h) | $C_{max}$ | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 4:2 diPAP | Male | 190 | Oral | 50 | 0.005 | 48.0 ± 2.4 | 6 | 39000 ± 4000 ng/g | D'eon and Mabury (2011a,b) |
| 4:2 diPAP | Male | 100 | IV | 15 | 0.5 | 38.4 ± 2.4 | 2 | 14000 + 1000 ng/g | D'eon and Mabury (2011a,b) |
| 6:2 diPAP | Male | 74 | Oral | 50 | 0.6 | 93.6 ± 16.8 | 4 | 1500 ± 500 ng/g | D'eon and Mabury (2011a,b) |
| 6:2 diPAP | Male | 100 | IV | 15 | 1 | 50.4 ± 7.2 | 2 | 2200 ± 200 ng/g | D'eon and Mabury (2011a,b) |
| 8:2 diPAP | Male | 5 | Oral | 50 | 9 | 57.6 ± 7.2 | 24 | 400 ± 60 ng/g | D'eon and Mabury (2011a,b) |
| 8:2 diPAP | Male | 100 | IV | 15 | 9 | 28.8 ± 24 | 24 | 490 ± 120 ng/g | D'eon and Mabury (2011a,b) |
| 10:2 diPAP | Male | 100 | IV | 15 | 8 | 79.2 ± 9.6 | 24 | 480 ± 110 ng/g | D'eon and Mabury (2011a,b) |
| 8:2 FTOH | Male | – | Oral | 5 | – | 0.9 ± 0.2 | 1.0 ± 0.0 | 63 ± 18 ng/mL | Fasano et al. (2006) |
| 8:2 FTOH | Female | – | Oral | 5 | – | 1.6 ± 0.7 | 0.9 ± 0.3 | 50 ± 35 ng/mL | Fasano et al. (2006) |
| 8:2 FTOH | Male | – | Oral | 125 | – | 4.9 ± 0.4 | 2.0 ± 0.0 | 376 ± 147 ng/mL | Fasano et al. (2006) |
| 8:2 FTOH | Female | – | Oral | 125 | – | 3.5 ± 2.0 | 3.8 ± 2.6 | 535 ± 119 ng/mL | Fasano et al. (2006) |
| 8:2 FTCA | Male | – | Oral | 5 | – | 15.2 ± 15.4 | 0.9 ± 0.3 | 170 ± 102 ng/mL | Fasano et al. (2006) |
| 8:2 FTCA | Female | – | Oral | 5 | – | 4.6 ± 3.6 | 1.0 ± 0.0 | 852 ± 198 ng/mL | Fasano et al. (2006) |
| 8:2 FTCA | Male | – | Oral | 125 | – | 17.8 ± 8.5 | 1.3 ± 0.5 | 2460 ± 565 ng/mL | Fasano et al. (2006) |
| 8:2 FTCA | Male | – | Oral | 125 | – | 3.9 ± 1.2 | 2.5 ± 2.4 | 1600 ± 2400 ng/mL | Fasano et al. (2006) |

## 3. Toxicity of industrial fluorotelomer building blocks: FTOHs, FTIs, and FTAcs

Exposure to FTOHs and their corresponding toxicity has received more attention compared to PAPs, FTIs, and FTAcs. Toxicity associated with FTOH was first reported by two studies that observed subchronic developmental and reproductive toxicities for 6:2, 8:2, and 10:2 FTOH (Ladics et al., 2005; Mylchreest et al., 2005a,b). However, subsequent studies in rats and mice found that 6:2 FTOH was not a selective reproductive or developmental toxicant, where adverse effects only occurred at dosages that induced maternal and/or paternal toxicity (125 mg/kg/day and 25 mg/kg/day in rats and mice, respectively) (Mukerji et al., 2015; O'Connor et al., 2014). Fasano et al. evaluated the pharmacokinetics, metabolism and corresponding toxicity of 8:2 FTOH in the rat following subchronic exposure (Table 3). Excess concentration of fluoride in plasma was detected following exposure, causing degeneration and disorganization of enamel organ ameloblast cells, leading to the formation of tooth lesions (Fasano et al., 2006). These investigators also observed renal tubule lesions, although mechanisms for this remain speculative (Fasano et al., 2006). Ladics et al. found that subchronic toxicity of an FTOH mixture led to tooth alterations, hepatocellular hypertrophy, elevated liver weights, and thyroid follicular hypertrophy in rats (Ladics et al., 2005). For 6:2 FTOH, the liver was also the primary target in rats and mice, where oral exposure (250 and 100 mg/kg/day in males and females, respectively) led to increased liver weights, histological changes and elevations in clinical chemistry parameters consistent with hepatotoxicity. During a 90-day subchronic oral exposure study in rats, the no-observed-adverse-effect level for 6:2 FTOH was 5 mg/kg/day, based on hematology and liver effects at higher doses (Serex et al., 2014). Liver weights also increased after rats were exposed to 100 ppm 6:2 FTOH over 6 weeks via inhalation. The incisor teeth of both male and female rats in this study had basophilic striation within the inner dentin, likely due to release of fluoride upon metabolism, although was not considered adverse because it lacked changes to the epithelium. Kudo et al. observed increased liver weights and peroxisome proliferation in mice administered 8:2 FTOH in their diet (Kudo et al., 2005). Another study characterized FTOHs as xenoestrogens *in vitro* (Maras et al., 2006).

The mechanism of FTOH on hormonal activity has been further explored by a few studies. A study by Liu et al. indicated that exposure to 6:2 or 8:2 FTOH significantly altered hormone plasma concentrations, including decreased testosterone and estradiol in zebrafish (Liu et al., 2009). They later proposed the cellular mechanisms underlying this steroidogenic disruption *in vitro*, by characterizing expression and activities of steroid enzymes and the effects of 8:2 FTOH on production of seven steroid hormones within a H296R cancer cell model (Liu et al., 2010). Inhibition of steroid hormones was dependent on 8:2 FTOH, due to decreased expression and protein abundance of several CYP enzymes. These effects were observed at concentrations similar to the PFCA serum concentrations required to initiate a similar testosterone response ($5 - 260\ \mu g/mL$). No PFCAs were observed in this study, indicating that metabolism did not occur in this model, suggesting that 8:2 FTOH itself has potential to depress steroidogenesis (Liu et al., 2010). The authors hypothesized that decreased production of these hormones after 8:2 FTOH incubation can potentially alter a variety of physiological processes including metabolism, stress responses, immune responses, vasoconstriction, growth, and development (Mommsen et al., 1999; Osborn, 1991). However, given that FTOHs regularly undergo oxidative metabolism and conjugation, it is unknown how steroidogenesis would be affected upon FTOH metabolism, or whether FTOH metabolites may have similar mechanisms of action.

Compared to PAPs and FTOHs, the toxicity from exposure to FTIs and FTAc is more limited. Though not exactly a toxicity study, one group examined 6:2–10:2 FTI estrogenic activity using MF-7 cancer cells. None of the FTIs had estrogenic effects within this system (Wang et al., 2012). Similarly, one study has examined the toxicity of FTAcs (Anand et al., 2012). In this study, the acute and subchronic toxicity of the 6:2 fluorotelomer methacrylate monomer was assessed, where low acute oral and dermal toxicity was reported (Anand et al., 2012). The no-observed-adverse-effect level in this study was >1000 mg/kg/day in rats. Changes in the liver, thyroid, and kidney weights occurred at some doses, although there were no associated histopathological or clinical pathology changes. Free fluoride affected teeth (altered mineralization, retention of basophilic material) and femur (increased mineralization) in all treated groups (Anand et al., 2012). The systemic effects of 6:2 FTMAC was attributed to either the 6:2 FTOH or its metabolites, since rat and mouse hepatocyte metabolism studies indicated that 6:2 FTMAC was rapidly metabolized to 6:2 FTOH and its metabolites ($T_{1/2} \leq 3$ min), where 20% and 0% of parent remained after 15 min in mouse and rat hepatocytes, respectively. (Anand et al., 2012). The FTAcs and FTIs have been shown transform to yield FTOHs *in vivo* and *in vitro*, respectively, therefore

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they may have similar mechanisms of toxicity compared with FTOHs, based on their transformation rates (Butt et al., 2010a,b; Ruan et al., 2014).

### 3.1. FTOH metabolism and bioactivation

FTOHs of varying chain length (6:2, 8:2, 10:2) have been shown to metabolize to produce PFCAs by a β-oxidation pathway upon activation from CYPs, although 8:2 FTOH has received most attention given its transformation to PFOA (Butt et al., 2014; Dinglasan et al., 2004; Fasano et al., 2006; Hagen et al., 1981; Martin et al., 2005; Nabb et al., 2007 Dinglasan et al., 2004; Fasano et al., 2006; Hagen et al., 1981; Martin et al., 2005; Nabb et al., 2007) (Fig. 1). Human CYP 2C19 and rat CYP 2E1 have been identified to metabolize FTOHs (Li et al., 2016; Martin et al., 2009). The transformation rates to PFCAs are low, making up 0.24–1.4 mol % of the total molar balance in rats (Himmelstein et al., 2012; Martin et al., 2005; Nabb et al., 2007). Reasons for this may be that FTOHs are either eliminated, mostly through feces (Fasano et al., 2006), or they undergo phase II conjugation (Fasano et al., 2006; Li et al., 2016; Martin et al., 2005; Nabb et al., 2007; Rand and Mabury, 2014). Nabb et al. compared the transformation rates of human, mouse, trout, and rat hepatocytes after incubation with 8:2 FTOH (Nabb et al., 2007). Transformation depended on the species, where mice and rat hepatocytes transformed 8:2 FTOH 22- and 9.5-times more efficiently than humans, respectively. Trace levels of PFCAs were detected in human hepatocytes after incubation (0.02 mol%). Despite low levels detected in human hepatocytes, evidence of FTOH transformation has been observed in human plasma, where elevated levels of PFCAs were found within occupationally exposed ski workers, along with intermediate metabolites, FTCAs and FTUCAs, suggesting that biotransformation from FTOHs in humans does occur, although perhaps not as efficiently compared to rodents (Nilsson et al., 2013, 2010).

Although the mass balance corresponding to FTOH oxidative metabolism is low, FTOHs, diPAPs, FTAcs, and FTIs all metabolize through a similar mechanism rather than bioaccumulate. Therefore, it is likely that the toxicity observed is through metabolic activation to a chemically or biologically active metabolite rather than through accumulation of the parent compound. No current studies have explicitly determined whether bioactivation is dependent on diPAP, FTAc, and FTI toxicity. Martin et al. used rat hepatocytes to examine whether FTOH toxicity was correlated to oxidative metabolism and formation of bioactive FTUAL and FTUCA metabolites (Martin et al., 2009). Toxicity was greatly correlated to metabolism of FTOH, since use of a P450 2E1 enzyme inhibitor diminished cytotoxicity, protein carbonylation, and lipid peroxidation for all FTOHs examined (Martin et al., 2009). This was the first evidence that linked FTOH toxicity to associated metabolites.

## 4. FTOH intermediate metabolite toxicity

### 4.1. FTUCAs/FTCAs

Since FTOHs degrade to PFCAs in the environment, a few studies examined the toxicity of intermediate FTCAs and FTUCAs to aquatic organisms (Phillips et al., 2007; Mitchell et al., 2011; Phillips et al., 2010). These intermediate acids were up to 10,000-times more toxic than PFCAs to freshwater invertebrates (Phillips et al., 2007). Generally, FTCAs were more toxic than corresponding FTUCAs, where toxicity depended on the length of the carbon chain (Phillips et al., 2007). Results from this study corroborated with a later study by Phillips et al., which examined toxicity of FTCAs and FTUCAs to two species of freshwater algae and one amphipod (Phillips et al., 2010). Toxicity differences between FTCAs and FTUCAs observed from both studies were suggested to be due to the additive effects of exposure to FTCAs and their metabolites, notably hydrogen fluoride (HF). As shown in Fig. 1, 8:2 FTCA can also metabolize to produce 8:2 FTUCA and PFOA, 7:3 FTCA, 7:3 FTUCAs, and PFHpA (Butt et al., 2010a,b). Further evidence that the toxicity differences between FTCAs and FTUCAs were caused in part by FTCA metabolism was noted in one study of ours (Rand et al., 2013). Here, human liver epithelial cells (THLE-2) were used to monitor cell viability after separate incubation with PFCAs and their metabolic precursors. FTCAs had similar toxicity thresholds to FTUCAs; there was no evidence of metabolism in these cells, indicating that FTCA toxicity may be enhanced upon biotransformation. Toxicity differences may also be due to the species in question, as each species had different sensitivities to each analyte, where *D. magna* was most sensitive to FTCAs (Phillips et al., 2007).

FTCA and FTUCA toxicity was also dependent on chain length, where shorter chain lengths resulted in reduced toxicity (Phillips et al., 2007; Mitchell et al., 2011; Phillips et al., 2010; Rand et al., 2013). The mechanism for this may be due to their short lifetime *in vivo*, and changes associated with biological processing; Kudo et al. showed slower urinary elimination rates for PFCAs having chain lengths of 7–10 carbons, while Ohmori et al. found that PFCAs having shorter chain lengths cleared faster from blood plasma than longer chain lengths, reflecting differences in binding to plasma proteins (Kudo et al., 2001; Ohmori et al., 2003). PFCAs having chain lengths >8 carbons also disrupt fatty acid metabolism, alter properties of cell membranes, and cause oxidative cell damage (Glauert et al., 1992; Goecke-Flora et al., 1995; Panaretakis et al., 2001; Starkov and Wallace, 2002). Those having 7–11 carbon chain lengths alter gap junction intercellular communication, and induce peroxisome proliferation (Intrasuksri and Feller, 1991; Upham et al., 1998). This noted reduction in toxicity and residence half-life of shorter chains was a reason for the industrial shift to manufacture fluorinated materials having shorter chain lengths. FTCAs and FTUCAs may exert toxicity through similar chain-length dependent mechanisms.

### 4.2. FTALs/FTUALs

Although longer chain length may be a factor in FTCA, FTUCA, and PFCA induced toxicity, FTALs and FTUALs were most toxic at shorter chain lengths in THLE-2 cells (Rand et al., 2013). They also exhibited greatest toxicity compared to the FTCAs, FTUCAs, and PFCAs, having $EC_{50}$ toxicity thresholds ranging from 90 to 99% less than their corresponding acid metabolites having the same chain length. Martin et al. suggested that FTOH toxicity may be related to formation of electrophilic aldehyde or acid intermediates through GSH depletion or protein adduct formation. It is generally known that toxicity may occur through formation of a chemically reactive metabolite, which if not detoxified, can affect covalent modification of biological macromolecules.

## 5. Covalent binding

### 5.1. Formation of GSH, protein, and DNA conjugates

Martin et al. first observed formation of GSH adducts with FTUALs, which indicated these transient intermediates to be reactive electrophiles (Martin et al., 2005). GSH conjugation and depletion was also observed (Fasano et al., 2006; Ladics et al., 2005). GSH conjugates have also been observed in rodents and fish, but have not been assessed in humans. Regardless, this implies that a fate for both FTUALs and FTUCAs, having half-lives of less than 1 and 9 h in rat hepatocytes and rainbow trout, respectively (Butt et al., 2010a,b; Martin et al., 2005), is their ability to react with biological nucleophiles. Gannon et al. found that of all the phase II metabolites formed from 6:2 FTOH transformation, GSH

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conjugation was the most significant (Gannon et al., 2010). Reactivity to GSH is dependent on chain length, where the shorter chain length (6:2 and 4:2) can be 10-times more reactive, than 8:2 length (Rand and Mabury, 2012b). Conjugation to GSH is important because it generally acts as a detoxifying mechanism, but also serves as a marker for the reactive intermediate to bind to other nucleophilic sites, like those on proteins and DNA. Our studies pursued this hypothesis, indicating that FTUALs not only readily react with GSH (Rand and Mabury, 2012b), but also thiol- and nitrogen-containing nucleophilic amino acids, and bovine blood and rat liver proteins (Rand and Mabury, 2012a). Furthermore, this covalent protein binding contributed a significant part to the mass balance ($26.1 \pm 3.0\%$) when 8:2 FTOH was incubated with liver microsomes (Rand and Mabury, 2013). Covalent protein binding was observed after rats received an oral dose (100 mg/kg) of 6:2 diPAP and 8:2 FTOH in plasma, liver, and kidney (Rand and Mabury, 2014). While protein binding was observed *in vitro* and *in vivo*, no DNA adduct formation was observed after FTAL and FTUAL (6:2 and 8:2) incubation with THLE-2 cells (Rand et al., 2013), or by the *umu* genotoxicity assay (Oda et al., 2007). Similarly, 6:2 FTOH has not been shown to be mutagenic in the bacterial reverse mutation test (Serex et al., 2014).

Although phase II conjugation is generally considered detoxifying, there are instances where it may contribute to the bioactivation of the parent compound. For example, diclofenac, an NSAID, becomes bioactived by both oxidation and conjugation (Park et al., 2005). In rat hepatocytes, the binding of diclofenac to protein appears to derive primarily from reactions of the acyl glucuronide phase II conjugate (Kretzrommel and Boelsterli, 1993). FTUAL and FTUCA binding to GSH may lead to toxicity, since the α-β conjugated system still exists in the GSH conjugates, and therefore may react with other nucleophiles, although this has never been examined.

### 5.2. Depletion of GSH an essential mechanism for toxicity

Toxicity from bioactivation of a compound is often dependent on depletion of GSH (Pompella et al., 2003). This has been observed for drug overdose of N-acetyl-para-aminophenol (APAP), where a proportion of the drug undergoes bioactivation to N-acetyl-*p*-benzoquinoneimine (NAPQI) by CYP2E1, CYP1A2, and CYP3A4 (Raucy et al., 1989; Thummel et al., 1993). At a dose of 4 g for example, NAPQI can be quenched by hepatic GSH. However, when an overdose of >10 g GSH depletion occurs, which leads to destruction of critical proteins that interrupts several signaling mechanisms, resulting in hepatic necrosis (Davis et al., 1974). Once GSH is depleted, NAPQI is free to migrate to different regions of the cell, binding and inactivating specific proteins in most intracellular compartments of the hepatocyte.

It is unlikely that the exposure to FTOHs will lead to the same loss of GSH as observed for acute APAP toxicity, where indoor air levels for FTOHs range are generally low ($<10\ ng/m^3$) in average households and offices (Fraser et al., 2011; Haug et al., 2011), up to $300\ ng/m^3$ in stores selling outdoor clothing (Schlummer et al., 2013), and even for the ski wax technicians exposed to FTOH levels between 830 and 250,000 $ng/m^3$ (Nilsson et al., 2010). Likewise, exposure to FTOH precursors are comparably low, where diPAPs are present up to low pg/g levels in food samples (Gebbink et al., 2015, 2013), although levels have been observed to be higher in dust (7637 ng/g mean total diPAP concentrations) (De Silva et al., 2012). However, continuous exposure to these compounds will lead to conjugation with GSH, which may trigger chronic toxicity through GSH depletion. For example, inhalation of naphthalene (NA, 2–5 ppm) resulted in cytotoxicity to the respiratory epithelium in mice via CYP metabolic activation and loss of GSH after forming the following reactive intermediates: 1,2-napthalene epoxide, NA diol epoxide, 1,2-napthoquinone, and 1,4-napthoquinone (Pham et al., 2012; West et al., 2001).

Although GSH depletion is a necessary step for cytotoxicity, it is not the only one. For example, diethyl maleate (DEM) also binds to GSH similarly to NA, but then binds to different proteins (Phimister et al., 2005; Spiess et al., 2010). One study identified NA as having 35 unique proteins compared to DEM, which led to increased membrane permeability, mitochondrial disruption, and cell death (Spiess et al., 2010). In contrast, DEM exposure did not lead to toxicity because the cell vulnerability to reactive oxygen species upon GSH depletion was corrected by intact proteins involved in antioxidant protection and protein folding (Spiess et al., 2010). With NA, several of these proteins were adducted, altering their ability to repair proteins undergoing thiol oxidation and unfolding during early stages of NA toxicity. After FTOH and diPAP exposure in rats, we observed that despite the GSH binding step, there was significant overall binding to proteins in the kidney, liver, and plasma after a one-time oral dose (100 mg/kg) (Rand and Mabury, 2014). Additionally, Fasano et al. suggested that GSH depletion was not the only driver of FTOH-induced toxicity, and the involvement of intermediate metabolites may be associated with other mechanisms of toxicity, independent from GSH depletion (Fasano et al., 2009). This indicates that protein modification takes place in rats despite GSH conjugation, at least at high concentrations. It will be necessary to assess the relative extent of protein binding compared to GSH conjugation at more relevant exposure concentrations. In addition, the specific proteins that are bound after FTOH bioactivation are unknown, therefore we cannot yet establish whether any toxicity observed for FTOH is related to protein binding. It will be necessary to determine the specific protein targets, since some are understood as being critical to the toxicity process whereas others are not. To understand whether FTOH bioactivation equates to toxicity, it will be necessary to understand the extent of GSH depletion, the specific proteins modified and how this alters their function. The toxicity will also depend on the extent of protein damage versus protein replacement and cellular repair. By understanding these mechanisms, we can predict the responses in sensitive populations and devise ways to attenuate health consequences after exposure to fluorotelomer-based substances. In addition, proteins identified can be used as potential biomarkers for studies in exposed populations, to achieve a specific marker of indirect exposure to PFCAs.

## 6. Conclusion

The route of exposure to PFCAs and their relative contribution to our total exposure influences their relative risk. Given the extent of direct exposure to PFCAs, it is important to establish their modes of action. But we also need to understand the consequence of indirect exposure, by characterizing toxicity of PFCA precursors. For PFOA, a tolerable daily intake of 1500 ng/kg body weight has been established by a UK committee on toxicity of chemicals in food, consumer products, and the environment (COT, 2009). The US EPA released a health advisory of 70 ng/L for PFOA in drinking water, accounting for adverse effects due to chronic exposure (US EPA, 2016). The German Human Biomonitoring Commission recently set a 1 μL/L limit for PFOA in human blood plasma (HBM Commission, 2016). Currently, there is relatively less toxicity data for PFCA precursors, making it difficult to assess risk of indirect exposure and define the total daily intake for these fluorotelomer-based substances. The risk corresponding to inhalation exposure for 6:2 FTOH has been extrapolated, based on ambient air concentrations and the hepatotoxicity observed from *in vivo* studies (Himmelstein et al., 2012; Serex et al., 2014). The human equivalent dose for chronic exposure was 0.7 mg/kg/bw/day, where the margin of exposure for indoor and outdoor air

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concentrations ranged from $1.1 \times 10^5 - 2.5 \times 10^7$ ng/m$^3$.This indicated that there is no human health risk after exposure to 6:2 FTOH at the highest reported air concentrations (Buck, 2015). But the presence of other components leading to exposure to reactive metabolites and accumulation of PFCAs may contribute to the overall PFAS burden observed within humans, not calculated within this risk assessment. The combined exposure from all fluorotelomer-based substances transforming to FTOHs *in vivo* may lead to increased risk due to increased exposure, although this remains to be determined. If levels of fluorinated substances in our blood are an indication of exposure, we may be exposed to many more fluorinated substances than the two primarily studied fluorinated classes: the PFCAs and perfluorosulfonates (PFSAs). Recent studies indicate that known PFCAs and PFSAs account for only 5–9% of the organofluorine in human and wild rat blood samples, suggesting that a relatively large proportion of the organofluorine remains unknown (Loi et al., 2013). It is argued that FTOHs may count as the most significant source of indirect PFCA exposure, calculated to have negligible contribution (<1%) to the total PFOA exposure to adults (Fromme et al., 2009; Vestergren and Cousins, 2009). Yet diPAPs, FTAcs, FTOHs, and FTIs may all contribute to the indirect PFCA load measured in humans. If we are to gain a truer measure of the impact PFAS has on our health, we must characterize these fluorinated species in terms of their mechanisms of toxicity and whether these mechanisms have effect at low yet constant levels of exposure.

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## References

3M, 2000. Phase-Out Plan for POSF-Based Products. .
Alexander, B.H., 2001. Mortality study of workers employed at the 3M cottage grove facility. Final Rep. Div. Environ. Occup. Health Sch. Public Health Univ. Minn. 143–146.
Anand, S.S., Serex, T.L., Carpenter, C., Donner, E.M., Hoke, R., Buck, R.C., Loveless, S.E., 2012. Toxicological assessment of tridecafluorohexylethyl methacrylate (6:2 FTMAC). Toxicology 292, 42–52.
Biegel, L.B., Liu, R.C., Hurtt, M.E., Cook, J.C., 1995. Effects of ammonium perfluorooctanoate on leydig-cell function: in vitro, in vivo, and ex vivo studies. Toxicol. Appl. Pharmacol. 134, 18–25.
Buck, R.C., 2015. Toxicology data for alternative short-chain fluorinated substances. Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, pp. 451–477.
Butt, C.M., Muir, D.C., Mabury, S.A., 2010a. Biotransformation of the 8: 2 fluorotelomer acrylate in rainbow trout. 1. In vivo dietary exposure. Environ. Toxicol. Chem. 29, 2726–2735.
Butt, C.M., Muir, D.C., Mabury, S.A., 2010b. Elucidating the pathways of poly-and perfluorinated acid formation in rainbow trout. Environ. Sci. Technol. 44, 4973–4980.
Butt, C.M., Muir, D.C., Mabury, S.A., 2014. Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: a review. Environ. Toxicol. Chem. 33, 243–267.
COT, 2009. COT update statement on the tolerable daily intake for perfluorooctanoic acid. COT Statement 2009/02, .
Calafat, A.M., Wong, L.-Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the US population: data from the national health and nutrition examination survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. Environ. Health Perspect. 1596–1602.
D'eon, J.C., Mabury, S.A., 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.
D'eon, J.C., Mabury, S.A., 2011a. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? Environ. Sci. Technol. 45, 7974–7984.
D'eon, J.C., Mabury, S.A., 2011b. 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.
D'eon, J.C., Crozier, P.W., Furdui, V.I., Reiner, E.J., Libelo, E.L., Mabury, S.A., 2009. Observation of a commercial fluorinated material, the polyfluoroalkyl phosphoric acid diesters, in human sera, wastewater treatment plant sludge, and paper fibers. Environ. Sci. Technol. 43, 4589–4594.
Davis, D.C., Potter, W.Z., Jollow, D.J., Mitchell, J.R., 1974. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. Life Sci. 14, 2099–2109.
De Silva, A.O., Allard, C.N., Spencer, C., Webster, G.M., Shoeib, M., 2012. Phosphorus-containing fluorinated organics: polyfluoroalkyl phosphoric acid diesters (diPAPs), perfluorophosphonates (PFPAs), and perfluorophosphinates (PFPIAs) in residential indoor dust. Environ. Sci. Technol. 46, 12575–12582.
DeWitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ. Health Perspect. 116, 644.
DeWitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit. Rev. Toxicol. 39, 76–94.
Dinglasan, M.J.A., Ye, Y., Edwards, E.A., Mabury, S.A., 2004. Fluorotelomer alcohol biodegradation yields poly-and perfluorinated acids. Environ. Sci. Technol. 38, 2857–2864.
Dinglasan-Panlilio, M.J.A., Mabury, S.A., 2006. Significant residual fluorinated alcohols present in various fluorinated materials. Environ. Sci. Technol. 40, 1447–1453.
Dreyer, A., Weinberg, I., Temme, C., Ebinghaus, R., 2009. Polyfluorinated compounds in the atmosphere of the Atlantic and Southern oceans: evidence for a global distribution. Environ. Sci. Technol. 43, 6507–6514.
Fasano, W.J., Carpenter, S.C., Gannon, S.A., Snow, T.A., Stadler, J.C., Kennedy, G.L., Buck, R.C., Korzeniowski, 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.
Fasano, W.J., Sweeney, L.M., Mawn, M.P., Nabb, D.L., Szostek, B., Buck, R.C., Gargas, M. L., 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.
Fenton, S.E., Lau, C., Hines, E.P., Thibodeaux, J.R., White, S.S., 2007. Long-term health effects of PFOA after prenatal and lactational exposure in mice. Toxicologist 96, 12.
Fraser, A.J., Webster, T.F., Watkins, D.J., Nelson, J.W., Stapleton, H.M., Calafat, A.M., Kato, K., Shoeib, M., Vieira, V.M., McClean, M.D., 2011. Polyfluorinated compounds in serum linked to indoor air in office environments. Environ. Sci. Technol. 46, 1209–1215.
Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds–exposure assessment for the general population in Western countries. Int. J. Hyg. Environ. Health 212, 239–270.
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. Toxicologist 114, 97 Abstract #450.
Gebbink, W.A., Ullah, S., Sandblom, O., Berger, U., 2013. Polyfluoroalkyl phosphate esters and perfluoroalkyl carboxylic acids in target food samples and packaging—method development and screening. Environ. Sci. Pollut. Res. 20, 7949–7958.
Gebbink, W.A., Glynn, A., Darnerud, P.O., Berger, U., 2015. Perfluoroalkyl acids and their precursors in Swedish food: the relative importance of direct and indirect dietary exposure. Environ. Pollut. 198, 108–115.
Gilliland, F.D., Mandel, J.S., 1993. Mortality among employees of a perfluorooctanoic acid production plant. J. Occup. Environ. Med. 35, 950–954.
Glauert, H.P., Srinivasan, S., Tatum, V.L., Chen, L.-C., Saxon, D.M., Lay, L.T., Borges, T., Baker, M., Chen, L.H., Robertson, L.W., 1992. Effects of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cellular antioxidants and lipid peroxidation in rats. Biochem. Pharmacol. 43, 1353–1359.
Goecke-Flora, C.M., Wyman, J.F., Jarnot, B.M., Reo, N.V., 1995. Effect of the peroxisome proliferator perfluoro-n-decanoic acid on glucose transport in the isolated perfused rat liver. Chem. Res. Toxicol. 8, 77–81.
H.B.M. Commission, 2016. HBM I values for perfluorooctanoic acid (PFOA) and perfluorooxtanesulfonic acid (PFOS) in blood plasma. Statement of the German Human Biomonitoring Commission (HBM Commission), .
Hagen, D.F., Belisle, J., Johnson, J.D., 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.
Haug, L.S., Thomsen, C., Becher, G., 2009. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ. Sci. Technol. 43, 2131–2136.
Haug, L.S., Huber, S., Schlabach, M., Becher, G., Thomsen, C., 2011. Investigation on per-and polyfluorinated compounds in paired samples of house dust and indoor air from Norwegian homes. Environ. Sci. Technol. 45, 7991–7998.
Himmelstein, M.W., Serex, T.L., Buck, R.C., Weinberg, J.T., Mawn, M.P., Russell, M.H., 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.
Intrasuksri, U., Feller, D.R., 1991. Comparison of the effects of selected monocarboxylic, dicarboxylic and perfluorinated fatty acids on peroxisome proliferation in primary cultured rat hepatocytes. Biochem. Pharmacol. 42, 184–188.
Kim, S.-K., Shoeib, M., Kim, K.-S., Park, J.-E., 2012. Indoor and outdoor poly-and perfluoroalkyl substances (PFASs) in Korea determined by passive air sampler. Environ. Pollut. 162, 144–150.
Kretzrommel, A., Boelsterli, U.A., 1993. Diclofenac covalent protein binding is dependent on acyl glucuronide formation and is inversely related to P450-

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mediated acute cell injury in cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 120, 155–161.
Kudo, N., Suzuki, E., Katakura, M., Ohmori, K., Noshiro, R., Kawashima, Y., 2001. Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem. Biol. Interact. 134, 203–216.
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.
Ladics, G.S., Stadler, J.C., Makovec, G.T., Everds, N.E., Buck, R.C., 2005. Subchronic toxicity of a fluoroalkylethanol mixture in rats. Drug Chem. Toxicol. 28, 135–158.
Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B., Strynar, M.J., 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol. Sci. 90, 510–518.
Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.
Lau, C., 2015. Perfluorinated compounds: an overview. Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, pp. 1–21.
Lee, H., Mabury, S.A., 2011. A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009. Environ. Sci. Technol. 45, 8067–8074.
Li, Z.-M., Guo, L.-H., Ren, X.-M., 2016. Biotransformation of 8:2 fluorotelomer alcohol by recombinant human cytochrome P450s, human liver microsomes and human liver cytosol. Environ. Sci. Process. Impacts 18, 538–546.
Liu, R.C., Hurtt, M.E., Cook, J.C., Biegel, L.B., 1996. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male CrI CD BR (CD) rats. Toxicol. Sci. 30, 220–228.
Liu, C., Yu, L., Deng, J., Lam, P.K., Wu, R.S., Zhou, B., 2009. Waterborne exposure to fluorotelomer alcohol 6:2 FTOH alters plasma sex hormone and gene transcription in the hypothalamic–pituitary–gonadal (HPG) axis of zebrafish. Aquat. Toxicol. 93, 131–137.
Liu, C., Zhang, X., Chang, H., Jones, P., Wiseman, S., Naile, J., Hecker, M., Giesy, J.P., Zhou, B., 2010. Effects of fluorotelomer alcohol 8:2 FTOH on steroidogenesis in H295R cells: targeting the cAMP signalling cascade. Toxicol. Appl. Pharmacol. 247, 222–228.
Liu, R., Ruan, T., Wang, T., Song, S., Yu, M., Gao, Y., Shao, J., Jiang, G., 2013. Trace analysis of mono-, di-, tri-substituted polyfluoroalkyl phosphates and perfluorinated phosphonic acids in sewage sludge by high performance liquid chromatography tandem mass spectrometry. Talanta 111, 170–177.
Loi, E.I., Yeung, L.W., Mabury, S.A., Lam, P.K., 2013. Detections of commercial fluorosurfactants in Hong Kong marine environment and human blood: a pilot study. Environ. Sci. Technol. 47, 4677–4685.
Maras, M., Vanparys, C., Muylle, F., Robbens, J., Berger, U., Barber, J.L., Blust, R., De Coen, W., 2006. Estrogen-like properties of fluorotelomer alcohols as revealed by MCF-7 breast cancer cell proliferation. Environ. Health Perspect. 100–105.
Martin, J.W., Mabury, S.A., O'Brien, P.J., 2005. Metabolic products and pathways of fluorotelomer alcohols in isolated rat hepatocytes. Chem. Biol. Interact. 155, 165–180.
Martin, J.W., Chan, K., Mabury, S.A., O'Brien, P.J., 2009. Bioactivation of fluorotelomer alcohols in isolated rat hepatocytes. Chem. Biol. Interact. 177, 196–203.
Mitchell, R.J., Myers, A.L., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2011. Toxicity of fluorotelomer carboxylic acids to the algae Pseudokirchneriella subcapitata and Chlorella vulgaris, and the amphipod Hyalella azteca. Ecotoxicol. Environ. Saf. 74, 2260–2267.
Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268.
Mukerji, P., Rae, J.C., Buck, R.C., O'Connor, J.C., 2015. Oral repeated-dose systemic and reproductive toxicity of 6:2 fluorotelomer alcohol in mice. Toxicol. Rep. 2, 130–143.
Mylchreest, E., Ladics, G.S., Munley, S.M., Buck, R.C., Stadler, J.C., 2005a. Evaluation of the reproductive and developmental toxicity of a fluoroalkylethanol mixture. Drug Chem. Toxicol. 28, 159–175.
Mylchreest, E., Munley, S.M., Kennedy Jr, G.L., 2005b. Evaluation of the developmental toxicity of 8–2 telomer B alcohol. Drug Chem. Toxicol. 28, 315–328.
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., Kärrman, A., Rotander, A., van Bavel, B., Lindström, G., Westberg, H., 2010. Inhalation exposure to fluorotelomer alcohols yield perfluorocarboxylates in human blood? Environ. Sci. Technol. 44, 7717–7722.
Nilsson, H., Kärrman, A., Rotander, A., van Bavel, B., Lindström, G., Westberg, H., 2013. Biotransformation of fluorotelomer compound to perfluorocarboxylates in humans. Environ. Int. 51, 8–12.
O'Connor, J.C., Munley, S.M., Serex, T.L., Buck, R.C., 2014. Evaluation of the reproductive and developmental toxicity of 6:2 fluorotelomer alcohol in rats. Toxicology 317, 6–16. doi:http://dx.doi.org/10.1016/j.tox.2014.01.002.
Oda, Y., Nakayama, S., Harada, K.H., Koizumi, A., 2007. Negative results ofumu genotoxicity test of fluorotelomer alcohols and perfluorinated alkyl acids. Environ. Health Prev. Med. 12, 217–219.
Ohmori, K., Kudo, N., Katayama, K., Kawashima, Y., 2003. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184, 135–140.
Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 1298–1305.
Olsen, G.W., Mair, D.C., Church, T.R., Ellefson, M.E., Reagen, W.K., Boyd, T.M., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., Rios, J.A., 2008. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American red cross adult blood donors, 2000–2006. Environ. Sci. Technol. 42, 4989–4995.
Osborn, J.L., 1991. Relation between sodium intake, renal function, and the regulation of arterial pressure. Hypertension 17, I91.
Panaretakis, T., Shabalina, I.G., Grandér, D., Shoshan, M.C., DePierre, J.W., 2001. Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma HepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid. Toxicol. Appl. Pharmacol. 173, 56–64.
Park, B.K., Kitteringham, N.R., Maggs, J.L., Pirmohamed, M., Williams, D.P., 2005. The role of metabolic activation in drug-induced hepatotoxicity. Annu. Rev. Pharmacol. Toxicol. 45, 177–202.
Parsons, J.R., Sáez, M., Dolfing, J., de Voogt, P., 2008. Biodegradation of perfluorinated compounds. Reviews of Environmental Contamination and Toxicology, vol. 196. Springer, pp. 53–71.
Pham, N.T., Jewell, W.T., Morin, D., Buckpitt, A.R., 2012. Analysis of naphthalene adduct binding sites in model proteins by tandem mass spectrometry. Chem. Biol. Interact. 199, 120–128.
Phillips, M.M., Dinglasan-Panlilio, M.J.A., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2007. Fluorotelomer acids are more toxic than perfluorinated acids. Environ. Sci. Technol. 41, 7159–7163.
Phillips, M.M., Dinglasan-Panlilio, M.J., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2010. Chronic toxicity of fluorotelomer acids to Daphnia magna and Chironomus dilutus. Environ. Toxicol. Chem. 29, 1123–1131.
Phimister, A.J., Williams, K.J., Van Winkle, L.S., Plopper, C.G., 2005. Consequences of abrupt glutathione depletion in murine clara cells: ultrastructural and biochemical investigations into the role of glutathione loss in naphthalene cytotoxicity. J. Pharmacol. Exp. Ther. 314, 506–513.
Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., Casini, A.F., 2003. The changing faces of glutathione, a cellular protagonist. Biochem. Pharmacol. 66, 1499–1503.
Rand, A.A., Mabury, S.A., 2012a. In vitro interactions of biological nucleophiles with fluorotelomer unsaturated acids and aldehydes: fate and consequences. Environ. Sci. Technol. 46, 7398–7406.
Rand, A.A., Mabury, S.A., 2012b. Assessing the structure–activity relationships of fluorotelomer unsaturated acids and aldehydes with glutathione. Cell Biol. Toxicol. 28, 115–124.
Rand, A.A., Mabury, S.A., 2013. Covalent binding of fluorotelomer unsaturated aldehydes (FTUALs) and carboxylic acids (FTUCAs) to proteins. Environ. Sci. Technol. 47, 1655–1663.
Rand, A.A., Mabury, S.A., 2014. Protein binding associated with exposure to fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate esters (PAPs) in rats. Environ. Sci. Technol. 48, 2421–2429.
Rand, A.A., Rooney, J.P., Butt, C.M., Meyer, J.N., Mabury, S.A., 2013. Cellular toxicity associated with exposure to perfluorinated carboxylates (PFCAs) and their metabolic precursors. Chem. Res. Toxicol. 27, 42–50.
Rankin, K., Lee, H., Tseng, P.J., Mabury, S.A., 2014. Investigating the biodegradability of a fluorotelomer-based acrylate polymer in a soil–plant microcosm by indirect and direct analysis. Environ. Sci. Technol. 48, 12783–12790.
Raucy, J.L., Lasker, J.M., Lieber, C.S., Black, M., 1989. Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. Arch. Biochem. Biophys. 271, 270–283.
Rosenmai, A.K., Nielsen, F.K., Pedersen, M., Hadrup, N., Trier, X., Christensen, J.H., Vinggaard, A.M., 2013. Fluorochemicals used in food packaging inhibit male sex hormone synthesis. Toxicol. Appl. Pharmacol. 266, 132–142.
Ruan, T., Sulecki, L.M., Wolstenholme, B.W., Jiang, G., Wang, N., Buck, R.C., 2014. 6:2 fluorotelomer iodide in vitro metabolism by rat liver microsomes: comparison with [1,2–14C] 6:2 fluorotelomer alcohol. Chemosphere 112, 34–41.
Schlummer, M., Gruber, L., Fiedler, D., Kizlauskas, M., Müller, J., 2013. Detection of fluorotelomer alcohols in indoor environments and their relevance for human exposure. Environ. Int. 57, 42–49.
Serex, T., Anand, S., Munley, S., Donner, E.M., Frame, S.R., Buck, R.C., Loveless, S.E., 2014. Toxicological evaluation of 6:2 fluorotelomer alcohol. Toxicology 319, 1–9.
Shoeib, M., Harner, T., M. Webster, G., Lee, S.C., 2011. Indoor sources of poly-and perfluorinated compounds (PFCS) in Vancouver, Canada : implications for human exposure. Environ. Sci. Technol. 45, 7999–8005.
Spiess, P.C., Morin, D., Williams, C.R., Buckpitt, A.R., 2010. Protein thiol oxidation in murine airway epithelial cells in response to naphthalene or diethyl maleate. Am. J. Respir. Cell Mol. Biol. 43, 316–325.
Starkov, A.A., Wallace, K.B., 2002. Structural determinants of fluorochemical-induced mitochondrial dysfunction. Toxicol. Sci. 66, 244–252.
Taxvig, C., Rosenmai, A.K., Vinggaard, A.M., 2014. Polyfluorinated alkyl phosphate ester surfactants–current knowledge and knowledge gaps. Basic Clin. Pharmacol. Toxicol. 115, 41–44.
Thummel, K.E., Lee, C.A., Kunze, K.L., Nelson, S.D., Slattery, J.T., 1993. Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem. Pharmacol. 45, 1563–1569.
Trier, X., Granby, K., Christensen, J.H., 2011. Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. Environ. Sci. Pollut. Res. 18, 1108–1120.
US EPA, 2016. Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). .

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Upham, B.L., Deocampo, N.D., Wurl, B., Trosko, J.E., 1998. Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int. J. Cancer 78, 491–495.
Vestergren, R., Cousins, I.T., 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environ. Sci. Technol. 43, 5565–5575.
Wang, C., Wang, T., Liu, W., Ruan, T., Zhou, Q., Liu, J., Zhang, A., Zhao, B., Jiang, G., 2012. The in vitro estrogenic activities of polyfluorinated iodine alkanes. Environ. Health Perspect. 120, 119.
West, J.A., Pakehham, G., Morin, D., Fleschner, C.A., Buckpitt, A.R., Plopper, C.G., 2001. Inhaled naphthalene causes dose dependent clara cell cytotoxicity in mice but not in rats. Toxicol. Appl. Pharmacol. 173, 114–119.
White, S.S., Calafat, A.M., Kuklenyik, Z., Villanueva, L., Zehr, R.D., Helfant, L., Strynar, M.J., Lindstrom, A.B., Thibodeaux, J.R., Wood, C., 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicol. Sci. 96, 133–144.
White, S.S., Fenton, S.E., Hines, E.P., 2011. Endocrine disrupting properties of perfluorooctanoic acid. J. Steroid Biochem. Mol. Biol. 127, 16–26.
Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklenyik, Z., Bryant, X.A., Thibodeaux, J., Das, K.P., White, S.S., Lau, C.S., 2007. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicol. Sci. 95, 462–473.
Yang, Q., Xie, Y., Depierre, J.W., 2000. Effects of peroxisome proliferators on the thymus and spleen of mice. Clin. Exp. Immunol. 122, 219–226.
Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X.-Y., Möller, G., Nelson, B.D., DePierre, J. W., 2002a. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. Int. Immunopharmacol. 2, 389–397.
Yang, Q., Xie, Y., Alexson, S.E., Nelson, B.D., DePierre, J.W., 2002b. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochem. Pharmacol. 63, 1893–1900.
Yeung, L.W., Robinson, S.J., Koschorreck, J., Mabury, S.A., 2013. Part I: a temporal study of PFCAs and their precursors in human plasma from two German cities 1982–2009. Environ. Sci. Technol. 47, 3865–3874.

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