Risk Evaluation of Select PFAS: Report to the Hawaii State Department of Health

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Executive Summary

A comprehensive literature search and review was conducted to assess the toxicity and potential health effects of specific emerging perfluoroalkyl and polyfluoroalkyl substances (PFAS) compounds. These compounds include those found in aqueous film forming foam or AFFF (4:2 FTTAoS, 6:2 FTTAoS, 8:2 FTTAoS and their partial breakdown products 4:2 FTS, 6:2 FTS, and 8:2 FTS), those found in food wrappers and containers (6:2 FTOH, 8:2 FTOH, 5: FTCA), and those found in toilet paper (6:2 diPAP). Compared to legacy PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), there was a dearth of literature on these lesser-known compounds. Twenty-seven unique studies were identified. Most of the studies examined 6:2 FTOH, 8:2 FTOH, and 5:3 FTCA (n=17) or 6:2 FTTAoS (n=7).

Most of the identified studies were toxicology studies using various animal models including mice, rats, amphibians, and fish. A focus of the report was the identification of Points of Departure (POD) including NOAELS, LOAELs, and BMD_{10}s for use in risk assessment guidance. PODs for 6:2 FTOH, 8:2 FTOH, and 6:2 FTTAoS, and measures of toxicity (i.e., lethal dose (LD_{50})) for 6:2 FTOH were derived in select studies; however, significant data gaps exist in the literature to draw a conclusion about the toxicity of these compounds.

Almost all rodent studies focused on exposure to the compounds of interest through ingestion or placental transfer; the concentration and frequency of dosing were similar among the studies. The endpoints analyzed and the study durations, however, were limited and differed greatly. While PODs for 6:2 FTTAoS were derived based on immunological outcomes, the PODs for fluorotelomer alcohols (FTOHs) were determined from reproductive, developmental, or systemic toxicity. Most studies only assessed acute exposure to the compounds of interest, but even among these studies, the study duration differed widely (e.g., 14 or 28 days). Only a couple of studies evaluated subchronic exposure (e.g., 90 and 112 days), and studies on chronic exposure were not identified in the literature. Differences in exposure duration make it challenging to directly compare the small number of toxicological studies, especially given the various endpoints and animal models used in these studies. Furthermore, to draw conclusions about long-term exposures to these compounds, which are more pertinent to the exposure pattern in humans, chronic exposure studies are required.

Four epidemiology studies on the selected PFAS compounds were identified 6:2 diPAP (n=2); 6:2 FTTAoS and 4:2 FTTAoS (n=1) and 8:2 FTOH (n=1); these studies examined health effects, including rate of infant weight gain, birthweight, preeclampsia, couple fecundity, infertility, and testicular function. A prospective cohort study found that 6:2 diPAP concentrations in blood in women were significantly associated with reduced fecundity and an increased risk of infertility. A case-control study found a significant association between serum concentrations of 4:2 FTS and lower odds of preeclampsia. There were too few epidemiology studies to draw conclusions regarding health effects.

To assist HDOH in its health risk assessments, Provisional Guidance Values (PGVs) were calculated for 6:2 FTOH, 8:2 FTOH, and 6:2 FTTAoS. The points of departure (PODs) for oral exposure to 6:2 FTOH and 8:2 FTOH are $BMDL_{10}$s; the POD for 6:2 FTTAoS is a NOAEL. The PODs were converted to human equivalency doses and divided by uncertainty factors to arrive at the PGVs for oral exposure. The PGVs for 6:2 FTOH and 8:2 FTOH were used to estimate PGVs for inhalation exposure.

Background

PFAS are synthetic chemical compounds that are used to make fluoropolymer coatings and a variety of products that are resistant to heat, oil, stains, grease, and water (CDC 2022). Products that contain PFAS include clothing, furniture, adhesives, heat-resistant non-stick cooking surfaces, insulation of electrical wire, firefighting foam, and food packaging (CDC 2022; Carnero et al. 2021). Although there are more than 4500 manufactured substances that can be defined as PFAS, research and legislation have only focused on a few of these substances (Carnero et al. 2021). PFAS, and their impurities, are released into the environment because of their production, use, and disposal (Carnero et al. 2021). PFAS contamination has been found in soil and groundwater from firefighter training, wastewater and sludge release from PFAS manufacturing facilities, leachate from unlined municipal landfills, biosolids containing PFAS as soil amendments, and treated wastewater used in irrigation (SHDH 2022).

PFAS exposure to the general population usually occurs via ingestion of PFAS-contaminated water or food but can also occur by using products that contain PFAS (CDC 2022). Indirect exposure can occur through consumption of food that has been in contact with PFAS-containing materials (Carnero et al. 2021). For example, paper-based food packaging materials can contain PFAS because of residues from recycled fiber and paperboard used in the manufacture of food packaging products (Curtzwiler et al. 2021). PFAS is also used in some food packaging, such as fast-food wrappers, microwave popcorn bags, and take-out paperboard containers, as a grease-proofing agent (FDA Undated). PFAS can migrate from the food container into the soil and water if the container ends up in a landfill (Loria 2022). Leachate from these landfills can then contaminate groundwater (SHDH 2022). There is evidence that PFAS concentrations are higher in wastewater plants that accept leachate from landfills and industrial wastewater (SHDH 2022). In general, the production of paper products is thought to be a major point source of PFAS contamination in water (Carnero et al. 2021).

The following subsections provide further background on the specific types of PFAS compounds examined in this report: fluorotelomer thioether amido sulfonates, fluorotelomer alcohols, and 6:2 fluorotelomer phosphate diester.

Fluorotelomer alcohols

Fluorotelomer alcohols (FTOHs) are major precursors of perfluoroalkyl acids (PFCAs), and FTOHs can ultimately degrade to form PFCAs through atmospheric oxidation and biodegradation (Liu et al. 2015). FTOHs are used to create fluorotelomer-based polymers, grease-proof coatings, carpet, and surfactants (Jin et al. 2020). A study of consumer products found that 6:2 FTOH was detected in carpet, commercial carpet-care liquids, treated home textiles and upholstery, treated medical garments, treated floor waxes and stone/wood sealants, treated food contact paper, membranes for apparel, and in thread sealant tapes and pastes (Liu et al. 2015). 8:2 FTOH was detected in carpet, commercial carpet-care liquids, household carpet/fabric-care liquids and foams, treated apparel, treated home textiles and upholstery, treated medical garments, treated floor waxes and stone/wood sealants treated food contact paper, membranes for apparel, and thread sealant tapes and pastes (Liu et al. 2015).

8:2 FTOH generally degrades to PFOA and PFNA but also to 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, 7:3 FTUCA, and 7:2 sFTOH, depending on the environment (DME 2015; Butt et al. 2014). See Figures 1 and 2 in Appendix A. On the other hand, 6:2 FTOH typically metabolizes

to PFHxA, PFBA, PFHpA, and 5:3 acid or 5:3 FTCA (Russell et al. 2015; Butt et al. 2014). See Figure 3 in Appendix A.

A study by Lang et al. (2017) found that 5:3 FTCA was the dominant PFAS compound in U.S. landfill leachate in 2013; 6:2 FTCA and 8:2 FTCA were also detected[1]. Lang and colleagues also reported that the mean 5:3 FTCA concentration was at least three times higher than PFOA and PFOS combined for leachate from landfills from all climates (Lang et al. 2017).

Fluorotelomer thioether amido sulfonates (FTTAoS)

The Strategic Environmental Research and Development Program (SERDP) reports that aqueous film forming foam, or AFFF, which is used in firefighting and firefighting training, contains PFAS compounds (SERDP 2017). The 3M Company manufactured AFFF using PFOS and PFOA until 2001 when these compounds were voluntarily phased out by 3M (Harding-Marjanovic et al. 2015; 3M 2019). At least three other manufacturers, Ansul, Chemguard, and Angus, used a different PFAS compound to produce AFFF: fluorotelomer thioether amido sulfonate (FTTAoS) (Harding-Marjanovic et al. 2015). FTTAoS was used in AFFF as early as 1984 (Harding-Marjanovic et al. 2015). 6:2 FTTAoS is believed to be the most common FTTAoS in AFFF formulations (Harding-Marjanovic et al. 2015; SERDP 2017). 4:2 FTTAoS and 8:2 FTTAoS have also been detected in some AFFF formulations (Harding-Marjanovic et al. 2015; ECHA 2018; SERDP 2017 [Table 3]).

Harding-Marjanovic et al. (2015) indicates that 4:2 FTTAoS ultimately transforms into 3:3 FtCA, and PFBA, that 6:2 FTTAoS ultimately transforms into PFBA, PFPeA, PFHxA and 5:3 FTCA, and that 8:2 FTTAoS ultimately transforms into PFHxA, PFHpA, PFOA, and 7:3 FTCA. See Appendix A, Figure 4.

According to SERDP (2017), AFFF waste is frequently released in surface-holding ponds and groundwater. The report also indicates that PFOA, PFOS, and fluorotelomer sulfonates (FTSAs) are transported in groundwater from the locations where the AFFF was released (SERDP 2017).

6:2 fluorotelomer phosphate diester

6:2 fluorotelomer phosphate diester (6:2 diPAP) is part of the polyfluoroalkyl phosphate esters (PAPs) class of PFAS. PAPs can be classified into mono-, di-, or triesters (i.e., monoPAP, diPAP, and triPAP) and by the number of fluorinated carbons and their two hydrogenated carbon atoms (e.g., 4:2, 6:2, 8:2, and 10:2) (Luo et al. 2022b). Luo et al. (2022b) indicate that diPAPs are used as the precursors of PFAS end products in the water- and grease-proofing industries, and in personal care products and cosmetics.

6:2 diPAP can metabolize into PFPeA, PFHxA, PFHpA, 6:2 FTOH, 6:2 FTCA, 6:2 FTUCA, and 5:3 FTCA (Butt et al. 2014; Lee et al. 2010). See Figure 5 in Appendix A.

[1] 5:3 FTCA and 6:2 FTCA are products of anaerobic 6:2 fluorotelomer alcohol (FTOH) degradation (Zhang et al. 2013; Lang et al. 2017). Zhang et al. (2013) state that 6:2 FTOH and 8:2 FTOH are the main polyfluorinated raw materials used to manufacture FTOH-based products.

Literature Search Methods

Fluorotelomers of interests

The following PFAS compounds were evaluated for this report: 1) 4:2 FTTAoS and its partial breakdown products; 2) 6:2 FTTAoS and its partial breakdown products; 3) 8:2 FTTAoS and its partial breakdown products; 4) 6:2 FTOH, 8:2 FTOH, and 5:3 FTCA; and 5) 6:2 diPAP.

PECO Criteria

The systematic literature search for health effects of the various compounds identified above followed the methodology described in the 2019 National Toxicology Program/Office of Health Assessment and Translation (NTP/OHAT) *Handbook for Conducting a Literature-Based Health Assessment Using the OHAT Approach for Systematic Review and Evidence Integration* (NTP 2019). For the search, the following population, exposure, comparator, and outcome of interest (PECO) criteria, described below, guided the inclusion/exclusion criteria and selection of terms. The aim of the literature search was to identify original, peer-reviewed epidemiologic studies based on the PECO criteria. Only studies in English were reviewed.

- **Population of Interest:** The population studied must be human (with no restrictions on age, life stage, sex, country of residence/origin, race/ethnicity, lifestyle, or occupation) or animal (with no restrictions on the species).
- **Exposure:** Any route of exposure was included.
- **Comparator:** Exposed or case populations must be compared to populations with low/no exposure or to non-cases to arrive at an effect estimate for a health outcome. Similarly, *in vivo* toxicology studies must compare effects in various dose groups, including a dose of zero.
- **Time Frame:** There are no restrictions on the publication date.
- **Outcome:** All reported health effects were included.

Search engines and number of hits

PubMed and Google Scholar were utilized to identify the relevant literature. The search terms used can be found in Appendix B ("Coded Literature Search Terms"). Table 1, below, provides the number of hits in each search engine for each PFAS compound and its partial breakdown products, if applicable. The literature search was completed on April 6, 2023. Along with the literature search in PubMed and Google Scholar, documents by ECHA, EFSA, EPA, FDA, WHO and OECD were also examined to gather additional information on these PFAS compounds.

Table 1. Literature search results by search engine and compound

Search Engine	Number of hits (including epidemiology and toxicology terms)				
	4:2 FTTAoS & partial breakdown products	**6:2 FTTAoS & partial breakdown products**	**8:2 FTTAoS & partial breakdown products**	**6:2 FTOH; 8:2 FTOH; 5:3 FTCA**	**6:2 diPAP**
PubMed	34 (27)	122 (86)	46 (33)	333 (226)	35
Google Scholar	104	167	147	496	325
PubMed Relevant	3	8	0	19	2
Google Scholar Relevant	3	9	2	24	8
Unique articles based on title and abstract	5	9	2	31 including 2 dissertations	9
Final number of studies reviewed	2	7	0	16	3

Results

Twenty-seven unique studies were identified in the literature search. The findings are described by PFAS compound, below. The toxicology studies that identify PODs such as a LOAEL or NOAEL or that utilized a benchmark dose (BMD) approach are included in the summary tables in this report. All studies are summarized in detail in an Excel document provided with this report ("Appendix C – Study Summaries").

6:2 FTOH, 5:3 FTCA, and 8:2 FTOH

We found 16 epidemiology or toxicology studies that evaluated 6:2 FTOH and 8:2 FTOH. Nine studies were identified for 6:2 FTOH and seven studies were found for 8:2 FTOH. One study looked at the toxicity of 5:3 FTCA; this study was identified and described in a review article by Rice et al. (2020). No additional studies regarding 5:3 FTCA were found through the literature search.

6:2 FTOH

All the 6:2 FTOH studies were animal toxicity studies. The most common evaluations were developmental toxicity (n=4) and subchronic toxicity (n=4), followed by reproductive toxicity (n=3), acute toxicity (n=1), genotoxicity (n=1), behavioral toxicity (n=1), and estrogenic effects (n=1).[2] The test animals were rats (n=6), mice (n=2), zebrafish (n=2), madeka fish (n=1), bacteria (n=1), and rabbits (n=1). The most common exposure route was ingestion (n=7),

[2] One study (Serex et al. 2014) examined acute, subchronic, and dermal toxicity of 6:2 FTOH.

followed by dermal absorption (n=1); the exposure routes studied in zebrafish included dermal exposure (through the gills) and ingestion (n=2).

One of two zebrafish studies evaluated lethal dose, morphometric, and behavioral effects at 120 hours post fertilization (Annunziato et al. 2019); the other focused on developmental endpoints (Shi et al. 2017). Shi et al. (2017) found that exposure to 6:2 FTCA could cause developmental toxicity, measured by hatching and survival percentages and malformation of zebrafish embryos. A study on male madeka fish by Ishibashi et al. (2008) concluded that 6:2 FTOH shows estrogenic potential based on hepatic vitellogenin (VTG) expression patterns, a widely used biomarker for screening estrogenic effects of compounds on aquatic organisms.

Reproductive, developmental, or systemic toxicity of 6:2 FTOH was evaluated in six rodent studies (2 mice, 4 rats) with mixed results. Mukerji et al. (2015) derived a NOAEL for systemic toxicity in CD-1 mice of 5 mg/kg/day in females and 25 mg/kg/day for males. Toxicity at higher doses included effects on mortality, clinical observations, body weight, nutritional parameters, hematology, clinical chemistry (liver-related), liver weights, and histopathology (liver, teeth, reproductive tract, and mammary gland). The mice were dosed between 40 and 109 days. The same study concluded that 6:2 FTOH was not a selective reproductive toxicant for CD-1 mice as no adverse reproductive outcome occurred, even in the highest treatment group (100 mg/kg/day); the NOAEL for reproductive toxicity was subsequently identified as >100 mg/kg/day. The NOAEL for viability and growth of the offspring was 25 mg/kg/day. Xia et al. (2023), however, observed adverse reproductive effects, such as impaired blood-testis barrier (BTB) formation and maturation and abnormal spermatogenesis among BALB/c male mice born to dams exposed to 6:2 FTOH at 5 mg/kg/day. This suggests that the NOAEL level derived by Mukerji et al. would not be protective against the adverse reproductive outcomes in the mice studied by Xia et al.

Kirkpatrick (2005) and Miyata (2007) evaluated systemic toxicity in Crl:CD(SD) rats exposed to 6:2 FTOH. While there appear to be sex differences, most adverse outcomes were observed in the highest treatment groups (above 125 mg/kg/day) in both studies. Kirkpatrick (2005) derived a NOAEL of 25 mg/kg/day for both sexes based on body weight and weight gain. The NOAEL identified by Miyata (2007) is 5 mg/kg/day based on discolored incisors in males and females, mottled teeth in males, and increased relative liver weight and enlargement of the liver in females. Serex et al. (2014) is the only study that evaluated systemic toxicity of 6:2 FTOH after subchronic exposure (i.e., 90 days) in rats (Crl:CD(SD) rats); the authors identified a NOAEL of 5 mg/kg/day in both sexes based on liver and hematologic endpoints. Serex et al. (2014) also evaluated the acute oral and dermal toxicities associated with 6:2 FTOH and determined that the oral and dermal lethal doses (LD_{50}) were 1750 mg/kg and > 5000 mg/kg, respectively. The same study did not find any evidence that 6:2 FTOH should be considered a dermal or eye irritant or dermal sensitizer.

The two studies that evaluated reproductive and developmental toxicity were done on Crl:CD(SD) rats. Kirkpatrick (2005) identified a NOAEL of 75 mg/kg/day for reproductive and neonatal toxicity based on mean number of pups born, postnatal survival, and pup weight. O'Connor et al. (2014) concluded that there was no evidence of either parental or developmental toxicity at doses of 5 and 25 mg/kg/day, and there were no effects on reproductive outcomes at any dose level. O'Connor et al. observed adverse effects (e.g., increased mortality in parental mice) only in the highest exposure groups (125 and 250 mg/kg/day).

Serex et al. (2014) derived a NOAEL of 5 mg/kg/day in rats for 6:2 FTOH for subchronic exposure based on liver and hematologic endpoints. Serex et al. (2014) did not find any mutagenic or genotoxic activity after 6:2 FTOH exposure or any evidence that 6:2 FTOH should be considered a dermal or eye irritant or dermal sensitizer.

Table 2, below, describes the studies that provide POD values derived in studies of 6:2 FTOH.

5:3 FTCA

For 5:3 acid, the LOAEL, identified by Rice et al. (2020), was 30 mg/kg/day based on kidney and liver adverse effects. Table 3 describes this study in more detail.

Table 2. Point of departure values identified in the literature for 6:2 FTOH

Potential POD	Exposure Route	Strain and species	Duration of Exposure (days)	Endpoint Evaluated	Critical effect(s)	Study authors
NOAEL: 25 mg/kg/day (males); 5 mg/kg/day (females)	Oral	CD-1 mice	Varied (40-109)	Systemic	Effects at higher doses on mortality, clinical observations, body weight, nutritional parameters, hematology clinical chemistry, liver weights, and histopathology	Mukerji et al. (2015)
NOAEL: >100mg/kg /day	Oral	CD-1 mice	Varied (40-109)	Reproductive toxicity	No effects on reproductive outcome were observed at any dosage	Mukerji et al. (2015)
NOAEL: 25 mg/kg/day	Oral	CD-1 mice	Varied (40-109)	Viability and growth of the offspring	Clinical signs of delayed maturation in pups, and reductions in pup survival and pup body weight during lactation at 100 mg/kg/day	Mukerji et al. (2015)
NOAEL: 5 mg/kg/day	Oral	Crl:CD (SD) (SPF) rats	28 days	Systemic toxicity	Discolored incisors in males and females, mottled teeth in males, and increased relative liver weight and enlargement of the liver in females	Miyata (2007)
NOAEL: 25 mg/kg.day	Oral	Crl:CD(SD) rats	Varied (14-52 days)	Systemic toxicity	Body weight and body weight gain	Kirkpatrick (2005)
NOAEL: 75 mg/kg/day	Oral	Crl:CD(SD) rats	Varied (39-44)	Reproductive and developmental toxicity	Mean number of pups born, postnatal survival, and pup weight	Kirkpatrick (2005)
NOAEL: 5 mg/kg/day (males) and 125	Oral	Crl:CD(SD) rats	90 days	Systemic toxicity	Hematology and liver effects	Serex et al. (2014)

mg/kg/day (females)						
$BMDL_{10}$: 18 mg/kg/day	Oral	Crl:CD(SD) rats	90 days	Systemic toxicity	Most sensitive endpoints from the 90-day oral gavage study (liver oval cell hyperplasia, pancreatic acinar apoptosis, and teeth ossification)	Serex et al. (2014)

Table 3. Point of departure value identified in the literature for 5:3 FTCA

Potential POD	Exposure Route	Strain and species	Duration of Exposure (days)	Endpoint Evaluated	Critical effect(s)	Study authors
LOAEL[a]: 25 mg/kg/day	Oral	Crl:CD (SD) rats	14 days	Systemic toxicity	Increased β-oxidation and bilirubin in females; Increased serum creatinine, β-oxidation and decreased urine total protein and thymus weight in males	DuPont (2012) (Identified in Rice et al. 2020)

[a] Rice et al. (2020) summarized LOAELs of previous studies; we do not have access to this original study.

8:2 FTOH

One epidemiologic study and six toxicology studies were identified evaluating exposure to 8:2 FTOH. The epidemiologic study (Jin et al. 2020) assessed the correlation between exposure to 8:2 FTOH via breastmilk and rate of infant weight gain. The animal studies assessed a variety of outcomes, including reproductive toxicity (n=2), immunotoxicity (n=1), behavioral toxicity (n=1), developmental toxicity (n=1), toxicokinetics (n=1), estrogenic effects (n=1) and hepatic effects (n=1). The routes of exposure in studies conducted in rats (n=3) and mice (n=2) included ingestion (n=4) and inhalation (n=1).

Jin et al. (2020) conducted a prospective cohort study of 174 mother-infant pairs in Hangzhou, China. The results provide limited information: the authors found a negative correlation between exposure to 8:2 FTOH via breastmilk and postnatal growth at 5 months but calculated Spearman correlation coefficients without adjustment for potential confounders.

In general, the toxicologic studies show adverse outcomes following 8:2 FTOH exposure, but effects are typically observed only in the highest treatment groups. Zebrafish exposed to 8:2 FTOH showed adverse reproductive effects, particularly in the highest exposure categories (90 and 270 µg/L) (Liu et al. 2010). Henderson and Smith (2007) also observed adverse maternal outcomes and offspring viability in a cross-fostering experiment[3] in mice. Mylchreest et al. (2005) derived a NOAEL of 200 mg/kg/day for both maternal and developmental toxicity based on a study in rats.

[3] Cross-fostering is transfer of young between mothers. In the Henderson and Smith (2007) study, four groups were created through cross-breeding: 1) no in utero and no exposure via lactation; 2) only in utero but not exposure via lactation; 3) no in utero but exposure via lactation; 4) exposure in utero and exposure via lactation.

Ladics et al. (2008) conducted a 90-day toxicity study in rats. The authors derived a NOAEL of 5 mg/kg for systemic toxicity based on elevated urine fluorine levels and mild hepatic necrosis. Based on the data provided by Ladics et al. (2008), Himmelstein et al. (2012) calculated a BMD_{10} and $BMDL_{10}$ of 6.3 and 3.7 mg/kg/day, respectively, for 8:2 FTOH based on mild hepatic necrosis. The calculated $BMDL_{10}$ is lower and thus would be more protective as a POD than the NOAEL identified by Ladics et al. (2008).

Studies also observed that exposure to 8:2 FTOH has estrogenic and immunotoxic potential (Ishibashi et al. 2008; Wang et al. 2019). Like 6:2 FTOH, exposure to 8:2 FTOH affected VTG expression in male medaka fish (Ishibashi et al. 2008). Adverse effects associated with immunotoxicity, including immune cell infiltration and downregulation of selected cytokines in the spleen and thymus, were observed in a 28-day oral gavage study in mice (Wang et al. 2019). However, the toxicological consequences of both study findings are unclear, and further studies are needed to understand the potential toxicity of 8:2 FTOH.

Table 4, below, summarizes the studies where potential points of departure values have been identified for 8:2 FTOH.

Table 4. Point of departure values identified in the literature for 8:2 FTOH

Potential POD	Exposure Route	Strain and species	Duration of Exposure (days)	Endpoint Evaluated	Critical effect(s)	Study authors
NOAEL: 5mg/kg/day	Oral	Crl:CD (SD)IGS BR rats	90 days	Systemic toxicity	Mild hepatic necrosis (males), chronic nephropathy (females)	Ladics et al. (2008)
$BMDL_{10}$: 3.7 mg/kg/day	Oral	Crl:CD (SD)IGS BR rats	90 days	Systemic toxicity	Hepatocellular necrosis in males and chronic nephropathy in females receiving 125 mg/kg/day	Himmelstein et al. (2012); the data is from Ladics et al. 2008
NOAEL: 200 mg/kg/day (females)	Oral	Crl:CD (SD)IGS BR rats	Gestational day 6 to gestational day 20	Developmental toxicity	Increased fetal skeletal variations (delayed pelvic bone ossification and wavy ribs)	Mylchreest et al. (2005)

4:2 FTTAoS

Only one toxicological study that examined exposure to 4:2 FTTAoS was identified. The study assessed developmental toxicity following embryonic exposure (Rericha et al. 2022). Another study, Narizzano et al. (2021a), mentions 4:2 FTTAoS in comparison to the concentration of 6:2 FTS found in the study but did not examine 4:2 FTTAoS directly. One epidemiological study was identified that examined the level of 4:2 FTS in the blood of women with and without pre-eclampsia (Tian et al. 2023).

Rericha et al. (2022) examined several different chemical exposures, including 4:2 FTS, in zebra fish embryos. 4:2 FTS was found to induce a small number of differentially expressed genes but no morphological effects. Additionally, 4:2 FTS had relatively low internal concentrations when the bioaccumulation of the chemical was studied in the tissue of the embryos (Rericha et al. 2022).

The epidemiological study, Tian et al. (2023), was a case-control study conducted in China that compared women with preeclampsia to healthy controls with respect to exposure to various PFAS including 4:2 FTS. The participants had blood taken a few days prior to delivery. Following delivery, Tian and colleagues examined the effect of various PFAS on both preeclampsia and low birth weight. Adjusted models found that higher maternal concentrations of 4:2 FTS were significantly inversely associated with preeclampsia. In multivariable regression, higher concentrations of 4:2 FTS was associated with higher birth weights, but this relationship was not statistically significant in a Cox proportional hazard regression that showed an inverse relationship between higher levels of 4:2 FTS and risk of low birth weight.

6:2 diPAP

One toxicological study and two epidemiological studies were identified that examined exposure to 6:2 diPAP. The toxicological study, Chen et al. (2020), examined thyroid toxicity of zebrafish embryos exposed to 6:2 diPAP. One of the epidemiologic studies, Luo et al. (2022a), examined couple fecundity and infertility. The other epidemiologic study, Luo et al. (2022b), examined testicular function.

Chen et al. (2020) examined the potential thyroid toxicity of 6:2 diPAP when zebrafish embryos were exposed post-fertilization. The study found that exposure to 6:2 diPAP was associated with decreases in five thyroid hormones.

Luo et al. (2022a) is a prospective preconception cohort study of 936 Chinese couples. Twenty-five different PFAS chemicals were examined in the blood samples taken from the couples at enrollment including 6:2 diPAP. Luo et al. (2022a) found that 6:2 diPAP levels in the women were significantly associated with reduced fecundity and an increased risk of infertility. Luo et al. (2022b) was a cross-sectional study examining 6:2 diPAP in the blood and semen of 902 men who were seeking preconception care. No association was found between 6:2 diPAP and semen quality.

6:2 FTTAoS

Six toxicology studies were identified that examined potential effects from exposure to 6:2 FTTAoS. These studies examined sublethal effects (n=1), developmental toxicity (n=4), and reproductive toxicity (n=1). The routes of exposure in the studies conducted on mice (n=2), fish (n=1), and amphibians (n=3) were ingestion (n=3), embryonic (n=1), dermal (n=1), and aquatic (n=1). One epidemiological study was identified that examined potential effects of maternal blood levels of 6:2 FTS and pre-eclampsia and low birth weight.

Of the toxicological studies, Abercrombie et al. (2021), found a lowest-observable-effect concentration (LOEC) between 50 and 120 ppb via dermal exposure[4] for the various amphibians examined. When examining the snout-vent length (SVL) of anurans, the authors found that exposure to 6:2 FTS reduced their SVL; however, the same exposure in salamanders caused an increased SVL. Abercrombie et al. concluded that effects depended on species and chemicals, but not on concentration. Flynn et al. (2022) also examined the effects of 6:2 FTS on several amphibian species. The authors noted several effects from 6:2, such as reduced survival among the salamanders exposed to 6:2 FTS versus the controls. It was also found that the toads and salamanders had increased SVL and that the toads exposed to the chemical also had increased

[4] The amphibians were dermally exposed via dosed sphagnum moss substrate (Abercrombie et al. 2021).

growth. “Body condition” was reduced for both the salamanders and frogs exposed to the 6:2 FTS. Another study, Narizzano et al. (2021a), found that of the six PFOS chemicals administered to white-footed mice, 6:2 FTS had the lowest serum to dose ratio. The authors indicate that the lowest serum to dose ratio means that 6:2 FTS had the lowest bioaccumulation potential of the six PFAS compounds studied. Fey et al. (2022) examined several outcomes among zebra fish embryos and rainbow trout embryos exposed to 6:2 FTS, such as mortality, hatching, and other developmental endpoints. Of the endpoints identified, none were significant for 6:2 FTS. Bohannon et al. (2023) examined reproduction, development, and immune function in white-footed mice exposed to 6:2 FTS. The immune function of the mice was decreased in both males and females in the two highest dose groups, but the authors found little evidence of effect upon reproduction or development. In a 30-day experiment, Flynn et al. (2021) fed salamanders crickets that had been exposed to 6:2 FTS through their food and watering gel. The authors found that the crickets showed bioaccumulation of 6:2 FTS; the salamanders did not. Body condition, hepatosomatic index, and change in mass of the salamanders were not significantly different from controls for the low, medium, or high 6:2 FTS dose groups. There was a significant decrease in SVL for the low and high 6:2 FTS dose groups but not for the medium dose group. The salamander liver burden of 6:2 FTS was not significantly correlated with the estimated dose rate.

Table 5, below, summarizes the studies that identified point of departure values for 6:2 FTTAoS.

One epidemiological study was identified that examined 6:2 FTS, Tian et al. (2023). This study, which was previously discussed with respect to 4:2 FTS, also examined the relationship between preeclampsia and 6:2 FTS exposure. There was no significant association between maternal blood levels of 6:2 FTS and preeclampsia or low birth weight.

Table 5. Point of departure values identified in the literature for 6:2 FTTAoS

Potential POD	Exposure Route	Strain and species	Duration of Exposure (days)	Endpoint Evaluated	Critical effect(s)	Study authors
LOEL: 50-120 ppb[a]	Dermal	Amphibians[b]	30 days	Relative body condition	Exposure influenced snout-vent length and scaled mass index. The effects observed were dependent on species and chemical, not concentration.	Abercrombie et al. (2021)
NOAEL: 1.0 mg/kg/day	Oral	White-footed Mice	112 days	Immune Function	Decreased plaque-forming cell (PFC) assay	Bohannon et al. (2023)
LOAEL: 5.0 mg/kg/day	Oral	White-footed Mice	112 days	Immune Function	Decreased plaque-forming cell (PFC) assay	Bohannon et al. (2023)
$BMDL_{10}$: 2.63 (males), 2.26 (females) mg/kg/day	Oral	White-footed Mice	112 days	Immune Function	Decreased plaque-forming cell (PFC) assay	Bohannon et al. (2023)

[a] Sphagnum moss concentrations

[b] Juvenile American toads (Anaxyrus Americanus), eastern tiger salamanders (Ambystoma tigrinum), and northern leopard frogs (Rana pipiens)

8:2 FTTAoS

No studies were identified for 8:2 FTTAoS.

Provisional Guidance Values

The U.S. EPA calculates Reference Doses (RfDs) as a "reference point from which to gauge the potential effects of the chemical at other doses" (EPA 1993). This report does not provide RfDs; that is the purview of the U.S. EPA. However, this report has made estimates of what we are calling Provisional Guidance Values (PGVs) for 6:2 FTOH, 8:2 FTOH, and 6:2 FTTAoS to assist HDOH in its health evaluation of exposure to these compounds. The PGVs for oral exposure (PGV_{OS}) for 6:2 FTOH and 8:2 FTOH are based on $BMDL_{10}$s for which human equivalency doses (HEDs) were calculated, and both use uncertainty factors like those used by U.S. EPA in its development of RfDs. The PGV_O for 6:2 FTTAoS is based on a NOAEL for which a human equivalency dose was calculated; uncertainty factors were used to develop the PGV_O.

$BMDL_{10}$s were calculated by Serex et al. (2014), Himmelstein et al. (2012), and Bohannon et al. (2023) for 6:2 FTOH, 8:2 FTOH, and 6:2 FTTAoS, respectively (See Tables 2, 4, and 5). The data on which Serex et al. (2014) estimated a $BMDL_{10}$ for 6:2 FTOH were not provided by the authors, and thus the $BMDL_{10}$ could not be confirmed. Data were available in Miyata et al. (2007), however, on which to calculate a $BMDL_{10}$. Miyata et al. (2007) was a 28-day study while Serex et al. 2014 was a 90-day study. The effects in both studies were similar, however, and the test animals were the same [Crl:CD (SD) rats]. The lowest $BDML_{10}$ calculated from Miyata et al. (2007) was for discolored teeth in the females (1.578 mg/kg/day), which is lower than the $BMDL_{10}$ of 18 mg/kg/day estimated by Serex et al. (2014). See Table 2.

Himmelstein et al. (2012) et al. estimated a $BMDL_{10}$ for 8:2 FTOH of 3.69 mg/kg/day based on the focal liver necrosis in Crl:CD (SD) male rats in Ladics et al. (2008). Himmelstein et al. excluded the highest dose group for the $BMDL_{10}$ estimate. To confirm the $BMDL_{10}$ estimate for 8:2 FTOH by Himmelstein et al. (2012), the Ladics et al. (2008) data were run excluding the highest dose group. The resulting $BMDL_{10}$, 1.368, was lower than that obtained by Himmelstein et al. (2012). A $BMDL_{10}$ using focal liver necrosis data (all dose groups) in Ladics et al. (2008) was also run. The result was 1.636, which was also lower than the $BMDL_{10}$ obtained by Himmelstein et al. (2012). Because the chronic progressive nephropathy results in female rats in Ladics et al. (2008) provided a relatively monotonic response, a $BMDL_{10}$ of 3.719 was estimated using the nephropathy data.

Bohannon et al. (2023) estimated $BMDL_{10}$s for 6:2 FTTAoS of 2.63 and 2.26 mg/kg/day in male and female mice, respectively, using a Bayesian Hill model. The estimates were based on the results of plaque-forming cells in the spleen, a measure of immune function. The authors reported that they used EPA's BMDS Version 3.2 for their $BMDL_{10}$ estimate. The EPA currently provides BMDS 3.3.2 on their website, which is a newer version of the BMDS 3.2 that Bohannon et al. (2023) used. There have been some substantial changes between the two versions; one change is that Bayesian models have been disabled in Version 3.3, and thus we are not able to confirm the Bohannon et al. (2023) $BMDL_{10}$ result. The NOAEL of 1 mg/kg/day for immune effects (See Table 5) was used as a POD.

The models used to calculate $BMDL_{10}$s for this report are the ones recommended by the EPA software (Version 3.3.2) based on AIC and other parameters of model fit. Human Equivalent Doses (HEDs) of the $BMDL_{10}$s were calculated using the dosimetric adjustment factor (DAF) described in EPA (2011):

$$HED = BMDL_{10} \times DAF$$

Where the $DAF = (BW_a / BW_h)^{1/4}$ and "BW_a" is the body weight of the animal and "BW_h" is the body weight humans. Here, we assumed BW_a to be 0.25 kg for rats[5] and 0.025 for mice[6] and BW_h to be 70 kg (EPA 2011). The DAFs were calculated to be 0.244462 for 6:2 FTOH and 8:2 FTOH and 0.137471 for 6:2 FTTAoS.

$BMDL_{10}$s and the HEDs for the $BMDL_{10}$s are described in Table 6. Because the $BMDL_{10}$s calculated for this report are all lower and thus more protective than the one calculated by Serex and colleagues, the Serex et al. (2014) $BMDL_{10}$ was not used to calculate a PGV_O. Similarly, because the $BMDL_{10}$s calculated for this report using the Ladics et al. (2008) data are lower and thus more protective than that calculated by Himmelstein et al. (2012), the $BMDL_{10}$ estimated by Himmelstein and colleagues was not used to calculate a PGV_O. As indicated above, a NOAEL for immune effects, rather than a $BMDL_{10}$ was used to calculate a PGV_O for 6:2 FTTAoS. The HED for the NOAEL is 0.137471 mg/kg/day [1 mg/kg/day (NOAEL) x 0.137471 (DAF)].

Table 6. Results of $BMDL_{10}$ analyses and HED calculations for 6:2 FTOH and 8:2 FTOH

PFAS	Study	Critical endpoint	BMD	$BMDL_{10}$ (mg/kg/day)	Model fit	HED (mg/kg/day)
6:2 FTOH	Miyata (2007)	Liver enlargement (females)	6.032	2.852	Multistage 1	0.6972
	Miyata (2007)	Discolored teeth (females)	3.344	1.578	Multistage 1	0.3858
	Miyata (2007)	Discolored teeth (males)	4.541	2.155	Multistage 1	0.5268
8:2 FTOH	Ladics et al. (2008)	Liver necrosis, focal (males)	5	1.636	Hill[7]	0.3999
	Ladics et al. (2008)	Liver necrosis, focal (males)	5.38	1.368	Log Probit[8]	0.3344
	Ladics et al. (2008)	Chronic progressive nephropathy (females)	22.464	3.719	Hill	0.9092

[5] The 6:2 FTOH and 8:2 FTOH studies (Miyata et al. 2007 and Ladics et al. 2008, respectively), used rats.

[6] The study of 6:2 FTTAoS (Bohannon et al. 2023) used mice.

[7] All dose groups were included; the Hill model was recommended; the Log Probit model was not recommended.

[8] The highest dose group was removed from this analysis to mimic the Himmelstein et al. (2012) analysis.

To determine the PGV_{OS}, uncertainty factors were applied to the HEDs of the $BMDL_{10}$s. According to the EPA, when dosimetric adjustments are applied and an HED is calculated, as was done above, the interspecies uncertainty factor should be set at 3 for calculation of a RfD (EPA 2011). An uncertainty factor of 10 was used for the variation in sensitivity in the human population. An uncertainty factor of 10 was also used for extrapolation from less than chronic exposure. A modifying factor (MF) may be used in the determination of the guidance valued; modifying factors range from 1-10 and depend on the professional assessment of scientific uncertainties of the study and database (EPA 1993). The default value of the MF is 1 (EPA 1993). Developmental studies have been done for all three compounds, which did not indicate effects below the $BMDL_{10}$s or the NOAEL (6:2 FTTAoS). It was concluded that the MF would be 1. The composite uncertainty factor for both 6:2 FTOH and 8:2 FTOH is 300 (10 for human variability x 3 for animal to human extrapolation x 10 for less than chronic exposure).

The PGV_{OS} were determined using the following equation where the UF are the uncertainty factors, and MF is the modifying factor:

$$PGV_O = \text{HED of the } BMDL_{10} \text{ (or NOAEL)/ (UF x MF)}$$

Route-to-route extrapolation was utilized to estimate Provisional Guidance Values for inhalation (PGV_I). The extrapolation was made following guidance from EPA on conversion of RfDs to RfCs (EPA Undated):

$$\text{Extrapolated RfC (mg/m}^3\text{)} = \text{RfD (mg/kg-day) x (70 kg/20 m}^3\text{/day)}$$

PGVs for oral and inhalation exposure are reported in Table 7. [9, 10]

Table 7. Provisional Guidance Values

PFAS	HED (mg/kg/day)	Critical endpoint	UF	MF	Provisional Guidance Values	
					Oral (µg/kg/day)	Inhalation (µg/m^3)
6:2 FTOH	0.3858	Discolored teeth (females)	300	1	1.3	4.55
8:2 FTOH	0.3344	Liver necrosis, focal (males)	300	1	1.1	3.85
6:2 FTTAoS	0.1375	Immune response [Decreased plaque-forming cell (PFC) assay]	300	1	0.5	-

[9] The PGVs for 6:2 FTOH and 8:2 FTOH are based on the lowest HEDs from Table 6; the PGV for 6:2 FTTAoS is based on the HED of the NOAEL from Bohannon et al. (2023).

[10] According to EPA (Undated a), the method to extrapolate from an oral guidance value to an inhalation guidance value provides a reasonable approximation when the substance is volatile but can be very uncertain when the substance is less volatile. Both 6:2 and 8:2 FTOH are volatile; 6:2 FTTAoS is much less volatile (EPA Undated b). Thus, a PGV_I was not estimated for 6:2 FTTAoS.

Conclusion

Limited data on the potential health risks of the PFAS compounds described in this report are available. The data were sufficient, however, to make estimates of PGVs for 6:2 FTOH, 8:2 FTOH, and 6:2 FTTAoS.

Only two studies on 4:2 FTTAoS were identified; no studies on 8:2 FTTAoS were found. Of the two epidemiological studies that evaluated 6:2 diPAP, one showed potential reduction in fecundity and increased infertility among women; the other study showed no association between 6:2 diPAP and semen quality. The results of a toxicity study of zebrafish and 6:2 diPAP showed a reduction in five thyroid hormones.

Of the six animal studies examining the effects of exposure to 6:2 FTTAoS, two studies, both amphibian, found effects. However, Abercrombie et al. (2021), the author of one of the amphibian studies, noted that the effects depended on the species exposed and the specific chemical rather than the concentration. The remaining toxicological studies generally did not find effects from 6:2 FTTAoS exposure.

As indicated, there are limited toxicity data on the PFAS compounds described in this report. Hence, the development of guidance values for these compounds must be viewed with caution. Nevertheless, the development of guidance values, even with limited data, should provide benefit to HDOH in its health assessments.

References

3M (2019). 3M to share record on PFAS with House Oversight Subcommittee. September 10, 2019. Available at: https://news.3m.com/3M-to-share-record-on-PFAS-with-House-Oversight-Subcommittee#

Abercrombie, S.A., C. de Perre, M. Iacchetta, R.W. Flynn, M.S. Sepúlveda, L.S. Lee and J.T. Hoverman (2021). Sublethal Effects of Dermal Exposure to Poly- and Perfluoroalkyl Substances on Postmetamorphic Amphibians. Environ Toxicol Chem 40(3): 717-726.

Annunziato, K.M., C.E. Jantzen, M.C. Gronske and K.R. Cooper (2019). Subtle morphometric, behavioral and gene expression effects in larval zebrafish exposed to PFHxA, PFHxS and 6:2 FTOH. Aquat Toxicol **208**:126-137.

Bohannon, M.E., A.M. Narizzano, B.A. Guigni, A.G. East and M.J. Quinn (2023). Next-generation PFAS 6:2 fluorotelomer sulfonate reduces plaque formation in exposed white-footed mice. Toxicol Sci 192(1):97-105.

Butt C.M., D.C. Muir, and S.A. Mabury (2014). Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: a review. Environ Toxicol Chem 33(2):243-267. doi:10.1002/etc.2407

Carnero, A.R., A. Lestido-Cardama, P.V. Loureiro PV, et al. (2021). Presence of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials (FCM) and Its Migration to Food. Foods 10(7):1443. Published June 22, 2021. doi: 10.3390/foods10071443.

CDC (2022). Per- and Polyfluorinated Substances (PFAS) Factsheet, Centers for Disease Control and Prevention (CDC). Page last reviewed: May 2, 2022. Available at: https://www.cdc.gov/biomonitoring/PFAS_FactSheet.html

Chen, P., J. Yang, G. Chen, S. Yi, M. Liu and L. Zhu (2020). Thyroid-disrupting effects of 6:2 and 8:2 polyfluoroalkyl phosphate diester (diPAPs) at environmentally relevant concentrations from integrated in silico and in vivo studies. Environmental Science & Technology Letters **7**(5):330-336.

Curtzwiler, G.W., P. Silva, A. Hall, A. Ivey, and K. Vorst (2021). Significance of Perfluoroalkyl Substances (PFAS) in Food Packaging. Integr Environ Assess Manag 17(1):7-12. doi:10.1002/ieam.4346

DME (2015). Short-chain Polyfluoroalkyl Substances (PFAS): A literature review of information on human health effects and environmental fate and effect aspects of short-chain PFAS (Environmental project No. 1707), Danish Ministry of the Environment (DME), Environmental Protection Agency.

DuPont (2012). [5:3 Acid]: Repeated-Dose Oral Toxicity 2-Week Gavage Study in Rats with Metabolism and Genetic Toxicology.

ECHA (2018). Committee for Risk Assessment (RAC), Committee for Socio-economic Analysis (SEAC), Background document to the Opinion on the Annex XV dossier proposing restrictions on C9-C14 PFCAs including their salts and precursors, European Chemicals Agency (ECHA). November 29, 2018. Available at: https://echa.europa.eu/documents/10162/02d5672d-9123-8a8c-5898-ac68f81e5a72

EPA (1993). Reference Dose (RfD): Description and Use in Health Risk Assessments, Background Document 1A, U.S. Environmental Protection Agency (EPA). March 15, 1993. Available at: https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments

EPA (2011). Recommended Use of Body Weight 3/4 as the Default Method in Derivation of the Oral Reference Dose (100-R11-00), U.S. Environmental Protection Agency (EPA). February 2011. Available at: https://www.epa.gov/sites/default/files/2013-09/documents/recommended-use-of-bw34.pdf

EPA (2012). Benchmark Dose Technical Guidance (EPA/100/R-12/001), U.S. Environmental Protection Agency (EPA). June 2012. Available at: https://www.epa.gov/sites/default/files/2015-01/documents/benchmark_dose_guidance.pdf

EPA (2023). Basic Information about the Integrated Risk Information System, U.S. Environmental Protection Agency (EPA). Last updated on May 18, 2023. Available at: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system

EPA (Undated a). Appendix B: Route-to-Route Extrapolation of Inhalation Benchmarks, U.S. Environmental Protection Agency (EPA). Available at: https://semspub.epa.gov/work/11/175214.pdf

EPA (Undated b). CompTox Chemicals Dashboard, U.S. Environmenatl Protection Agency (EPA). Available at: https://comptox.epa.gov/dashboard/

FDA (Undated). Authorized Uses of PFAS in Food Contact Applications, U.S. Food and Drug Administration (FDA). Available at: https://www.fda.gov/food/process-contaminants-food/authorized-uses-pfas-food-contact-applications

Fey, M.E., P.E. Goodrum, N.R. Razavi, C.M. Whipps, S. Fernando and J.K. Anderson (2022). Is Mixtures' Additivity Supported by Empirical Data? A Case Study of Developmental Toxicity of PFOS and 6:2 FTS in Wildtype Zebrafish Embryos. Toxics 10(8).

Flynn, R.W., G. Hoover, M. Iacchetta, S. Guffey, C. de Perre, B. Huerta, W. Li, J.T. Hoverman, L. Lee and M.S. Sepúlveda (2022). Comparative Toxicity of Aquatic Per- and Polyfluoroalkyl Substance Exposure in Three Species of Amphibians." Environ Toxicol Chem 41(6): 1407-1415.

Flynn, R.W., T.D. Hoskins, M. Iacchetta, C. de Perre, L.S. Lee, J.T. Hoverman and M.S. Sepulveda (2021). Dietary exposure and accumulation of per- and polyfluoroalkyl substances alters growth and reduces body condition of post-metamorphic salamanders. Sci Total Environ 765:142730.

Harding-Marjanovic, K.C., E.F. Houtz, S. Yi, J.A. Field, D.L. Sedlak, and L. Alvarez-Cohen (2015). Aerobic Biotransformation of Fluorotelomer Thioether Amido Sulfonate (Lodyne) in AFFF-Amended Microcosms. Environmental science & technology 49(13):7666–7674. https://doi.org/10.1021/acs.est.5b01219

Henderson, W.M. and M.A. Smith (2007). Perfluorooctanoic acid and perfluorononanoic acid in fetal and neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol. Toxicol Sci 95(2):452-461.

Himmelstein, M. W., T.L. Serex, R.C. Buck, J.T. Weinberg, M.P. Mawn and M.H. Russell (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(1-3):122-132.

Ishibashi, H., R. Yamauchi, M. Matsuoka, J. W. Kim, M. Hirano, A. Yamaguchi, N. Tominaga and K. Arizono (2008). Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (Oryzias latipes). Chemosphere 71(10):1853-1859.

Jin, H., L. Mao, J. Xie, M. Zhao, X. Bai, J. Wen, T. Shen and P. Wu (2020). Poly- and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. Sci Total Environ 713:136417.

Kirkpatrick, J.B. (2005). A combined 28-day repeated dose oral toxicity study with the reproduction/developmental toxicity screening test of perfluorohexanoic acid and 1H, 1H, 2H, 2H-Tridecafluoro-1-octanol in rats, with recovery. WIL Research Laboratories, LLC, Ashland, OH Study # WIL-534001. Downloaded from fluorocouncil's website

Ladics, G.S., G.L. Kennedy, J. O'Connor, N. Everds, L.A. Malley, S.R. Frame, S. Gannon, R. Jung, T. Roth, H. Iwai and S. Shin-Ya (2008). 90-day oral gavage toxicity study of 8-2 fluorotelomer alcohol in rats. Drug Chem Toxicol 31(2):189-216.

Lang, J.R., B.M. Allred, J.A. Field, J.W. Levis, and M.A. Barlaz (2017). National Estimate of Per- and Polyfluoroalkyl Substance (PFAS) Release to U.S. Municipal Landfill Leachate. Environmental science & technology, 51(4):2197–2205. https://doi.org/10.1021/acs.est.6b05005

Lee, H., J. D'eon, and S.A. Mabury (2010). Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. Environmental Science & Technology 44(9): 3305-3310. https://doi.org/10.1021/es9028183

Liu, C., J. Deng, L. Yu, M. Ramesh and B. Zhou (2010). Endocrine disruption and reproductive impairment in zebrafish by exposure to 8:2 fluorotelomer alcohol. Aquat Toxicol 96(1):70-76.

Liu, X., Z. Guo, E.E. Folk, 4th, and N.F. Roache (2015). Determination of fluorotelomer alcohols in selected consumer products and preliminary investigation of their fate in the indoor environment. Chemosphere 129:81–86. https://doi.org/10.1016/j.chemosphere.2014.06.012

Loria. 2022. Dangerous PFAS Chemicals Are in Your Food Packaging. Consumer Reports. March 24, 2022.

Luo, K., X. Liu, W. Zhou, M. Nian, W. Qiu, Y. Yang and J. Zhang (2022a). Preconception exposure to perfluoroalkyl and polyfluoroalkyl substances and couple fecundity: A couple-based exploration. Environment International 170:107567.

Luo, K., X. Meng, X. Liu, M. Nian, Q. Zhang, Y. Tian, D. Chen and J. Zhang (2022b). Environmental Exposure to 6:2 Polyfluoroalkyl Phosphate Diester and Impaired Testicular Function in Men. Environ Sci Technol 56(12): 8290-8298.

Miyata, K. (2007). Twenty-eight day repeated dose oral toxicity study of the 13F-EtOH in rats. #B11-0839. Hita Laboratory, Japan Downloaded from Fluorocouncil's website. https://www.daikinchemicals.com/library/pb_common/pdf/sustainability/C6-2Alcohol/28_C6-2Alcohol_E.pdf on 1/26/18.

Mukerji, P., J.C. Rae, R.C. Buck and J.C. O'Connor (2015). Oral repeated-dose systemic and reproductive toxicity of 6:2 fluorotelomer alcohol in mice. Toxicol Rep 2: 130-143.

Mylchreest, E., S.M. Munley and G.L. Kennedy, Jr. (2005). Evaluation of the developmental toxicity of 8-2 telomer B alcohol. Drug Chem Toxicol 28(3):315-328.

Narizzano, A. M., M.E. Bohannon and M.J. Quinn Jr (2021). Comparative Serum Pharmacokinetics of Per-and Polyfluoroalkyl Substances (PFAS) in White-Footed Mice (Peromyscus leucopus), October 2017-July 2018, Army Public Health Center.

NTP (2019). Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. March 4, 2019. Available at: https://ntp.niehs.nih.gov/sites/default/files/ntp/ohat/pubs/handbookmarch2019_508.pdf

O'Connor, J.C., S.M. Munley, T.L. Serex and R.C. Buck (2014). Evaluation of the reproductive and developmental toxicity of 6:2 fluorotelomer alcohol in rats. Toxicology 317: 6-16.

Rericha, Y., D. Cao, L. Truong, M.T. Simonich, J.A. Field and R.L. Tanguay (2022). Sulfonamide functional head on short-chain perfluorinated substance drives developmental toxicity. iScience 25(2):103789.

Rice, P.A., J. Aungst, J. Cooper, O. Bandele and S.V. Kabadi (2020). Comparative analysis of the toxicological databases for 6: 2 fluorotelomer alcohol (6: 2 FTOH) and perfluorohexanoic acid (PFHxA). Food and Chemical Toxicology **138**: 111210.

Russell, M.H., M.W. Himmelstein, and R.C. Buck (2015). Inhalation and oral toxicokinetics of 6:2 FTOH and its metabolites in mammals. Chemosphere 120:328-335. doi:10.1016/j.chemosphere.2014.07.092

SERDP. 2017. Final Report: Characterization of the Fate and Biotransformation of Fluorochemicals in AFFF-Contaminated Groundwater at Fire/Crash Testing Military Sites, Strategic Environmental Research and Development Program (SERDP). April 2017.

Serex, T., S. Anand, S. Munley, E.M. Donner, S.R. Frame, R.C. Buck and S.E. Loveless (2014). Toxicological evaluation of 6:2 fluorotelomer alcohol. Toxicology **319**: 1-9.

SHDH (2022). Interim Soil and Water Environmental Action Levels (EALs) for Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs), State of Hawai'i Department of Health (SHDH). December 29, 2022.

Shi, G., Q. Cui, Y. Pan, N. Sheng, Y. Guo, and J. Dai (2017). 6:2 fluorotelomer carboxylic acid (6:2 FTCA) exposure induces developmental toxicity and inhibits the formation of erythrocytes during zebrafish embryogenesis. Aquatic Toxicology 190, 53–61. https://doi.org/10.1016/j.aquatox.2017.06.023

Tian, Y., Q. Zhou, L. Zhang, W. Li, S. Yin, F. Li and C. Xu (2023). In utero exposure to per-/polyfluoroalkyl substances (PFASs): Preeclampsia in pregnancy and low birth weight for neonates. Chemosphere 313:137490.

Wang, X., B. Kong, B. He, L. Wei, J. Zhu, Y. Jin, Y. Shan, W. Wang, C. Pan and Z. Fu (2019). 8:2 Fluorotelomer alcohol causes immunotoxicity and liver injury in adult male C57BL/6 mice. Environ Toxicol 34(2):141-149

Xia, Y., L. Hao, Y. Li, Y. Li, J. Chen, L. Li, X. Han, Y. Liu, X. Wang and D. Li (2023). Embryonic 6:2 FTOH exposure causes reproductive toxicity by disrupting the formation of the blood-testis barrier in offspring mice. Ecotoxicol Environ Saf 250: 114497.

Appendix A: Proposed Degradation Pathways of Selected PFAS

Figure 1. Proposed biodegradation pathway of 8:2 FTOH in mixed microbial systems (Figure 1 from Butt et al. 2014)

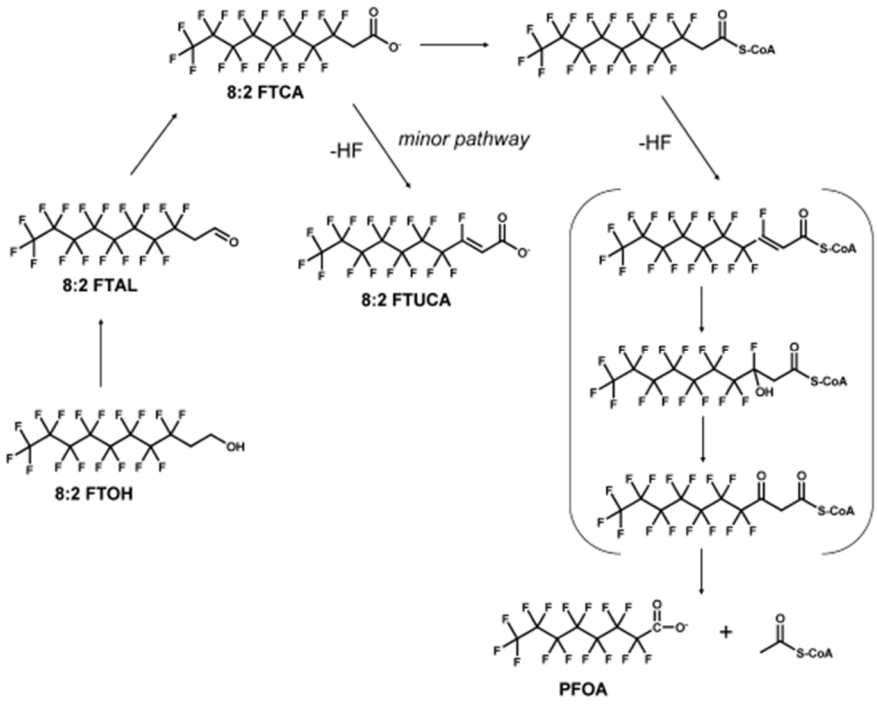


Figure 2. Proposed degradation pathway of 8:2 FTOH in soil and activated sludge (Figure 2 from Butt et al. 2014)

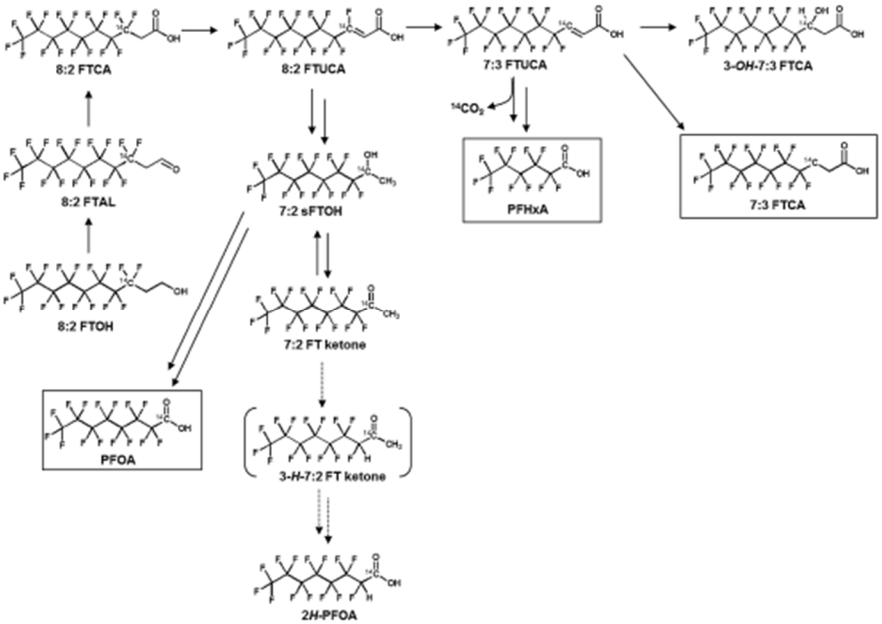


Figure 3. Proposed aerobic degradation pathway of 6:2 FTOH (Figure 3 from Butt et al. 2014)

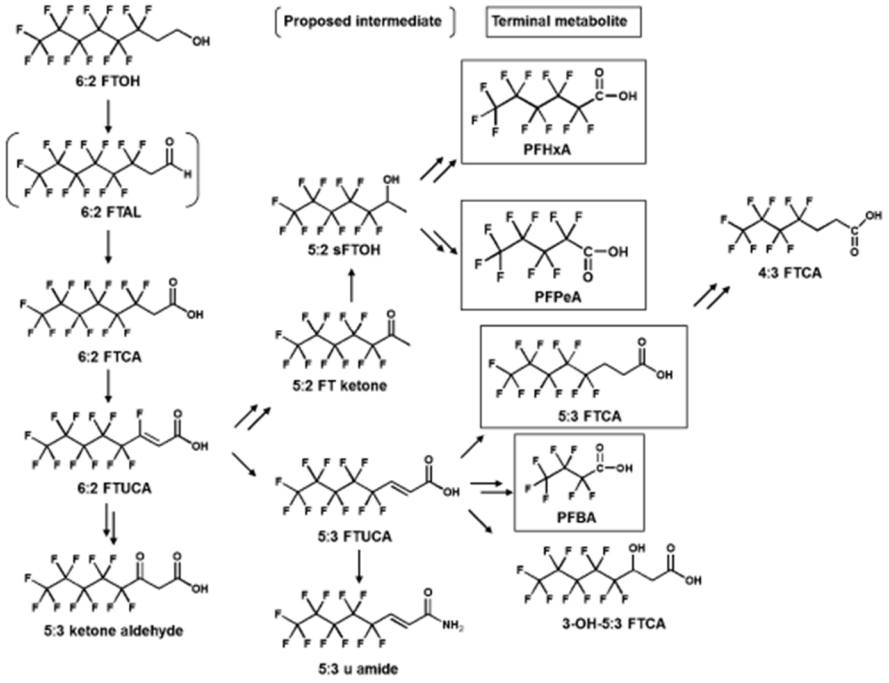


Figure 4. Proposed biotransformation pathways of 4:2, 6:2, 8:2 FTTAoS (Figure 4 from Harding-Marjanovic et al. 2015)

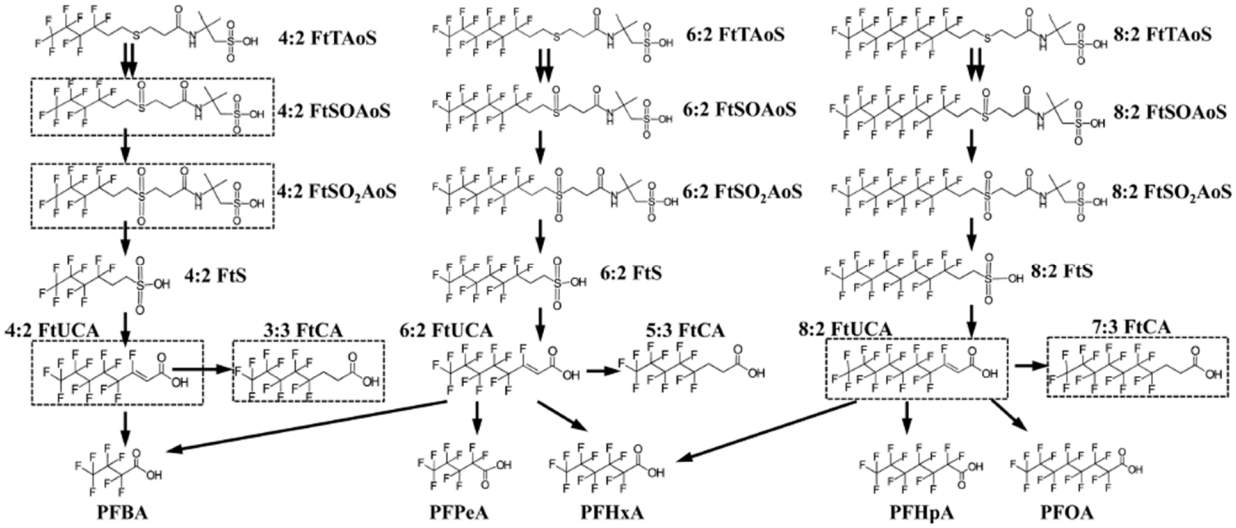
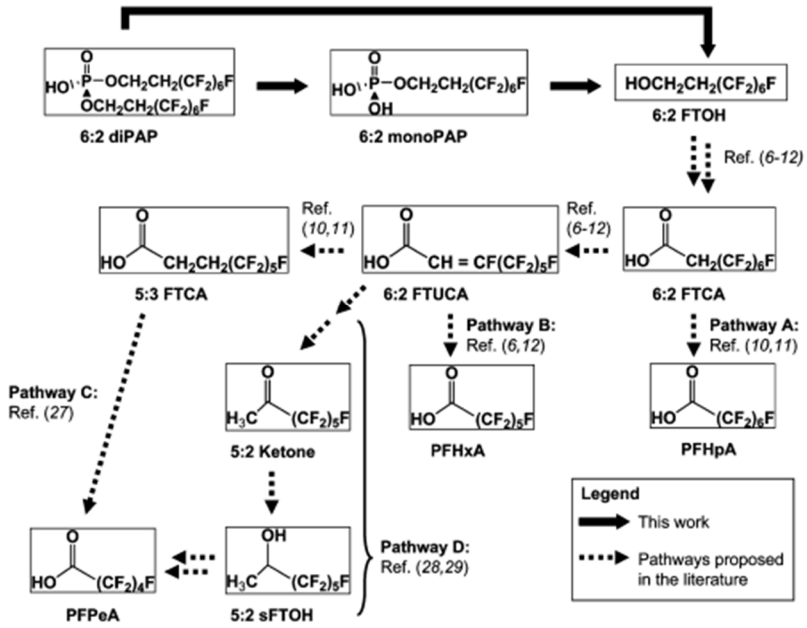


Figure 5. Proposed degradation pathway of 6:2 diPAP and 6:2 monoPAP (Figure 1 from Lee et al. 2010)



APPENDIX B

	PubMed / PubMed Central	Google Scholar
4:2 FTTAoS	(("fluorotelomer"[All Fields] OR "fluorotelomers"[All Fields]) AND ("sulfides"[MeSH Terms] OR "sulfides"[All Fields] OR "thioether"[All Fields] OR "thioethers"[All Fields]) AND "amido"[All Fields] AND "sulfonate*"[All Fields]) OR ("fluorotelomer thioether amido sulfonate"[Supplementary Concept] OR "fluorotelomer thioether amido sulfonate"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "4 2 fluorotelomer sulfonate"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "4 2 fts"[All Fields])	4:2 Fluorotelomer thioether amido sulfonate* or 4:2 FTTAoS OR 4:2 fluorotelomer sulfonate OR 4:2 FTS
6:2 FTTAoS	(("fluorotelomer"[All Fields] OR "fluorotelomers"[All Fields]) AND ("sulfides"[MeSH Terms] OR "sulfides"[All Fields] OR "thioether"[All Fields] OR "thioethers"[All Fields]) AND "amido"[All Fields] AND "sulfonate*"[All Fields]) OR ("fluorotelomer thioether amido sulfonate"[Supplementary Concept] OR "fluorotelomer thioether amido sulfonate"[All Fields] OR "6 2 fttaos"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "6 2 fluorotelomer sulfonate"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "6 2 fts"[All Fields])	6:2 Fluorotelomer thioether amido sulfonate* or 6:2 FTTAoS OR 6:2 fluorotelomer sulfonate OR 6:2 FTS
8:2 FTTAoS	("8 2"[All Fields] AND ("fluorotelomer"[All Fields] OR "fluorotelomers"[All Fields]) AND ("sulfides"[MeSH Terms] OR "sulfides"[All Fields] OR "thioether"[All Fields] OR "thioethers"[All Fields]) AND "amido"[All Fields] AND "sulfonate*"[All Fields]) OR ("fluorotelomer thioether amido sulfonate"[Supplementary Concept] OR "fluorotelomer thioether amido sulfonate"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "8 2 fluorotelomer sulfonate"[All Fields]) OR ("fluorotelomer sulfonic acids"[Supplementary Concept] OR "fluorotelomer sulfonic acids"[All Fields] OR "8 2 fts"[All Fields])	8:2 Fluorotelomer thioether amido sulfonate* OR 8:2 FTTAoS OR 8:2 fluorotelomer sulfonate OR 8:2 FTS
6:2 fluorotelomer alcohols (6:2 FTOH) **8:2 fluorotelomer alcohols (8:2 FTOH)**	("6 2"[All Fields] AND ("fluorotelomer alcohols"[Supplementary Concept] OR "fluorotelomer alcohols"[All Fields])) OR ("fluorotelomer alcohols"[Supplementary Concept] OR "fluorotelomer alcohols"[All Fields] OR "6 2 ftoh"[All Fields]) OR ("8 2"[All Fields] AND ("fluorotelomer alcohols"[Supplementary Concept] OR "fluorotelomer alcohols"[All Fields])) OR ("fluorotelomer alcohols"[Supplementary Concept] OR "fluorotelomer alcohols"[All Fields] OR "8 2 ftoh"[All Fields]) OR	6:2 fluorotelomer alcohols OR 6:2 FTOH OR 8:2 fluorotelomer alcohols OR 8:2 FTOH OR 5:3 fluorotelomer carboxylic acid OR 5:3 FTCA
5:3 fluorotelomer carboxylic acid (5:3 FTCA)	("5 3"[All Fields] AND ("fluorotelomer"[All Fields] OR "fluorotelomers"[All Fields]) AND ("carboxylic acids"[MeSH Terms] OR ("carboxylic"[All Fields] AND "acids"[All Fields]) OR "carboxylic acids"[All Fields] OR ("carboxylic"[All Fields] AND "acid"[All Fields]) OR "carboxylic acid"[All Fields])) OR ("5 3"[All Fields] AND "FTCA"[All Fields])	
6:2 diPAP	"fluorotelomer phosphate diesters"[Supplementary Concept] OR "fluorotelomer phosphate diesters"[All Fields] OR "6 2 dipap"[All Fields]	6:2 diPAP
Epi and Tox Terms	epidemiologic methods [MeSH Terms] OR epidemiologic studies[MeSH Terms] OR incidence[MeSH Terms] OR epidemiology[MeSH Subheading] OR epidemiolog* OR cross-sectional OR case control OR cohort OR longitudinal OR retrospective OR prospective OR occupational stud* OR environmental stud* OR community stud* OR health survey* OR toxicity OR toxicolog* OR in vivo	
Exclusion Terms (Title)	plant*	

Appendix C: Toxicologic Studies

4:2 FTTAoS Toxicologic Studies

4:2 FTTAoS Toxi

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results	Applicable Guideline (*it is either OECD #### or TG-##)
Rericha et al. 2022	Embryonic zebrafish (Danio rerio)		Adjusted exposure concentrations calculated from analytically measured stock concentrations of 4:2 FTS were 0.9, 2.2, 5.5, 14, 30, 64, 85 µM		Developmental toxicity assessed 24 and 120 hours postfertilization (hpf)	4:2 FTS exposures did not induce morphological effects	

Epidemiologic Studies

Epidemiologic S

Author and Year	Type of Study	Study location	Study population	PFAS studied	Exposure Assessment	PFAS Concentration	Health Outcome(s)
Tian et al. 2023	Case-control	Hangzhou, China	n=82 pregnant women with preeclampsia (cases), and n=169 healthy pregnant women (controls)	4:2 FTS	Maternal serum 1-2 days before delivery	see Figure 1. Box plot	Preeclempsia and low birth weight

4:2 FTTAoS cologic Studies

Author and Year	Notes
Rericha et al. 2022	

Epidemiolog tudies

Author and Year	Health outcome assessment	General Findings	Adjusted for	Notes
Tian et al. 2023	Preeclampsia was defined as the onset of hypertension (systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg after 20 gestational weeks accompanied by proteinuria and/or maternal organ dysfunction and/or uteroplacental dysfunction. The birth weight (kg), birth length (cm), and fetal gender were extracted from the birth records. Low birth weight (LBW) was defined as a birth weight <2500 g. Small for gestational age (SGA) was defined as weight below the 10th percentile	Higher maternal concentrations of 4:2 FTS was inversely associated with preeclempsia in adjusted models: OR=0.282 (95% CI 0.09, 0.86). In mutivaraible regression, higher concentrations of 4:2 FTS was associated with higher birth weight: β=216.9 (95% CI 14.1, 419.8). In Cox proportional hazard regression, higher 4:2 FTS was inversely associated with risk of low birth weight (LBW), but the results were not significant: HR=0.308 (95% CI 0.096, 1.069).	Maternal age, pre-pregnancy BMI, education, occupation, smoking, alcohol drinking, ethnicity, menstrual cycle, menarche age, and parity	Exposure obtained obtained only a few days prior to delivery.

6:2 FTTAoS Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
Abercrombie et al. 2021	Juvenile American toads, eastern tiger salamanders, and northern leopard frogs	52 experimental units, with 12 animals in each unit. 13 treatments were replicated 4 times.	0, 80, 800, or 8000 ppb on a moss dry weight basis	30 days	Sublethal effects (survival and growth)	Anurans expososed to 6:2 FTS demonstrated reduced snout-vent length (SVL) compared to controls, whereas salamanders exposed to 6:2 FTS showed increased SVL. Survival was not affected
Fey et al. 2022	Zebrafish embryo (Danio rerio) 2 hours post-fertilization (HPF)	6 dosing groups (5 treatment groups and 1 control group)	6:2 treatment solutions were added at approximately 26, 50, 74, 98 hpf at doses 0.0544, 1.71, 14.8, and 36.3 µM	5 days	Developmental toxicity	No significant results for mortality, hatching, and developmental endpoints, including swim bladder inflation, yolk sac area, and larval body length
Flynn et al. 2021	Post-metamorphic tiger salamanders (Ambystoma tigrinum)	4 dosing groups (3 treatment groups and 1 control group)	6:2 FTS was incorporated into common house crickets, at three doses: low (<1.0), medium (2–5), or high (16–62) ng PFAS/g/d (wet weight).	30 days	Growth, body condition, and hepatosomatic index	Body condition tended to be 3 to 8% higher in the 6:2-FTS treatments compared to the control, but none of the results were statistically significant
Flynn et al. 2022	Larval northern leopard frogs, American toads, and eastern tiger salamanders	4 dosing groups (3 treatment groups and 1 control group) for each species	6:2 FTS measured mean concentrations in water: 10, 100, or 1000 µg/L for	30 days	Mortality, morphological (snout-vent length [SVL], mass, and body condition) and developmental (Gosner stage and time-to- metamorphosis)	Salamander survival was reduced in the 6:2 FTS group compared to the controls. Toad SVL was affected by 6:2 FTS in being 5% larger, and SVL of salamanders was also increased. Toad growth was also increased by 6:2 FTS. Body condition was reduced in frogs and salamanders greatest with 6:2.
Bohannon et al. 2023	Adult white-footed mice (n=114 males, n=121 females)	5 dosing groups (control [n=25 mating pairs] and 4 treatment groups [n=23 mating pairs in each group])	6:2 FTS was administered daily via oral gavage at 10 ml/kg body weight for total dosing exposures of 0.2, 1, 5, and 25 mg/kg-day; first filial generation was exposed in utero and via lactation	112 days (4 weeks premating, plus at least 4 weeks mating exposure)	Pregnancy and fertility indices, litter production, total litter size, live litter size, stillbirths, litter loss, average pup weight, and pinna unfolding, sex steroid and thyroid hormone serum levels, body weight, histopathology, and immune function	Reproductive endpoints were not significantly altered in response to 6:2 FTS. Immune function determined via a plaque-forming cell (PFC) assay was decreased in both male and female mice in the 2 highest dose groups.
Narizzano et al. 2021	Adult male and female white-footed mice (P. leucopus)	4 dosing groups (3 treatment groups and 1 control group) 9/sex/dose group	6:2 FTS solutions of 0.25, 1.0, and 2.0 mg/mL were prepared, corresponding to 2.5, 10, and 20 mg/kg-d, administered daily via oral gavage	28 days	Body weight, serum concentrations, detailed gross necropsy, sperm analysis	All outcomes were unaffected by exposure to 6:2 FTS

6:2 FTTAoS	6:2 FTTAoS Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
Abercrombie et al. 2021		A NOAEL could not be generated; a LOAEL concentration was determined to be between 50 and 120 ppb
Fey et al. 2022		Results from dose-response analysis indicate that the assumption of additivity using conventional points of departure (e.g., NOAEL, LOAEL) is not supported for critical effect endpoints with these PFAS mixtures, and that the interactions vary as a function of the dose range.
Flynn et al. 2021		
Flynn et al. 2022		Toxic effects were highly species-dependent, with species having prolonged larval development (frogs and salamanders) being more sensitive to PFAS than more rapidly developing species (toads).
Bohannon et al. 2023		A low benchmark dose was calculated based on PFCs as the critical effect and was found to be 2.63 and 2.26 mg/kg-day 6:2 FTS in male and female mice, respectively.
Narizzano et al. 2021		The quantified concentrations of 4:2 and 8:2 FTS were 3 times greater than that of 6:2 FTS in the dosing solution, indicating impurities of the 6:2 FTS powder.

6:2 FTTAoS Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results

Epidemiologic Studies

Author and Year	Type of Study	Study location	Study population	PFAS studied	Exposure Assessment	PFAS Concentration
Tian et al. 2023	Case control	Hangzhou, China	n=82 pregnant women with preeclampsia (cases), and n=169 healthy pregnant women (controls)	6:2 FTS	Maternal serum 1-2 days before delivery	see Figure 1. Box plot

6:2 FTTAoS	6:2 FTTAoS Toxicologic Studies	
Author and Year	Applicable Guideline (*it is either OECD #### or TG-##)	Notes

Epidemiolog	Epidemiologic Studies				
Author and Year	Health Outcome(s)	Health outcome assessment	General Findings	Adjusted for	Notes
Tian et al. 2023	Preeclampsia and low birth weight	Preeclampsia was defined as the onset of hypertension (systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg after 20 gestational weeks accompanied by proteinuria and/or maternal organ dysfunction and/or uteroplacental dysfunction. The birth weight (kg), birth length (cm), and fetal gender were extracted from the birth records. Low birth weight (LBW) was defined as a birth weight <2500 g. Small for gestational age (SGA) was defined as weight below the 10th percentile of the gestational age and gender.	No significant association was found between maternal 6:2 FTS levels and preeclampsia or low birth weight in neonates	Maternal age, pre-pregnancy BMI, education, occupation, smoking, alcohol drinking, ethnicity, menstrual cycle, menarche age, and parity	Exposure obtained at later time than preeclempsia - reverse causality

8:2 FTTAoS Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated
No studies were identified for 8:2 FTTAoS					

8:2 FTTAoS	8:2 FTTAoS Toxicologic Studies		
Author and Year	**Results**	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
No studies were identified for 8:2 FTTAoS			

6:2 diPAP Toxicologic Studies

6:2 diPAP Toxic

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results	Applicable Guideline (*it is either OECD #### or TG-##)
Chen et al. 2020	Zebrafish embryos	4 dosing groups (1 solvent control, 3 treatment	6:2 diPAP at concentrations 0.5, 5, and 50 ng/L in 96-well plates	6 hours post fertilization (hpf to 7 day post	Thyroid toxicity	Exposure to 6:2 diPAP was associated with decreases in five thyroid hormones	

Epidemiologic Studies

Epidemiologic S

Author and Year	Type of Study	Study location	Study population	PFAS studied	Exposure Assessment	PFAS Concentration	Health Outcome(s)
Luo et al. 2022a	ɔspective cohort stu	China	936 couples	Total of 25 PFAS were measured, including 6:2 diPAP	Blood plasma at preconception	N/A	Couple fecundity
Luo et al. 2022b	cross-sectional	China	902 men	6:2 diPAP and 8:2 diPAP	Blood plasma at preconception	N/A	Semen quality

6:2 diPAP Tologic Studies

Author and Year	Notes
Chen et al. 2020	

Epidemiologtudies

Author and Year	Health outcome assessment	General Findings	Adjusted for	Notes
Luo et al. 2022a	Couple fecundity was measured by time to pregnancy (TTP) and infertility (probability of getting pregnant within a specific menstrual cycle)	Higher levels of 6:2 diPAP at preconception in women was signifcantly associated with reduced fecundity. A one unit increase in female 6:2 diPAP was associated with a 15% lower odds of couple fecundity and	Age, BMI, education status, smoking, drinking, annual household income	Study only included couples who conceived within a year
Luo et al. 2022b	Semen samples were collected preconception at the same time with blood plasma	No association was found between exposure to 6:2 or 8:2 diPAP and semen quality	Age and BMI	

6:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
Annunziato et al. 2019	Zebrafish, strain AB		Embryos were exposed to 0.02 to 20 µM concentrations of 6:2 FTOH	~3 hours post fertilization until 120 hours post fertilization (hpf), and exposed larvae were observed until 14 days post fertilization (dpf)	Lethal dose, morphometric analysis, behavioral analysis	LC50 at 5 days post fertilization was 830 µM. Exposure to 6:2 FTOH caused no morphometric effects at 120 hpf. However, gene expression of both *tgfb1a* and *bdnf* were increased by greater than 2 fold change at this time point. Effects also persisted to 14 dpf where a significant increase in distance traveled and velocity were observed in the behavioral assay
Ishibashi et al. 2008	Male Japanese medaka fish (Oryzias latipes)	6 adult males for analysis of hepatic vitellogenin (VTG) protein; 3 adult males for analyis of estrogen-responsive genes in the liver	Exposed to 6:2 FTOH nominal concentrations of 0.01, 0.1, 1, and 10 µM for VTG protein study; exposed to 6:2 FTOH nominal concentrations of 10 and 100 µM for expression analysis of estrogen-responsive genes in the liver	3 days for the VTG protein experiment and 8 hours for the estrogen-responsove genes	Estrogen receptor activation	Expression analysis of hepatic VTG protein showed estrogenic potential with 6:2 FTOH, indicative of the induction of VTG synthesis in the livers of male medaka.
Kirkpatrick 2005	10-week old Crl:CD(SD) rats, 12-week old at mating	15/sex group (control & high-dose), 10/sex/group in the low and mid-dose. 10/sex/group in control and high-dose sacrificed at the end of treatment.	6:2 FTOH was administered in corn oil at concentrations of 0, 25, 75, or 225 mg/ kg bw/day	Males: 14 daily doses prior to mating, dosed throughout mating period, until day prior to euthanasia; total = 32–34 doses. Females: 14 daily doses prior to pairing; dosed through lactation day (LD) 3; total = 39–44 doses; euthanized on LD 4. Females with no evidence of mating or that failed to deliver dosed for total of 39–52 doses.	Systemic, reproductive and developmental toxicity	**Female:** Mortality (225, 11 rats) ↑liver weights (75); ↑total protein (225); ↑ALT (225);↑bilirubin (225) ↑BUN, creatinine, K (225); ↑wts (75); proximal tubule degeneration/dilation (225); collecting tubule vacuolation (225); RT mineralization (225); ↓Na & Cl (225) ↑globulin (225); bone marrow hyperplasia & erythroid depletion (225, moribund); lymphoid tissue necrosis &/or depletion (225, moribund) **Male:** Mortality (225, 1 rat); ↓BW (225) ↑liver weights (25), HC hypertrophy (225); ↑AST (225); ↑bilirubin (225) ↑kidney weights (75); proximal tubule degeneration/dilation (225); RT mineralization (225); chronic progressive nephropathy (225) ↓thyroid weights (75) bone marrow hyperplasia & erythroid depletion (225, moribund); lymphoid tissue necrosis &/or depletion (225, moribund); ↓thymus wts (225) **Reproductive and neonatal:** NOAEL 75 mg/kg/day for reproductive and neonatal toxicity (mean number of pups born, postnatal survival, and pup weight)

6:2 FTOH To	6:2 FTOH Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
Annunziato et al. 2019	Modified OECD 212	
Ishibashi et al. 2008		
Kirkpatrick 2005	OECD 422	NOAEL for systemic toxicity is 25 mg/kg/day (body weight and body gain); NOAEL 75 mg/kg/day for reproductive and neonatal toxicity (mean number of pups born, postnatal survival, and pup weight)

6:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
Miyata 2007	5-week old Crl:CD (SD) (SPF) rats	5/sex/group	6:2 FTOH was administered by oral gavage at concentrations of 0, 5, 25, or 125 mg/kg/day	28 days	Systemic toxicity	**Female:** Liver: increased weight (25mg/kg/day), increased ALT, GGT, HC hypertrophy, each at 125 mg/kg/day Kidney: increased weight at 25 mg/kg/day ↑Mottled teeth (125); discolored incisors (25); ↓ameloblast pigment (125); irregular ameloblast alignment (125); glandular stomach submucosa edema (125) **Male:** Liver: ↑GGT (125); ↑wights (25); Hepatocellular hypertrophy (125), ↑ALT (125) Kidney: weight (25) ↑Mottled teeth (25); discolored incisors (25); ↓ ameloblast pigment (125); irregular ameloblast alignment (125); glandular stomach submucosa edema (125); necrosis of fundic mucosa (125)
Mukerji et al. 2015	Male and female Crl:CD1 mice. Males were approximately 50 days old at study start. Females (nulliparous and not siblings of the males) were approximately 75 days old at study start.	15 mice/sex/group	6:2 FTOH was administered by intragastric intubation at dosage levels of 0, 1, 5, 25, and 100 mg/kg body weight/day (mg/kg/day).	Parental (P1) male mice were dosed for approximately 70 days prior to mating, in order to encompass the entire spermatogenic cycle; and throughout the cohabitation period (≤2 weeks), up until the day before scheduled euthanasia. P1 female mice were dosed for approximately 14 days prior to mating, and throughout the cohabitation period (≤2 weeks), gestation, and lactation, up until the day before scheduled euthanasia. F1 males and females that were selected for developmental landmarks were dosed from postnatal day (PND) 21 until the day before scheduled euthanasia.	Systemic, reproductive, developmetal toxicity	6:2 FTOH was not a selective reproductive toxicant. The NOAEL for reproductive toxicity was >100 mg/kg/day; no effects on reproductive outcome were observed at any dosage. The NOAEL for viability and growth of the offspring was 25 mg/kg/day, based on clinical signs of delayed maturation in pups, and reductions in pup survival and pup body weight during lactation at 100 mg/kg/day. The overall NOAELs were identical in both sexes, 5 mg/kg/day for systemic toxicity and 25 mg/kg/day for offspring viability/growth. 6:2 FTOH was not a selective reproductive toxicant in either species; no effects on reproductive outcome occurred at any dose level, and any effects observed in offspring occurred at dose levels that induced mortality and severe toxicity in maternal animals.

6:2 FTOH To	6:2 FTOH Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
Miyata 2007	OECD 2008, TG-407	NOAEL 5mg/kg/day based on discolored incisors in males and females, mottled teeth in males, and increased relative liver weight and enlargement of the liver in females
Mukerji et al. 2015	OPPTS 870.3550	

6:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
O'Connor et al. 2014	Male and female Sprague-Dawley (Crl:CD(SD)) rats. Nulliparous, time-mated females were approximately 67 days old at study start.	22 rats/group; for one-generation study: 20 males, 20 females / group	6:2 FTOH was administered by intragastric intubation at dosage levels of 0 (vehicle alone), 5, 25, 125, or 250 mg/kg/day	Pregnant dams were dosed once daily from gestation day (GD) 6 to 20	Developmental and one-generation reproductive toxicity	For the developmental toxicity study, adverse maternal toxicity was observed at 250 mg/kg/day; included reductions in body weight parameters and food consumption. Evidence of developmental toxicity was limited to increases in skeletal variations (ossification delays in the skull and rib alterations) at 250 mg/kg/day. There were no adverse maternal or developmental effects observed at 5, 25, or 125 mg/kg/day and there were no effects on reproductive outcome or quantitative litter data at any dose level. For the one-generation reproduction toxicity study, systemic parental and developmental toxicity were observed at 125 and 250 mg/kg/day. At 250 mg/kg/day, there was increased mortality among male and female parental rats, effects on body weight parameters, food consumption, and clinical signs, and there were effects on offspring survival indices and body weights. At 125 mg/kg/day, there was an increase in mortality in parental males only, and parental toxicity was limited to effects on body weight gain, food consumption (lactation), and clinical signs. Uterine weights were decreased at 125 and 250 mg/kg/day, although there were no corroborative histopathological changes. At 125 mg/kg/day, pup mortality was increased on lactation day 1, and body weights of the offspring were decreased during the second half of lactation. There was no evidence of either parental or developmental toxicity at 5 or 25 mg/kg/day, and there were no effects on reproductive outcome at any dose level.
Serex et al. 2014 (Acute toxicity: oral, dermal)	Crl:CD(SD) rats	5 rats/sex	A single dose of 175, 550, 1750, or 5000 mg/kg was administered orally to fasted female rats, which were observed for up to 14 days. For dermal toxicity, a single dose of 5000 mg/kg was applied as neat material to shaved skin of 5 male and 5 female rats.	Single dose	Acute toxicity - oral, dermal	In the oral up/down study, death occurred in 0/1, 0/3, 2/4, and 2/2 rats dosed at 175, 550, 1750, and 5000mg/kg, respectively. This resulted in an LD50 of 1750 mg/kg. Transient clinical signs of toxicity (wet fur, diarrhea, ear twitch, hair loss, stained fur/skin, lethargy, leaning, reduced respiratory rate, high or prostrate posture, ataxia, hyperreactivity, vocalization, and/or moribund condition) were observed in most rats. In the dermal study, one male and one female rat were found dead the day after application of 6:2 FTOH at 5000 mg/kg. Thus, the acute dermal LD50 is >5000 mg/kg.
Serex et al. 2014 (Dermal irritation and sensitization, and eye irritation)	Young adult New Zealand White rabbits and CBA/JHsd mice		6:2 FTOH was applied as neat material to shaved skin		Dermal irritation and sensitization, and eye irritation	In the dermal irritation study, erythema, but no edema, was observed in one rabbit only at the 60-min evaluation. In the murine LLNA dermal sensitization study, stimulation indices (SIs) of less than 3.0 were observed at all test concentrations up to 100% 6:2 FTOH. Therefore, 6:2 FTOH was not found to be a dermal irritant or dermal sensitizer. Instillation of 0.1 ml 6:2 FTOH produced iritis, conjunctival redness, conjunctival chemosis, and discharge when instilled into the rabbit eye. The treated eyes appeared normal by 24 or 48h after instillation. Fluorescein stain examinations were negative for corneal injury in the treated eyes of the rabbits throughout the study. Thus, 6:2 FTOH is not an eye irritant.

6:2 FTOH To	6:2 FTOH Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
O'Connor et al. 2014	OECD 414	
Serex et al. 2014 (Acute toxicity: oral, dermal)	OECD Section 4 (Part 425): Acute Oral Toxicity – Up-and-Down-Procedure (2001). The dermal toxicity study conformed to OECD Section 4 (Part 402): Acute Dermal Toxicity (1987).	Oral, death - resulting in an LD50 of 1750 mg/kg. Acute dermal LD50 is >5000 mg/kg.
Serex et al. 2014 (Dermal irritation and sensitization, and eye irritation)	The dermal irritation study conformed to OECD Section 4 (Part 404): Acute Dermal Irritation/Corrosion (2002). The dermal sensi- tization study conformed to OECD Section 4 (Part 429): Local Lymph Node Assay (LLNA; 2003) and the eye irritation study conformed to OECD Section 4 (Part 405): Acute Eye Irritation/Corrosion (2002).	

6:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
Serex et al. 2014 (*genotoxicity - included because we describe the toxicological components of this study)	Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA			dose level, up to a maximum of 5000 µg/plate, with any tester strain in either the presence or the absence of S9 metabolic activation in the Bacterial Reverse Mutation (Ames) Test.	Genotoxicity	None of the genotoxicity studies showed any indication of mutagenic or ge
Serex et al. 2014 (90-day subchronic oral tox)	7-to-8-week-old Crl:CD(SD) rats	95 male and 95 female rats were randomly assigned to 5 dosage groups 10 rats/sex in the control and highest exposed groups were assigned to a 1 month recovery phase 5 rats/sex from control and highest exposed groups were assigned to a 3-month recovery phase	6:2 FTOH was administered to rats by oral gavage at doses of 0, 5, 25, 125, 250 mg/kg/day	90-days	Systemic toxicity	Compound-related mortality was seen at 125 mg/kg (1/25 females at day 62) and 250 mg/kg (6/25 males and 13/25 females from days 22 to 84), with the majority of the deaths due to kidney degeneration and necrosis. In male rats, at the end of 91 days of dosing, changes in serum chemistry related to treatment with 125 and 250 mg 6:2 FTOH/kg/day included an increase in total protein, albumin, total bilirubin, inorganic phosphorus and potassium and a reduction in blood urea nitrogen (BUN). Among female rats, treatment-related changes included increases in bilirubin and cholesterol at 125 and 250 mg/kg/day. After 90 days of dosing, statistically significant reductions of up to 10% in red blood cell counts, hemoglobin, and hematocrit levels were observed at 25 mg/kg/day and above (in males) and at 125 and 250 mg/kg/day (in females). However, no effects on hematology were found at 5 mg/kg/day in males or at 5 and 25 mg/kg/day in females. Following 90 days of dosing, effects on organ weights were present in the testes, liver and kidney of males and in livers and kidneys of females
Shi et al. 2017	Adult wild-type zebrafish (Tuebingen strain), transgenic line Tg (gata1:DsRed)		The 6:2 fluorotelomer carboxylic acid (6:2 FTCA) and was dissolved in 100% dimethyl sulfoxide (DMSO) at 4 concentrations of 0, 4, 8, and 12 mg/L	6 to 120 h post-fertilization (hpf)	Developmental toxicity	6:2 FTCA exposure decreased the hatching and survival percentages, reduced the heart rate, and increased the malformation of zebrafish embryos. The median lethal concentration of 6:2 FTCA was 7.33 mg/L at 120 hpf. The most common developmental malformation was pericardial edema, which appeared in the 8 and 12 mg/L 6:2 FTCA-exposed embryos from 60 hpf.
Xia et al. 2023	9-week old female (n = 24) BALB/c mice	6 female mice/group	0, 5, 25, or 125 mg/kg b.wt./day dose of 6:2 FTOH (dissolved in corn oil) administered daily by oral gavage to female mice	Gestational day 12.5 to gestational day 21.5.	Developmental toxicity	Embryonic exposure to 6:2 FTOH resulted in the following in mice offspring: disrupted testicular structure, low expression of tight junction protein between Sertoli cells (SCs), impaired blood-testis barrier (BTB) formation and maturation, reduced sperm viability and increased malformation, and induced testicular inflammation.

Tox Studies from Rice et al. 2020

6:2 FTOH To	6:2 FTOH Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**
Serex et al. 2014 (*genotoxicity - included because we describe the toxicological components of this study)	OECD Section 4 (Part 471)	
Serex et al. 2014 (90-day subchronic oral tox)	Effects Test Guidelines OPPTS 870.3100 (1998).	NOAEL in the subchronic study was 5 mg/kg/day based on hematology and liver effects. Benchmark Dose Analysis was performed on the most sensitive endpoints from the 90-day oral gavage study and these levels were all above the study NOAEL of 5 mg/kg/day. For risk assessment purposes, the recommended point of departure is the more conservative study NOAEL of 5 mg/kg/day.
Shi et al. 2017		Taken together, 6:2 FTCA exposure decreased the erythrocyte number and disrupted erythroid differentiation during zebrafish embryonic development. Our results suggest that 6:2 FTCA can cause developmental toxicity in zebrafish embryos, and that FTCAs exhibit greater toxicity than that of PFCAs.
Xia et al. 2023		

Tox Studies	Tox Studies from Rice et al. 2020

6:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results
DuPont 2012	6-week old Crl:CD (SD) rats	5/sex/group	5:3 acid was administered daily by gavage at concentrations: 0, 30, 300, or 900 mg/kg/d	14 days	Systemic toxicity	**Female:** Mortality (300 mg/kg, 2 rats; 900 mg/kg, 5 rats); ↓Body weight (300 mg/kg)↓ thymus weightt (300 mg/kg) ↓spleen weight (300 mg/kg) ↑Thyroid weights (300 mg/kg); Follicular cell hypertrophy (300 mg/kg) Renal tubule vacuolation (300 mg/kg); ↓serum Na (300 mg/kg) ↑Serum triglyceride (300mg/kg); ↑liver weight (300 mg/kg); hepatocellular hypertrophy (300 mg/kg); ↑β-oxidation (30 mg/kg); ↑bilirubin (30 mg/kg); ↑Alkaline phosphatase (300 mg/kg) **Male:** Mortality (300 mg/kg, 5 rats; 900 mg/kg, 5 rats); ↓body weight (300 mg/kg); erosions of glandular and nonglandular stomach (300 mg/kg) ↓thymus weight (30 mg/kg) Follicular cell hypertrophy (30 mg/kg) ↑creatinine (30 mg/kg); ↑Cl (30 mg/kg). ↑urine volume (30 mg/kg), ↓urine total protein (30 mg/kg) ↓Total cholesterol (30 mg/kg); hepatocellular hypertrophy (300 mg/kg); hepatocellular necrosis (300 mg/kg); ↑β-oxidation (30 mg/kg)

6:2 FTOH To	6:2 FTOH Toxicologic Studies	
Author and Year	**Applicable Guideline (*it is either OECD #### or TG-##)**	**Notes**

DuPont 2012

8:2 FTOH Toxicologic Studies

8:2 FTOH Toxicologic Stu

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results	Applicable Guideline (*it is either OECD #### or TG-##)
Henderson and Smith 2007	Timed-pregnant CD-1 mice	**Gestational experimnet** For the in utero exposure study, mice (n =41) were divided into two groups, control (n=15) and treated (n=26) **Cross-fostering experiment T**imed- pregnant mice were divided into control (n = 34) and treated groups (n =36)	Mice received a gavage dose of 30 mg 8-2 FTOH/kg BW in a propylene glycol/water (1:1) vehicle or vehicle control on gestational day (GD) 8.	Single dose on GD 8 Immediately after birth, pups were cross-fostered with dams that had been treated during gestation with 8-2 FTOH (T) or vehicle (C) resulting in four treatment groups in which the first letter represents in utero (fetal) exposure and the second represents lactational (neonatal) exposure: C/C, T/C, C/T, T/T.	Maternal and neonatal serum and liver outcomes, and gestational outcomes	No apparent treatment-related effects on maternal body weight (BW) gain, 8-2 FTOH treatment did result in significant increases in the maternal liver weight (LW) to BW ratio (relative LW; LW:BW) and transient increase in absolute LW at GD18. Maternal LW:BW was significantly elevated in the 8-2 FTOH treated groups at GD13 through GD18. It was found that this effect continued throughout the postnatal period (PND1-PND15; p< 0.05). Average litter size in the gestational study and the cross-foster study were not significantly different between control and treated groups. In the cross-fostering experiment, 31% of treated dams had at least one nonviable neonate and 27% of the nonviable neonates had anencephaly or exencephaly.	
Himmelstein et al. 2012	7-8 weeks old Sprague-Dawley Crl:CD(SD) rats	5 rats/sex/group	Nose only inhalation to 8:2 FTOH at concentrations of 3 or 30 mg/m^3 (0.16 or 1.6 ppm, as vapor). For the 3.0 mg/m^3 exposure, 8:2 FTOH vapors were delivered to the nose-only system using a 40 L Tedlar® bag placed in a heated (73°C) containment box that was under pressure to drive the vapor contents in the bag toward the exposure system. For the 30 mg/m^3 exposure, 8:2 FTOH vapors were delivered to the nose-only system by utilizing a heated (30°C) 500-mL glass vaporization bulb that contained the solid 8:2 FTOH.	Nose only inhalation to 8:2 FTOH for 6 hours on one day at concentrations of 3 or 30 mg/m^3 (0.16 or 1.6 ppm, as vapor).	Comparison of plasma metabolites after 8:2 FTOH inhalation to oral data at 1, 3 and 6 h during exposure and 6 and 18 h post exposure	A BMDL 10% (3.7 mg/kg/day) was derived for mild hepatic necrosis observed in male rats following a 90-day oral dose study with 8:2 FTOH. The corresponding human equivalent air concentrations (HECs) were 1.8 and 3.7 mg/m3 , which provided margin of exposure (MOE) values ranging from 1.8 × 10^4 to 6.1 × 10^6 based on reported ambient air concentrations of 0.3–209 ng/m^3.	
Ladics et al. 2008	Crl:CD®(SD)IGS BR rats, approximatly 50 days of age at the beginning of the study	Each group of study rats consisted of 10 rats/sex/dose designated for an evaluation of subchronic toxicity; five rats/sex/dose designated as satellite bleed animals; and five rats/sex/dose designated for hepatic biochemical analysis following a 10-day exposure to the test substance (8:2 FTOH). In addition, control and high-dose groups contained 10 rats/sex for the three-month recovery period.	The test substance was administered daily to the rats by oral gavage to achieve dosage levels of 1, 5, 25, or 125 mg/kg body weight/day. Similarly, control rats were treated with 0.5% aqueous methyl-cellulose vehicle at the same dose volume as used in the respective high-dosage group. Dosing suspensions were prepared two times/week to day 48, then daily through the remainder of the dosing period.	90-day oral gavage	Complete toxicological profile, including neurobehavioral assessments and hepatic β-oxidation	No test-substance-related mortality occurred at any 8:2 FTOH dose levels. Rats at 125 mg/kg developed striated teeth; these animals were switched to ground chow at 77 days. No treatment-related alterations in body weight, food consumption, neurobehavioral parameters, or hematology/clinical chemistry were observed. Hepatic β-oxidation was increased in males at 125 mg/kg and in females at 25 and 125 mg/kg. In both males and females, plasma fluorine levels were increased at 125 mg/kg and urinary fluorine was elevated at ≥5 mg/kg. Degeneration/disorganization of enamel organ ameloblast cells was found at 125 mg/kg in males, but not among female rats. Liver weight increases with focal hepatic necrosis were observed at both 25 and 125 mg/kg, and chronic progressive nephrotoxicity occurred in female rats at 125 mg/kg. Except for hepatocellular necrosis in males at 125 mg/kg and the increased incidence and severity of chronic progressive nephropathy in females at 125 mg/kg, all other changes showed evidence of reversibility.	(OECD) test guideline 408

8:2 FTOH Tœdies

Author and Year	Notes
Henderson and Smith 2007	
Himmelstein et al. 2012	The exposure concentrations were targeted to give inhaled doses (in units of mg/kg bw) that were in the lower range of those used in a 90-day oral gavage study
Ladics et al. 2008	NOAEL was 5 mg/kg.

8:2 FTOH Toxicologic Studies

Author and Year	Species, sex, strain, age	N/group	Test article, doses, vehicle	Dosing regimen	Endpoint evaluated	Results	Applicable Guideline (*it is either OECD #### or TG-##)
Liu et al. 2010	4-month old zebrafish		Zebrafish were exposed to to 8:2 FTOH concentration at levels of 0, 10, 30, 90 and 270 µg/L in semi-static systems.	4 weeks	Reproductive toxicity: cumulative egg production, egg diameter, eggshell thickness, egg protein content, sperm density, sperm production, gonadal histology, and plasma T and E2 levels of adult zebrafish	Female fish exposed to 90 and 270 µg/L 8:2 FTOH caused a significant decrease in cumulative egg production of 41.4% and 46.0%; eggshell thickness was reduced by 14.3% and 19.0%; and total protein content of egg was decreased by 8.9% and 18.7%, respectively. Compared to the control group, egg diameter was also significantly decreased by 7.4%, 10.0% and 12.1% after 30, 90 and 270 µg/L 8:2 FTOH exposure, respectively. Exposure of female fish to 90 and 270 µg/L 8:2 FTOH significantly increased the gonadosomatic index (GSI) by 106.7% and 122.0% compared to the control group. In the female fish, exposure to 90 and 270 µg/L 8:2 FTOH significantly increased the plasma testosterone level by 25.0% and 39.1%, respectively. 8:2 FTOH exposure significantly increased the plasma E2 level by 10.9%, 13.1% and 11.3% in 30, 90 and 270 µg/L treatment groups, respectively. In the male fish, the plasma testosterone level was significantly decreased by 14.7%, 12.5% and 23.7% after	
Wang et al. 2019	6-week old male C57BL/6 mice	N/A	8:2 FTOH was delivered by oral gavage at concentrations of 0, 10, 30, and 100 mg/kg/d	28-day	Immunotoxicity	Exposure to 8:2 FTOH caused liver injuries such as cell swelling, vacuolation, karyopyknosis, nuclear swelling, and immune cell infiltration, as well as the down-regulation of selected cytokines in the spleen and thymus. The authors posit	
Mylchreest et al. 2005	time-mated female Crl:CD (SD)IGS BR rats	22 female rats/ groups	8:2 Telomer B Alcohol was delivered daily by oral gavage at doses of either 0, 50, 200, or 500 mg/kg from day	Gestational day 6 to gestational day 20	Developmental toxicity	Adverse maternal toxicity (maternal mortality, decreased body weights and body weight gains, and increased clinical observations of toxicity) was found at 500 mg/kg/day. Developmental toxicity (increased fetal skeletal variations [delayed pelvic bone ossification and wavy ribs]) was observed at 500 mg/kg/day. At 200 and 500 mg/kg/day, there were transient reductions in	
Ishibashi et al. 2008	Male japanese medaka fish (Oryzias latipes)	6 adult male for hepatic VTH protein 3 adult male for analyis if estrogen-responsive genes in the liver	Exposed to 6:2 FTOH nominal concentrations of 0.01, 0.1, 1, and 10 µM for VTG protein study; exposed to 6:2 FTOH nominal concentrations of 10 and 100 µM for expression analysis of estrogen-responsive genes in the liver	3 days for the VTG protein experiment and 8 hours for the estrogen-responsove genes	Estrogen receptor activation	Expression analysis of hepatic vitellogenin (VTG) protein showed estrogenic potentials with 8:2 FTOH which is indicative of the induction of VTG synthesis in the livers of male medaka fish.	

Epidemiologic Studies

Author and Year	Type of Study	Study location	Study population	PFAS studied	Exposure Assessment	PFAS Concentration	Health Outcome(s)
Jin et al. 2020	Prospective cohort (established between June	Hangzhou, China	174 monther-infant pairs, infants were exclusivley breastfeed	16 PFAS including 8:2 FTOH	Concentration in breast milk, collected from mothers in the hospital within 1 week of delivery	8:2 FTOH were detected in half of breast milk samples, with the mean concentration of 9.0 pg/mL	Postnatal growth

8:2 FTOH Tœdies

Author and Year	Notes
Liu et al. 2010	
Wang et al. 2019	
Mylchreest et al. 2005	The no-observed-adverse- effect level (NOAEL), defined as the highest dose at which adverse effects attributable to the test substance were not detected, for both maternal and developmental toxicity, is considered to be 200 mg/kg/day
Ishibashi et al. 2008	

Epidemiolog

Author and Year	Health outcome assessment	General Findings	Adjusted for	Notes
Jin et al. 2020	Infants underwent the follow-up investigation (mean 5.2 months after their birth) to obtain postnatal growth data	Infant weight gain rate (IWGR) was negatively associated with breast milk concentration of 8:2 FTOH	No adjustment	Only Spearman correlation was used to examine the relationship between 8:2 FTOH exposure and postnatal growth