Curr Envir Health Rpt (2015) 2:33–40
DOI 10.1007/s40572-014-0039-3

FOOD, HEALTH, AND THE ENVIRONMENT (KE NACHMAN, SECTION EDITOR)

C6-Perfluorinated Compounds: The New Greaseproofing Agents in Food Packaging

Penelope A. Rice

Published online: 28 January 2015

Abstract Due to their oleophobic and hydrophobic properties and stability, perfluorinated compounds (PFCs) are used in many applications, particularly as greaseproofing agents for food contact. However, PFCs 8-carbons in length or greater (C8-PFCs) have raised concerns regarding environmental biopersistence, bioaccumulation in humans, and potent toxicity that have resulted in their gradual phase-out for food contact use. Industry has replaced C8-PFCs with shorter-chained C6-based greaseproofing agents, which are intended to have the same favorable physicochemical properties without the problematic toxicological effects in humans and wildlife. Compared with the large body of data available for C8 compounds, however, the available database on toxicity and exposure to the C6 compounds is fairly limited. This article summarizes the information in this database, focusing on aspects of human exposure and potential health risks associated with two types of C6 PFCs found in food packaging: perfluorohexanoic acid (PFHxA) and 6–2 fluorotelomer alcohol (C6-FTOH).

Keywords Perfluorinated · Fluorotelomer · PFHxA · Perfluoroalkyl · Perfluorohexanoic · Perfluorohexylethanol · C6-FTOH · Perfluorocarboxylates · Food packaging

Introduction

Perfluorinated compounds (PFCs) are composed of an alkyl chain with all of the hydrogens replaced by fluorine. This perfluorinated alkyl chain is bonded to another functional group, such as an acid, in the case of perfluorocarboxylic acids (PFCAs), or an alcohol (FTOH). Their hydrophobic and hydrophilic properties make them useful as surfactants in emulsion reactions, as reactants to make low-molecular weight perfluorinated products, and as monomers in higher molecular weight polymers. These products are used in microwave popcorn bag susceptors and greaseproofing films for paper and paperboard used in contact with oily foods, such as fast food containers and pizza boxes, as well as for other applications. While PFCAs and FTOHs are not specifically regulated by the US Food and Drug Administration (FDA), PFCAs are regulated as indirect food additives for food contact use by the FDA as surfactants and in the polymerization of high-molecular weight food contact substances (FCSs) under the 21 Code of Federal Regulations (CFR) sections 177.1380, 177.1550, 177.1615, 177.2400, and 177.2510. The use of PFCAs in the manufacture of low-molecular weight perfluorinated paper coatings was authorized in several listings for FCSs in 21 CFR 177.170 and 177.180 and Food Contact Notifications (FCNs) [1]. FTOHs are components of high-molecular weight polymeric FCSs used as coatings, which are the subject of several effective FCNs [1] for their use as greaseproofing agents in food-contact paper and paperboard.

Residual PFCAs and FTOHs derived from the manufacture of perfluorinated polymeric FCSs are present in these FCSs, and migration of these compounds into food has been demonstrated to occur as a result of the regulated uses of those FCSs [2]. As such, the FDA has historically considered the safety of PFCAs and FTOHs in the regulation of those FCSs at the dietary exposures expected to result from their migration into food. Although PFCs of eight carbons in length or greater (C8-PFCs) have a long history of regulated use since the 1960s, recent epidemiological and in vivo studies in animal models have identified concerns for persistence in serum and

This article is part of the Topical Collection on *Food, Health, and the Environment*

P. A. Rice (✉)
5100 Paint Branch Parkway, HFS-275, College Park, MD 20740, USA
e-mail: Penelope.rice@fda.hhs.gov

other bodily fluids and the environment and potent systemic and reproductive toxicity for C8-PFCs as a class [3•]. Beginning in 2006, these concerns led to regulatory actions by several agencies, including the FDA [3•] and US Environmental Protection Agency (EPA) [4, 5], resulting in voluntary agreements with industry to phase out C8-PFCs from all uses, particularly those involving direct contact with food. In the US EPA agreement, industry pledged to eliminate C8-PFCs from emissions and products by 2015. In 2013, the FDA reached a voluntary agreement with the manufacturers of five perfluorinated FCSs to eliminate production of these compounds. As a result of these agreements, industry has replaced the C8-PFC FCSs with FCSs using shorter-chained PFCs (carbon chain lengths of 6 carbons; C6-PFCs), and applications for the use of over 150 of these C6-PFC compounds have been submitted to the US EPA; these compounds are used as grease- and waterproofing paper and paperboard additives for use in contact with food and other items, anti-stain textile and carpet treatments, and tile surface treatments [6]. However, the database for the C6-based compounds is still much less extensive than that for the C8-PFCs, and these data have, as yet, not been considered as a whole in the public literature database. This article discusses the available toxicity data for C6-PFCs in the public database that are relevant for human health safety assessment, focusing on the 6–2 fluorotelomer alcohol (C6-FTOH; Fig. 1) starting material for these polymeric FCSs and perfluorohexanoic acid (PFHxA; Fig. 2), a common impurity derived from the FCS manufacturing process, and placing these data in context with data on levels in food, water, and human biological fluids. Of note, perfluorohexane sulfonate, a biopersistent C6-PFC containing a sulfonate group, is not discussed herein, as there are no C6-sulfonated PFCs authorized for use in food contact applications in the United States.

Uses and Routes of Exposure

As stated above, C6-perfluorinated telomers have similar uses to their long-chain counterparts. However, unlike the long-chain PFCs, the C6-PFCs do not appear to be used in the manufacture of non-stick cookware. It should also be noted that long-chain PFCs usually comprise a mixture of fluorotelomers varying in perfluorinated carbon length from C6 to C12 [2]; additionally, these mixed-chain-length telomers can be transformed in mammals [7] and in the

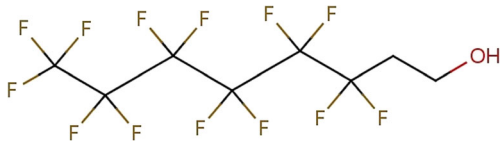

Fig. 1 PFHxA, CASRN: 307-24-4

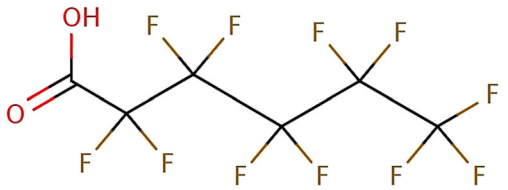

Fig. 2 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octanol (C6-FTOH), CASRN: 647-42-7

environment [8, 9] to PFHxA and to perfluoroheptanoate (PFHpA). As such, it should be emphasized that levels of PFHxA measured in various media, including human bodily fluids and tissues, could originate from a variety of sources, including C6- and long-chain PFCs and FTOHs, and the presence of PFHxA in these media can only rarely be directly extrapolated to direct exposure to PFHxA itself. Therefore, the recent replacement of the long-chain PFCs with C6-PFCs is unlikely to be reflected in bodily fluid PFHxA levels from human biomonitoring studies, as exposure to PFHxA from biotransformation of the C6-PFCs would simply substitute for exposure to PFHxA from biotransformation of the long-chain PFCs. Regarding C6-FTOH, studies have rarely attempted to quantify C6-FTOH content in environmental media or human tissues, except for indoor air, due to its high volatility. However, since C6-FTOH was likely present as a contaminant and biotransformation product of long-chain PFCs, C6-FTOH tissue levels are also unlikely to change significantly as a result of the replacement of long-chain PFCs with C6-PFCs.

Studies have detected PFHxA in surface waters of Victoria Harbor in Hong Kong [10•] and European riverine discharge [11] at levels of 0.15–2.24 ng/L and 2.2–32 ng/L, respectively. There are conflicting data concerning the presence of PFHxA in foods and beverages, with one critical review concluding that C6 compounds were not found at detectable levels in any type of food analyzed [12] except for low levels of PFHpA in pizza (2 ng/g), microwave popcorn (1.5 ng/g), tap water (0.64–3.02 mg/g), and bottled water (0.4 ng/g). Likewise, a recently published total diet study conducted in France [13] reported measurable levels of PFHxA at levels less than 1 ng/g in all of the food types sampled, with the highest reported levels in 'sweet and savory biscuits and bars' (0.915 ng/g), pastry and cakes (0.791 ng/g), and dairy-based desserts (0.583 ng/g); and an European Food Safety Authority (EFSA) scientific report summarizing the results of an analysis of 4,881 samples derived from various foodstuffs collected during the period 2000–2009 reported detectable levels of PFHxA in 0.9 % of samples [14]. In contrast, Zhang et al. (2011) reported levels of <0.1–0.97 ng/g PFHxA in freshwater fish and seafood [15•], and a study conducted in Catalonia, Spain reported levels of ~0.1 ng/g PFHxA in veal, fried chicken nuggets, and frankfurters [16]. There are no data on levels of possible precursors to PFHxA (FTOH, PFCs) in food and

drinking water. As may be seen from the discussion in the next section, exposure to these compounds may also contribute to PFHxA body burden.

Pharmacokinetics

PFHxA is rapidly and completely absorbed from the gut after oral administration, with no saturation of absorption noted at high doses, and is rapidly eliminated from the body via the urine without being metabolized [17••] in rats, mice, and monkeys, with only negligible percentages of the dose excreted in feces. Notably, and unlike the long-chain PFCA compounds, PFHxA is not a substrate for the renal tubule organic anion transporters (OATs) and thus is not reabsorbed from the renal filtrate, which accounts for the high efficiency of urinary elimination of PFHxA compared with longer-chain PFCAs [18]. PFHxA does not significantly accumulate in any tissue examined, except for the liver [19•], which had PFHxA levels 4- to 8-fold greater than plasma levels in mice in one study. Systemic half-lives of PFHxA in male and female rats were estimated to be 1.5–1.7 hours and 0.5–0.7 hours [17••], respectively, with elimination half-lives of 1–2 hours in both mice and rats [20••]. Elimination half-lives in cynomolgus monkeys and humans were calculated to be 1–2 days [21] and 14–49 days [20••], with no apparent gender differences. Moreover, the elimination half-lives for PFHxA were proportional to bodyweights, indicating similar volumes of distribution and elimination mechanisms between species [20••]. The C6-FTOH similarly has extremely short elimination half-lives in vivo, with 75–90 mol-% of the oral dose eliminated within 24 hours [22]. Studies conducted in rat liver microsomes observed that PFHxA and the 5:3 acid were the primary stable metabolites produced from the C6-FTOH [23••]; other stable metabolic products included the 4:3 acid, perfluorobutanoate, perfluoropentanoate, and PFHpA, all of which would be rapidly excreted in the urine. The 6–2 fluorotelomer iodide and the 6–2 fluorotelomer methacrylate were rapidly metabolized to C6-FTOH in rat liver microsomes [23••] and rat hepatocytes [24•], respectively. Perfluorinated FCSs, such as the perfluoroalkyl phosphate surfactants, can also be metabolized in vivo to their FTOH components and the corresponding FTOH metabolites [25]. Collectively, these data indicate that direct dietary exposure to perfluorinated FCSs or their perfluorinated monomeric precursors may produce toxic effects similar to exposure to the FTOH itself. As such, data from toxicity studies conducted with PFCAs and particularly with the FTOHs are directly relevant to the safety assessment of direct dietary exposure to perfluorinated polymeric FCSs.

PFHxA Biomonitoring Data

Several studies have examined serum levels of PFCs in various populations; most of the available data concern levels of C8-PFCs in biological fluids, with very few papers reporting levels of C6 compounds. There are several papers that have reported that PFHxA levels in human biological fluids (serum, milk) are below methodological limits of detection. For instance, a survey of PFC levels in umbilical cord blood samples from hospital deliveries in Ottawa, Canada reported that PFHxA levels were below the limit of detection in the majority of tested samples [26•]. Another study conducted in the general population in Hong Kong only found detectable levels of PFHxA in 40 % of the serum samples [10•], and PFHxA was not detected in any of the serum samples derived from primiparous Swedish women sampled during pregnancy and nursing [27•]. Other studies, however, have reported extremely low, but measureable, levels of PFHxA in bodily fluids from the general population ranging from 0.25 to 3 ng/ml [28•, 15•], with most samples <1 ng/ml. There is little difference in age range or limit of detection between studies that find PFHxA in sera/bodily fluids versus those that do not, and the studies were conducted over a similar time period. Thus, these differences in detection of PFHxA between studies should not be attributed to changing environmental levels. PFHxA was not detected in samples of breast milk [29•] but was detected in serum and urine taken from 5- to 13-year-old South Korean children and serum from adults of the same population [30•]. Autopsy samples of brain, lung, and liver from the general population in Catalonia, Spain reported PFHxA at mean levels of 180, 50.1, and 115 ng/g tissue, respectively [31]. The relatively high tissue levels found in the Spanish study are difficult to reconcile with the extremely low levels found in serum in most studies and with the data from pharmacokinetic studies in animals, which did not find significant accumulation of PFHxA in any tissue examined, except for low levels in liver. It is possible that the high levels of PFHxA found in this study reflect exposure to PFHxA precursors that were metabolized to PFHxA *in situ*, rather than to PFHxA itself. In fact, the extremely high levels of PFHxA found in the brain and lung in that study are consistent with possible inhalation exposure to the C6-FTOH, which has been shown to be present in ambient air at levels of up to 196 pg/m^3 [32]. Given the fact that the subjects in the Spanish study were co-exposed to PFHxA and to both C6-PFC and long-chain PFC during life, untangling the contributions of each of these exposures to the observed tissue levels would be extremely difficult and would confound any comparison with pharmacokinetic studies conducted with purified PFHxA in animals.

In conclusion, most studies report extremely low or undetectable levels of PFHxA in serum and other bodily fluids in the general population and, with a few exceptions, in foodstuffs consumed in the diet. Biomonitoring data for the C6-

FTOH and its metabolic byproducts, with the exception of PFHxA and PFHpA, are unavailable, however.

Systemic and Reproductive Toxicology

Compared with the extensive toxicological database available for the long-chain PFCs, relatively few mammalian toxicity studies have been conducted with the C6 compounds, and all of these have been conducted in rodents. For PFHxA, 90-day [33] subchronic and 2-year [34••] oral toxicity studies have been conducted in rats with the free acid, at dose ranges of 0, 10, 50, and 200 mg/kg/day for the subchronic study and doses of 0, 2.5, 15, and 100 mg/kg/day (males) and 0, 5, 30, and 200 mg/kg/day (females) in the chronic study. Ninety-day oral studies have also been conducted in rats with sodium PFHxA [35] and C6-FTOH [36••] at dose ranges of 0, 20, 100, and 500 mg/kg/day for Na PFHxA and 0, 5, 25, 125, and 250 mg/kg/day of C6-FTOH. One-generation reproductive toxicity and teratogenicity studies have been conducted with sodium PFHxA [35] and C6-FTOH [37••] in rats using the same dose ranges as the subchronic studies cited above, and a one-generation reproductive toxicity study has been conducted with PFHxA ammonium salt in mice [38••] using dose ranges of 0, 100, 350, or 500 mg/kg/day (phase I) or 0, 7, 35, or 175 mg/kg/day (phase II). A 14-day study conducted with the 6–2 methacrylate in rats is also available [24•]. The subchronic [39••], chronic [40], and developmental [41] toxicity studies conducted with PFHxA and C6-FTOH were all compliant with their respective guidelines in the FDA's Redbook. In particular, the subchronic and chronic studies gavaged rats with the test compound for 90 days and 104 weeks, respectively, and measured the following endpoints: bodyweight and feed consumption; biochemical parameters in serum and urine; hematological parameters; organ weights and histopathology; ophthalmology; and neurological function via conduct of a Functional Observational Battery (FOB).

The subchronic [33, 35, 36••] and developmental/reproductive toxicity [35, 37••, 38••] studies demonstrate that the C6 compounds share some similarities in their toxicological profiles with the C8 compounds (see below), except that PFHxA appears to be at least an order of magnitude less potent than perfluorooctanoate (PFOA). Common findings in the above-cited 90-day studies included mortality and/or decreased bodyweights, hepatocellular hypertrophy and increased liver weights, increased kidney weights, and hematological changes indicative of mild anemia at the high doses tested in these studies. However, unlike PFOA, PFHxA did not induce neoplastic effects in any organ in the chronic study [34••].

Studies noted either decreased survival or decreased bodyweights with PFHxA or C6-FTOH administration [33, 35, 36••]. Decreased survival and early mortality was noted at the highest doses tested in the chronic study [34••] with PFHxA of 100 mg/kg/day (males) and 200 mg/kg/day (females), with early mortality noted in both sexes and decreased survival noted in females only. The early mortality was associated with renal papillary necrosis and renal tubular degeneration. The C6-FTOH also induced mortality in both sexes at 250 mg/kg/day in the subchronic study, the highest dose tested, and in one female at 125 mg/kg/day [36••]. Bodyweights were not affected by treatment in either study. In contrast, significantly decreased bodyweights were noted in the subchronic studies conducted with PFHxA in males at doses of ≥50 mg/kg for the free acid and 500 mg/kg for the Na salt [33, 35], with no mortality noted.

Hepatocellular hypertrophy with increased liver weight parameters was one of the most sensitive effects noted in the subchronic studies [33, 35, 36••] and in the 14-day study [24•]; lowest observed effect levels (LOELs) from 90-day studies for this effect were 25 mg/kg/day for C6-FTOH and 100–200 mg/kg/day for PFHxA in males and 125 mg/kg/day for C6-FTOH and 500 mg/kg/day for PFHxA in females. In the chronic study [34••], hepatocellular hypertrophy was not evident at doses of up to 100 mg/kg/day (males) and 200 mg/kg/day (females); however, hepatocellular necrosis and hepatic congestion were noted in high-dose males and females. The hepatocellular necrosis observed after 104 weeks of PFHxA administration is likely the result of the enzyme induction and peroxisomal proliferation that was noted in the 90-day study. The C6-FTOH also induced single-cell hepatocellular necrosis, oval cell/biliary hyperplasia, and periportal inflammation at ≥25 mg/kg/day in males and ≥125 mg/kg/day in females after 90 days of administration [36••]. In neither study were these changes accompanied by elevations in biochemical indicators of liver injury. Interestingly, while Loveless et al. [35] noted induction of peroxisomal proliferation at the same dose levels that induced hepatocellular hypertrophy, these changes were not accompanied by alteration in serum cholesterol or triglycerides. Indeed, while all of the studies noted hepatocellular hypertrophy with C6 administration, no consistent effects on serum cholesterol profiles were reported. Supporting this finding, recent studies observed that the potency of PFHxA at the human peroxisome-proliferator activated receptor (PPAR)α, activation of which is associated with decreased blood lipid levels, is approximately half the potency of PFOA in the human hepatocellular carcinoma cell line HepG2 [42•] and approximately six-fold less potent than PFOA in both mouse and human PPARα in transiently transfected COS cells [43•]. Additionally, the decreased retention of PFHxA in the liver compared with the longer-chained compounds such as PFOA greatly decreases its potency to induce hepatic peroxisomal proliferation in vivo [44]. Concomitant with these findings, there

was also no association of PFHxA serum levels (0.03 ng/ml median) with blood lipids in a Chinese population [45•].

Subchronic studies conducted with both PFHxA and C6-FTOH noted increased kidney weight parameters [33, 35, 36••]. Kidney weight parameters were increased only in males administered PFHxA for 90 days at ≥10 mg/kg [33], whereas Na PFHxA increased kidney weight parameters in both sexes at ≥100 mg/kg/day [35]; these increases occurred in the absence of histopathological changes. However, after administration for 104 weeks, PFHxA induced renal tubular degeneration and papillary necrosis accompanied by increased urine volume and decreased specific gravity in females at 200 mg/kg/day, indicating significant adverse functional alterations in renal concentrating ability [34••]. Therefore, it would appear that the increased kidney weights in males noted in the subchronic studies represented adaptive changes to PFHxA administration, whereas the free acid of PFHxA induced renal injury in females only after chronic exposure. The reasons for this gender-specific effect of PFHxA and difference in kidney-weight response in females between the free acid and the Na salt are not apparent. For the C6-FTOH, the increased kidney weights noted in the subchronic study were accompanied by adverse histopathological changes, and the kidney appeared to be as sensitive as the liver to the adverse effects of C6-FTOH in females, with renal tubular degeneration and necrosis evident at ≥125 mg/kg/day C6-FTOH in females and at 250 mg/kg/day in males [36••].

Decreased erythrocyte parameters (erythrocyte number, hematocrit, hemoglobin) and increased reticulocyte counts were noted in both subchronic and chronic toxicity studies conducted with PFHxA at the highest doses tested, indicative of mild anemia with concomitant regenerative responses [33, 35, 36••]. While this was noted in both sexes in the subchronic study [33], anemia was only noted in females in the chronic study [34••], and the decreased erythrocyte parameters did not persist through the study duration. In contrast, the C6-FTOH induced adverse changes in the same erythrocyte parameters in the same dose range and gender pattern as was observed for hepatotoxicity [36••]. This disparity in dose ranges for this effect between PFHxA and the C6-FTOH may reflect differences in mechanism of action, the additive effect of the adverse hematopoietic effects of the various metabolites of the FTOH, and/or mechanisms secondary to the renal toxicity of the FTOH.

Ameloblast degeneration and altered tooth mineralization were noted with both the C6-FTOH [36••] and the 6–2 methacrylate [24•]; the study authors speculated that fluoride released from metabolism of the test compounds was the causative agent for the observed changes, and increased urinary fluoride levels were noted at the same doses as the adverse effects on the teeth [36••]. These effects were not seen in any of the studies conducted with PFHxA [33, 35, 34••].

No observed adverse effect levels (NOAELs) for systemic toxicity of PFHxA in the 90-day studies were 20 mg/kg/day for the sodium salt [35] and 50 and 200 mg/kg/day for the free acid in males and females, respectively [33], which are considerably higher than the 90-day no observed effect level (NOEL) of 0.06 mg/kg/day reported for the ammonium salt of PFOA in rats [46]. Similarly, the NOAEL levels for systemic toxicity in male and female rats for PFHxA in the chronic study [24•] were 15 and 30 mg/kg/day, respectively, whereas the bioassay conducted with ammonium PFOA in rats noted liver damage in treated rats down to the lowest dose tested of 1.5 mg/kg/day [47]. In contrast, both the 90-day study conducted with the C6-FTOH [36••] and an oral 90-day study conducted with the C8 fluorotelomer alcohol (8–2 FTOH) [48] reported NOAELs of 5 mg/kg/day for systemic toxicity, indicating that decreased perfluorinated chain length of the FTOH did not decrease the toxic potency under the test conditions in short-term studies.

Reproductive toxicity studies were conducted with sodium PFHxA [35] and C6-FTOH [37••] in rats and ammonium PFHxA in mice [38••]. The studies conducted with PFHxA salt and C6-FTOH gavaged male and female CD rats for ~70 days prior to mating, and pregnant females through to lactation day (LD) 22. Separate teratology studies conducted with the sodium PFHxA and C6-FTOH gavaged pregnant rats on gestation days (GD) 6–20, with terminal necropsy on GD 21. The PFHxA study in mice gavaged pregnant ICR dams from GD 6 through LD 22.

There were no effects of sodium PFHxA [35] or C6-FTOH [37••] on any reproductive indices in rats at doses of up to 500 mg/kg/day and 250 mg/kg/day, respectively; decreased maternal bodyweights and bodyweight gains were noted in the teratology [35], but not the reproductive, cohort at 500 mg/kg/day PFHxA and in both cohorts at 250 mg/kg/day C6-FTOH [37••]. There were no effects of sodium PFHxA on reproductive organ weights or histopathology in the P0 generation. The only developmental effects of sodium PFHxA noted were a 17–18 % decrease in mean pup weight throughout the lactation period in the F1 generation in the one-generation study and 10 % decreased fetal weight in the teratology study at 500 mg/kg/day, the highest dose tested in both studies. For the C6-FTOH [37••], the reproductive study noted increased pup mortality and decreased pup weights at ≥125 mg/kg/day; increased incidences of delayed ossification and wavy ribs were noted in the teratology study at these doses. The NOELs for prenatal and postnatal toxicity in rats from these studies were 300 mg/kg/day PFHxA and 25 mg/kg/day C6-FTOH. In contrast, mice were far more sensitive to the effects of PFHxA. Decreased bodyweight gains during postnatal days (PNDs) 0–4 (≥350 mg/kg/d) and the entire lactation period (500 mg/kg/d) were noted in dams [38••]. Significant litter observations (mostly at≥350 mg/kg/d) included increased incidences of stillbirths, increased whole

litter loss on PNDs 0–3, decreased pup survival during lactation, decreased pup bodyweights, delayed eye opening, and reduced terminal bodyweights in F1 females and terminal bodyweight : liver weight ratios in F1 males. At 175 mg/kg/day, significant findings included increased numbers of stillborn pups and pups dying on PND 1 and decreased pup weight at PND 1. The NOEL for developmental toxicity of PFHxA salt in mice from the study is 35 mg/kg/day. The adverse effects noted on pup bodyweight, postnatal survival, and attainment of developmental landmarks are consistent with effects noted in mice after PFOA administration; however, the LOELs for these effects in the study conducted with PFHxA are at least two orders of magnitude greater than the respective LOELs for PFOA in mice, the most sensitive species [49], of 0.6 mg/kg/day and 1 mg/kg/day, respectively, emphasizing the decreased potency of the C6 compound compared with PFOA.

In summary, subchronic and chronic oral toxicity studies conducted with PFHxA (free acid and sodium salt) reported an array of toxicological effects that are broadly similar to those noted with PFOA: decreased bodyweights, hepatocellular hypertrophy and peroxisomal proliferation, and anemia. Kidney effects were more pronounced with PFHxA versus PFOA; but the data overall demonstrate that PFHxA is much less toxic than PFOA, with LOELs at least an order of magnitude higher for PFHxA than PFOA. Moreover, PFHxA was non-carcinogenic in rats and did not display the potent postnatal toxicity noted with PFOA in either rats or mice.

In contrast, the toxicological profile for the C6-FTOH is not as well characterized. Subchronic studies conducted with the C6-FTOH identify similar toxic endpoints to those identified for the 8–2 FTOH, with adverse effects on the teeth, the kidneys, the liver, and red blood cell homeostasis. However, the mortality noted during the C6-FTOH study was not seen with the 8–2 FTOH, and the adverse effects on the kidney were more severe in the C6-FTOH study. As such, while the toxicological profile for PFHxA itself appears less concerning than that for long-chain PFCAs, the toxicological profile and potency for the C6-FTOH may be similar to the long-chained FTOHs. Future studies are needed to confirm whether this is the case.

Conclusions

PFCAs and FTOHs have been used in a variety of applications, including food packaging. Human exposure to these compounds has been demonstrated, with diet as a significant contributor, although the significance of exposure from food packaging has not been elucidated. Due to concerns regarding the toxicological profile of C8-PFCs, industry has phased out use of this class of compounds, replacing them with C6-based PFCs. Although the existing toxicological database for the C6-PFCs is, as yet, comparatively sparse, these compounds do not appear to possess the biopersistence and potent systemic and reproductive toxicity that are characteristic of C8-PFCs as a class. Instead, data from animal and epidemiological studies indicate that C6-PFCs are rapidly and completely excreted and do not appear to accumulate in biological fluids. Of the two C6 compounds discussed in this article, PFHxA has been well characterized in rodent models. PFHxA has a similar profile of toxicological effects to PFOA based on in vivo subchronic studies in rodents; however, the lack of bioaccumulation in the liver significantly decreases the potency of PFHxA, leading to NOAEL values that are at least an order of magnitude higher than the respective NOAEL values for long-chain PFCs. Moreover, PFHxA has been demonstrated to be non-carcinogenic in rodents, unlike PFOA, and appears to be a far less potent postnatal toxicant. In contrast, significant data gaps remain in the toxicological profile of C6-FTOH. The pharmacological profile of this compound in humans and rodents in vivo is not well characterized, and data from biomonitoring studies determining levels of this compound or its metabolites in human biological fluids are lacking. Data on the chronic, reproductive, and developmental toxicity of this compound are also scanty, as there are no available studies examining the toxicological profile of the C6-FTOH in mice, which have been shown to be more sensitive to the toxicological effects of PFCs than rats. Given the fact that toxicity data for the FTOHs are highly pertinent to the safety evaluation of dietary exposure to perfluorinated PFCs, confirmation that the C6-PFC compounds are a safer alternative to the long-chain PFCs awaits data from appropriately designed studies conducted with the C6-FTOH that address these data gaps.

Compliance with Ethics Guidelines

Conflict of Interest Penelope A. Rice declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest and published recently are highlighted below as:
- • Of importance
- •• Of major importance

1. US FDA (2013) Inventory of effective food contact substance (FCS) notifications. http://www.fda.gov/Food/IngredientsPackagingLabeling/PackagingFCS/Notifications/ucm116567.htm. Accessed 2 Aug 2014.

2. Begley TH, White K, Honigfort P, et al. Perfluorochemicals: potential sources of and migration from food packaging. Food Addit Contam. 2005;22(10):1023–31.
3.• US FDA (2012), Update on perfluorinated grease-proofing agents. http://www.fda.gov/Food/IngredientsPackagingLabeling/PackagingFCS/Notifications/ucm308462.htm. Accessed 2 Aug 2014. *Voluntary agreement between US FDA and industry phasing out use of 3 long-chain perfluorinatedFCSs.*
4. US EPA (2009) Long-chain perfluorinated chemicals action plan. http://www.epa.gov/oppt/existingchemicals/pubs/pfcs_action_plan1230_09.pdf. Accessed 2 Aug 2014.
5. US EPA (2013) 2010/2015 PFOA Stewardship Program. http://www.epa.gov/oppt/pfoa/pubs/stewardship/index.html. Accessed 2 Aug 2014.
6. US EPA (2014) Perfluorooctanoic acid and fluorinated telomers: New chemical review of alternatives for PFOA and related chemicals. http://www.epa.gov/oppt/pfoa/pubs/altnewchems.html#3. Accessed 18 Nov 2014.
7. Fasano WJ, Sweeney LM, Mawn MP, et al. 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. 2009;180(2):281–95.
8. Wang N, Szostek B, Buck RC, et al. 8–2 Fluorotelomer alcohol aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. Chemosphere. 2009;75:1089–96.
9. Lee H, D'eon J, Mabury SA. Biodegradation of perfluoroalkyl phosphates as a source of perfluorinated acids to the environment. Environ Sci Technol. 2010;44(9):3305–10.
10.• Loi EIH, Yeung LWY, Mabury SA, et al. Detections of commercial fluorosurfactants in Hong Kong marine environment and human blood: A pilot study. Environ Sci. 2013;47:4677–85. *Only 40 % of human blood samples had detectable PFHxA; one of predominant perfluorinated compounds in water samples from Victoria Harbor.*
11. McLachlan MS, Holmstrm KE, Reth M, et al. Riverine discharge of perfluorinated carboxylates from the European continent. Environ Sci Technol. 2007;41:7260–5.
12. Pico Y, Farre M, Llorca M, et al. Perfluorinated compounds in food: A global perspective. Crit Revs Food Sci Nutr 2011; 1, doi: 10.1080/10408391003721727.
13. Riviere G, Sirot V, Tard A, et al. Food risk assessment for perfluoroalkyl acids and brominated flame retardants in the French population: Results from the second French total diet study. Sci Total Environ. 2014;491–492:176–83.
14. European Food Safety Authority Results of the monitoring of perfluoroalkylated substances in food in the period 2000–2009. EFSA J 2011; 9(2):2016.
15.• Zhang T, Sun H, Lin Y, et al. Perfluorinated compounds in human blood, water, edible freshwater fish, and seafood in China: Daily intake and regional differences in human exposures. J Agric Food Chem. 2011;59(20):11168–76. *Mean (detection frequency) human blood and freshwater fish and seafood PFHxA levels in four locations in China: 0.27 ng/ml blood (39 % of samples) and 0.07 ng/g wet weight (3 % of samples).*
16. Jogsten IE, Perilio G, Llebaria X, et al. Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. Food Chem Toxicol. 2009;47:1577–83.
17.•• Gannon SA, Johnson T, Nabb DL, et al. Absorption, distribution, metabolism, and excretion of [1-14C]-perfluorohexanoate ([14C]-PFHx) in rats and mice. Toxicology. 2011;243:55–62. *Maximum plasma concentration after oral dosing: 30 min (male rats), 15 min (female rats), 15–30 min (mice). No metabolism in rat or mouse hepatocytes; 100 % urinary excretion within 24 h.*
18. Yang C-H, Glover KP, Han X. Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. Toxicol Sci. 2010;117(2):294–302.
19.• Iwai H. Toxicokinetics of ammonium perfluorohexanoate. Drug Chem Toxicol. 2011;34(4):341–6. *Recovery 90 % of oral dose within 24 h in mice and rats; major route of elimination in urine (≥85 %). Tissue levels of PFHxA below LOD 168 h after dosing, except for liver. No difference in toxicokinetics between single and repeated dosing.*
20.•• Russell MH, Nilsson H, Buck RC. Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat, and monkey. Chemosphere. 2013;93(10):2419–25. *Apparent elimination half-life 14–49 d in humans, based on ski-wax workers exposed via inhalation to 6–2 FTOH.*
21. Chengelis CP, Kirkpatrick JB, Myers NR, et al. Comparison of the toxicokinetic behavior of perfluorohexanoic acid (PFHxA) and nonafluorobutane-1-sulfonic acid (PFBS) in cynomolgus monkeys and rats. Reprod Toxicol. 2009;27(3–4):400–6.
22. Gannon SA, Mawn MP, Munley SW, et al. Toxicokinetic evaluation of 6:2 fluorotelomer alcohol and metabolites in rats following 90 days or oral exposure. Toxicol-Off J Soc Toxicol. 2012;126(1):402.
23.•• Ruan T, Sulecki LM, Wolstenholme BW, et al. 6:2 fluorotelomer iodide in vitro metabolism by rat liver microsomes: Comparison with [1,2-14C] 6:2 fluorotelomer alcohol. Chemosphere. 2014;112:34–41. *Six stable biotransformation products: 5:3 acid, 4:3 acid, PFBA, PFPeA, PFHxA, PFHpA; Five transient products: 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, and 5:3 Uacid. Similar metabolic profile for 6:2 FTI.*
24.• Anand SS, Serex TL, Carpenter C, et al. Toxicological assessment of tridecafluorohexylethyl methacrylate (6:2 FTMAC). Toxicology. 2012;292:42–52. *6:2 FTMAC non-mutagenic in Ames test and mouse lymphoma assay; induced structural aberrations in absence of S9 in human lymphocytes, but did not induce numerical aberrations and was non-clastogenic in vivo. Test substance-related effects in 14-day oral toxicity test in Sprague–Dawley rats: increased liver, kidney, and thyroid weights with minimal hepatocyte hypertrophy and altered mineralization of teeth and femur.*
25. D'eon JC, Mabury SA. Production of perfluorinated carboxylic acids (PFCAs) from the biotransformation of perfluoroalkyl phosphate surfactants (PAPS): Exploring routes of human contamination. Environ Sci Technol. 2007;41:4799–805.
26.• Arbuckle TE, Kubwabo C, Walker M, et al. Umbilical cord levels of perfluoroalkyl acids and polybrominated flame retardants. Int J Hyg Environ Health. 2013;216:184–94. *PFHxA levels less than limit of detection in the majority of umbilical cord blood samples from hospital deliveries in Ottowa, Canada.*
27.• Glynn A, Berger U, Bignert A, et al. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: Serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environ Sci Technol. 2012;46:9071–9. *PFHxA not detected in any sample.*
28.• Guo F, Zhong Y, Wang Y, et al. Perfluorinated compounds in human blood around Bohai Sea, China. Chemosphere. 2011;85:156–62. *Mean and median whole blood PFHxA levels in Chinese cities in Bohai Sea area: 0.51 and 0.4 ng/ml, detection frequency: 99 %. No gender difference or age difference in levels.*
29.• Kubwabo C, Kosarec I, Lalonde K. Determination of selected perfluorinated compounds and perfluoroalkyl phosphate surfactants in human milk. Chemosphere. 2013;91:771–7. *PFHxA not detected.*
30.• Kim D-H, Lee M-Y, Oh J-E. Perfluorinated compounds in serum and urine samples from children aged 5–13 years in South Korea. Environ Pollut. 2014;192:171–8. *Mean (detection frequency) PFHxA in urine (adults and children) and serum (children): 1.38 (5 %), 0.731 (11 %), and 0.353 (8 %) ng/ml, respectively, in South Korean survey.*

31. Perez F, Nadal M, Navarro-Ortega A, et al. Accumulation of perfluoroalkyl substances in human tissues. Environ Intl. 2013;59:354–62. *Postmortem tissue levels of PFHxA in 20 subjects from Tarragona County in Spain: 68.3 ng/g liver, main compound in brain (10.1–486 ng/g), 207 ng/g lung.*
32. Fromme H, Tittlemier SA, Volkel W, et al. Review: Perfluorinated compounds—Exposure assessment for the general population in western countries. Int J Environ Health. 2009;212(3):239–70.
33. Chengelis CP, Kirkpatrick JB, Radovsky A, et al. A 90-day repeated dose oral (gavage) toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and motor activity determinations). Reprod Toxicol. 2009;27:342–51.
34.•• Klaunig JE, Shinohara M, Iwai H, et al. Evaluation of the chronic toxicity and carcinogenicity of perfluorohexanoic acid (PFHA) in Sprague–Dawley rats. Toxicol Pathol. 2014;20:1–12. *PFHA did not induce tumors at any dose; treatment related effects: decreased survival (females), early mortality (both sexes), hepatocellular necrosis and congestion (both sexes), erosion/ulceration of glandular and nonglandular stomach (females), renal tubular necrosis and papillary degeneration (females), pulmonary congestion/hemorrhage and increased alveolar macrophages (both sexes).*
35. Loveless SE, Slezak B, Serex T, et al. Toxicological evaluation of sodium perfluorohexanoate. Toxicology. 2009;264:32–44.
36.•• Serex T, Anand S, Munley S, et al. Toxicological evaluation of 6:2 fluorotelomer alcohol. Toxicology. 2014;319:1–9. *Subchronic NOAEL 5 mg/kg/d based on hematological and liver effects; mortality and renal toxicity observed at higher doses. Nonmutagenic in Ames and mouse lymphoma; nonclastogenic in human lymphocyte chromosome aberration assay.*
37.•• O'Connor JC, Munley SM, Serex TL, et al. Evaluation of the reproductive and developmental toxicity of 6:2 fluorotelomer alcohol in rats. Toxicology. 2014; 317: 6–16. *Reproductive NOEL >250 mg/kg (highest dose tested). Parental NOELs 25 mg/kg/d, for decreased feed consumption and bodyweight parameters at ≥125 mg/kg/d. Developmental NOEL of 25 mg/kg/d for increased delayed ossification and wavy ribs (teratology study) and pup mortality and decreased bodyweights (reproductive study) at ≥125 mg/kg/d.*
38.•• Iwai H, Hoberman AM. Oral (gavage) combined developmental and perinatal/postnatal reproduction toxicity study of ammonium salt of perfluorinated hexanoic acid in mice. Int J Toxicol. 2014;33:219–37. *Maternal and reproductive NOAEL = 100 mg/kg/d in ICR mice. Maternal effects at ≥350 mg/kg/d: mortality, excess salivation, decreased bodyweight gains. Developmental effects: stillbirths, decreased viability indices, delayed physical development.*
39. US FDA Toxicological Principles for the Safety Assessment of Food Ingredients–*Redbook 2000.* Chapter IV.C.4.a. Subchronic Toxicity Studies with Rodents, 11/03. Accessed at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm078345.htm on 12/4/14.
40. US FDA Toxicological Principles for the Safety Assessment of Food Ingredients–*Redbook 2000.* Chapter IV.C.7. Combined Chronic Toxicity/Carcinogenicity Studies with Rodents, 11/03. Accessed at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm078393.htm on 12/5/14.
41. US FDA Toxicological Principles for the Safety Assessment of Food Ingredients–*Redbook 2000.* Chapter IV.C.9.b. Guidelines for Developmental Toxicity Studies, 7/00. Accessed at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm078399.htm on 12/4/14.
42.• Buhrke T, Kibellus A, Lampen A. In vitro toxicological characterization of perfluorinated carboxylic acids with different chain lengths. Toxicol Lett. 2013;218:97–104. *Concentration of 20 % effect (C20 %) values for PFHxA ~10-fold, ~20-fold, and 2-fold respective values for PFOA in cytotoxicity, proliferation, and PPARα-activation assays in HepG2 cells; negative in V79 micronucleus and Ames assays.*
43.• Wolf CJ, Schmid JE, Lau C, et al. Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds. Reprod Toxicol. 2012;33:546–51. *10-fold higher no observed effect concentration for PFHxA versus PFOA activation of human and mouse PPARα in transfected COS cells.*
44. Kudo N, Suzuki-Kakajima E, Mitsumoto A, et al. Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice: In relation to induction of hepatomegaly, peroxisomal β-oxidation, and microsomal 1-acylglycerophosphocholine acyltransferase. Bio Pharm Bull. 2006;29(9):1952–7.
45.• Fu Y, Wang T, Fu Q, et al. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf. 2014;106:246–52. *PFHxA detected in ~77 % samples, 0.03 ng/ml (median).*
46. Perkins RG, Butenhoff JL, Kennedy GL, et al. 13-Week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug Chem Toxicol. 2004;27(4):361–78.
47. Butenhoff JL, Kennedy GL, Chang S-C, et al. Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague–Dawley rats. Toxicology. 2012;298:1–13.
48. Ladics GS, Kennedy GL, O'Connor J, et al. 90-Day oral gavage toxicity study of 8–2 fluorotelomer alcohol in rats. Drug Chem Toxicol. 2008;31(2):189–216.
49. Abbott BD, Wolf CJ, Schmid JE, et al. Perfluorooctanoic acid – induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor alpha. Toxicol Sci. 2007;98(2):571–81.