MICHIGAN DEPARTMENT OF ENVIRONMENT, GREAT LAKES, AND ENERGY

INTEROFFICE COMMUNICATION

September 24, 2020

TO: File for 6:2 Fluorotelomer Sulfonic Acid (CAS No. 27619-97-2)

FROM: Michael Depa, Toxics Unit, Air Quality Division

SUBJECT: Screening Level Evaluation

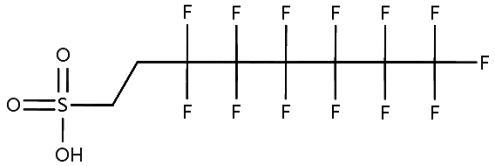
The initial threshold screening level (ITSL) for 6:2 fluorotelomer sulfonic acid (or 6:2 FTS) is 1 µg/m³, with annual averaging time.

The literature was searched to find relevant data to assess the toxicity of 6:2 FTS and its salts. The following references or databases were searched: U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS), Registry for Toxic Effects of Chemical Substances (RTECS), American Conference of Governmental and Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs), National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Levels (RELs), International Agency for Research on Cancer (IARC) Monographs, Chemical Abstract Service (CAS) SciFinder, European Chemical Agency (ECHA), and the U.S. National Toxicology Program (NTP).

The EPA has not established a reference dose (RfD) or a reference concentration (RfC) for 6:2 FTS. There are no occupational exposure limits for 6:2 FTS.

The molecular weight of 6:2 FTS is 428.16 g/mol, and the molecular formula is $C_8H_5F_{13}O_3S$. The molecular structure is shown in Figure 1. According to the International Union of Pure and Applied Chemistry, the name for this compound is called 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-sulfonic acid.

Figure 1. Molecular Structure of 6:2 FTS



Physical Data
Color/ Physical State: Light brown solid at 20°C and 760 mmHg (ECHA, 2020)
Water solubility: 658 g/L at 20°C (Danish EPA, 2015; ECHA, 2020) (This water solubility value is characterized as “moderately water soluble” by EPA, 2019.)
Vapor Pressure: 0.015 mmHg[1] at 20°C (ECHA, 2020; Danish EPA, 2015)

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[1] “moderately volatile,” as defined by EPA. 1985.

Absorption, Metabolism, and Excretion

No specific information was available about the absorption of 6:2 FTS via the respiratory track. ATSDR (2018) stated that:

> Perfluoroalkyls are absorbed following oral, inhalation, and dermal exposure. No quantitative estimates of the fractional absorption of perfluoroalkyls following inhalation or dermal exposure were identified.

6:2 FTS is moderately water soluble and given its surfactant properties indicates that it is likely to be fully absorbed via the inhalation route as are other PFAS (Hintiliter et al., 2006; Himmelstein et al., 2012).

After absorption via the respiratory tract, 6:2 FTS is very likely to accumulate in the blood. The Agency for Toxic Substances and Disease Registry (ATSDR) (2018) states, "there is presently no evidence that perfluoroalkyls undergo metabolism." Based on the structural similarity of 6:2 FTS to perfluorooctane sulfonic acid (PFOS; CAS No. 1763-23-1) it is likely to be slowly excreted, predominantly in urine and to a lesser extent, feces. Regarding absorption, metabolism and excretion of 6:2 FTS, ECHA (2020) reported the following:

> *in vitro* metabolism screening (no guideline followed): Two replicates for test substance and heat-inactivated control, 1 replicate for positive control (4-nonylphenol). The screen of the test substance (35% purity in water) in male rat liver S9 for the amount of parent compound (concentration 2.5 µM) remaining after 2 hour incubation compared to heat inactivated controls indicated, based on the results, no metabolism is anticipated.
>
> *in vivo* toxicokinetics: Groups of three male and female rats (Crl:CD(SD)) were dosed with single gavage dose of 10 mg/kg bw (total dose) or 30 mg/kg bw (total dose) of parent compound (purity: 35.6% wt; vehicle = water). It was reported that there were no control rats. Time from dose to sacrifice was not reported. Fat and liver were analyzed for parent compound to provide an estimate of tissue:plasma ratio.
>
> The tissue:plasma ratio at sacrifice:
> Fat: Females all below LOQ.
> Male at low dose = <0.1, at high dose = 0.1
> Liver: Female plasma values below LOQ so no T:P ratio calculated.
> Male at low dose = 3.0, at high dose = 3.1
>
> *in vivo* toxicokinetics - excretion: 65-68% of the test substance (Purity: 35.6% wt) was recovered in the urine of three male rats (Crl:CD(SD)) 96 hours after a single gavage dose of 73 µM/kg. Half-times for urinary excretion were 20.9 and 23.75 hours via NMR and LC/MS, respectively.

ECHA (2020) reported that the reliability of the absorption, metabolism and excretion data (see above) was "4 (not assignable)." The rationale for the reliability rating was given as, "documentation insufficient for assessment."

The anion of 6:2 FTS (CAS No. 425670-75-3) is likely the toxicologically important molecule. Some of 6:2 FTS's commercially important salts are:

> Sodium Salt (CAS No. 27619-94-9)
> Potassium Salt (CAS No. 59587-38-1)
> Ammonium Salt (CAS No. 59587-39-2)
> Lithium Salt (CAS No. 59587-40-5)
> Barium Salt (CAS No. 1807944-82-6)

Toxicity Data

Genotoxicity (as reported by ECHA, 2020)

- In vitro gene mutation study in bacteria - Organisation [*sic*] for Economic Cooperation and Development (OECD) 471; Ames mutation in *S. typhimurium*. **Negative**.
- In vitro cytogenicity/chromosome aberration study in mammalian cells. OECD 473; Chromosome aberrations in CHO. **Positive** for the induction of structural chromosome aberrations in cells treated 4-hours in the presence and absence of metabolic activation. **Negative** for the induction of structural chromosome aberrations in cells treated 20-hours in the absence of metabolic activation. **Negative** for the induction of numerical chromosome aberrations in cells under all exposure conditions.
- In vivo mammalian somatic cell study. OECD 475; bone marrow chromosome aberrations in mouse. **Negative**.
- In vivo mammalian somatic cell study: OECD 474; bone marrow micronucleus induction in mouse. **Negative**.
- In vivo mammalian somatic cell study: OECD 486, DNA repair (UDS) in liver of rat. **Negative**.
- In vivo mammalian somatic cell study: OECD 489, DNA damage (Comet) in liver and stomach of rat. **Negative**.

Acute Toxicity

An oral gavage LD50 for 6:2 FTS in (female Wistar RccHan®:WIST) rats was determined to be between 300 and 2000 mg/kg body weight (BW) (ECHA, 2020).

Repeated Dose Toxicity

Two reports were identified that contained toxicity data relevant for deriving a screening level. A published 28-day continuous oral dosing study in mice was available for review for 6:2 FTS ammonium salt (Sheng et al., 2017). The second report is unpublished, and was performed according to OECD 422 guideline for Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. It was performed in male and female Wistar rats. ECHA (2020) reported that the study period was from Nov. 4, 2016 to Mar. 7, 2017. The combined subchronic, developmental and reproductive studies (summarized below) have not been published in a peer reviewed journal; only the summaries are found on-line (Study report dated 2018 as cited in ECHA 2020).

Note about peer review: Publishing to a peer-reviewed journal is preferable to unpublished studies because during the publishing process draft reports undergo a review process by independent experts in the field. Furthermore, most journals insist that the authors identify potential financial interests, which are then attached to the study. Peer review helps ensure the study follows acceptable scientific protocols, the characterization of the results is valid, and the conclusions of the report are supported by the data. ECHA (2020) gave the study summaries the highest reliability score of “1 (reliable without restriction),” and referenced the test guideline of the (OECD, 2015) Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test). Because there were enough details of the study protocols in the summaries provided by ECHA (2020), the Air Quality Division of Michigan Department of Environment, Great Lakes and Energy (EGLE) deemed these summaries are adequate to assess potential health risks from exposure to 6:2 FTS, although there were limitations (see text below).

90-Day Subchronic Study (ECHA, 2020)

In an unpublished subchronic oral study summarized on a webpage administered by ECHA (2020)(hereafter referred to as "the summary"), groups of twelve male and female Wistar rats (Crl:WI(Han)) were dosed by gavage with 0, 5, 15, or 45 mg/kg BW/day 6:2 FTS (CAS No. 27619-97-2) (purity: 97.1%) once daily for 90 days. Male rats were dosed during a 10-week premating period, during mating, and up to sacrifice. The female rats were dosed with the test substance during a 10-week premating period, and during mating, gestation, and lactation up to the day before sacrifice (approximately day 14 of lactation). Reproductive and developmental endpoints were evaluated as per protocol; however, the guidelines do not require skeletal or visceral examinations, and only gross pathology for the pups and reproductive indices were examined. According to the protocol for the 90-day study microscopic examination was performed on the preserved organs of all animals of the control group and high-dose group. The summary stated that upon treatment-related changes in the kidney observed in the high-dose group, the evaluation of this organ was extended to the intermediate-dose groups.

Results of the Subchronic Study (ECHA, 2020)

From the summary: The oral administration of 6:2 FTS was well tolerated. Clinical signs observed were related to the skin (encrustations, sparsely haired areas, and encrustations around the eyes). There were no mortalities or changes in neurobehavioral observations, growth, food intake, red blood cell variables, clotting potential or results on macroscopy.

Body Weights: Mean body weight was comparable in all groups in males and females during premating, mating, gestation, and lactation. Mean body weight change was statistically significantly lower in the high-dose group males (45 mg/kgBW/day) from treatment day 49 to 70, and in the high-dose females (45 mg/kgBW/day) at the start of dosing (interval treatment day 0-7) and from treatment days 21-28 and 56-70. The stated that this was concluded to be treatment related. However, no body weight values or % changes were reported.

Organ Weights: No specific values were reported. The summary stated that mean absolute kidney weight was statistically significantly increased in the low dose males. Mean relative kidney weight was statistically significantly increased in the low and high dose males. In females no changes were observed in absolute or relative mean kidney weight. Mean relative heart weights in mid- (15 mg/kgBW/day) and high-dose (45 mg/kgBW/day) female rats were statistically significantly lower than control animals. No effects were observed on absolute mean heart weights. Other than increased kidney weights in male rats and decreased heart weight in female rats, the summary did not specifically name other organ weight changes, including organ/body weight ratios in both male and female rats.

Clinical Chemistry: The summary stated that the higher mean serum urea level in high-dose males was related to treatment. No changes in thyroxine (T4) hormone levels were observed in males (females not tested). The summary reported that a treatment-relationship could not be ruled out for lower mean total serum protein levels and mean albumin levels in the low-dose (5 mg/kgBW/day) and high-dose males. However, in the absence of a dose-response relationship and in view of the limited effect, this was not considered adverse.

Urinalysis was not performed. Immunological endpoints were also not examined.

Hematological Results: The mean corpuscular hemoglobin was statistically elevated in the mid-dose males (15 mg/kgBW/day dose group).

Neurological Testing: Functional Observational Battery (FOB) and motor activity testing were performed in 5 adult animals/sex/group shortly prior to sacrifice of the male and female rats. The summary reported that the results of the neurobehavioral observations and motor activity assessment did not indicate a neurotoxic potential of the substance.

Histopathological Results: The summary reported that microscopic examination was performed on the organs of all animals of the control groups and high-dose groups. If there were microscopic effects in the organ of a high-dose group rat, the microscopic examination was extended to the low- and mid-dose groups. The changes in the kidney were characterized by mild to moderate (multi)focal tubular dilatation in 5 of 12 high-dose males and in 1 of 12 high-dose females. Because of this finding, the kidneys of the low- and mid-dose animals were also processed and examined microscopically. In the low- and mid-dose groups, tubular dilatation was not observed. No other histopathological results were reported.

Although not specifically reported, the histopathology of the heart was probably evaluated in the control and high dose level female rats as specified in the protocol. Because the heart weight relative to body weight was statistically significantly lower in the mid- and high-dose female rats, the histopathology of the heart tissues in all female rats should have been reported. The relative heart-to-body weight ratio is especially significant because the body weight was also reduced in the high-dose females. The omission of cardiac histopathology is a significant shortcoming to understanding the potential adverse effects induced by 6:2 FTS. The mid-dose of 15 mg/kgBW/day was identified as a 90-day lowest observable adverse effect level (LOAEL) based on a decrease in the relative heart weights of female rats. In contrast, the authors of the summary identified the no-observed-adverse-effect-level (NOAEL) for the 90-day study (ECHA, 2020) was identified at 15 mg/kgBW/day.

Developmental and Reproductive Study and Results

In the developmental/reproductive[2] study (ECHA, 2020), twelve females in each group were placed with males and all animals except for one female in the control group were mated. According to the summary, one female in the low dose group (animal 35) was misjudged to be not mated but was pregnant. Therefore, the mating date was not exactly known for this female. One mated female in the mid dose group was not pregnant. This resulted in mating indices of 91.7% for the control group and 100% for the treatment groups. The male fertility index was 91.7% for the control group and mid-dose group, and 100% for the low-dose and high-dose group, respectively. The mean number of mating days until successful copulation was comparable in all groups. Mean gestational length was also comparable in all groups. The in-life parameters during the reproductive study included clinical observations, body weight, food consumption, mating, gestation, and delivery parameters. The authors stated that there was no effect of the test substance on male and female fertility or reproductive performance.

In the developmental phase of the screening study the pups were examined. At necropsy, animals were macroscopically examined (as per protocol), and the thyroid was also

[2] OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

examined. Macroscopic examination confirmed the stillbirth of one pup in the high dose group (lungs not distended). No macroscopic changes were observed in the 13-day old pups.

The summary stated that no effects were observed on absolute or relative mean thyroid weight in male and female pups on lactation day 13. In the 13-day old pups no statistically significant differences were observed in T4 levels in female pups. In 13-day old male pups the T4 levels were statistically significantly higher in the low- and mid-dose group pups. Compared to control male pups, no statistical significance was reached between T4 levels when compared to the high dose male pups. The summary stated, "The increased mean levels could be attributed to one or two high values per group." Unfortunately, the study protocol resulted in only examining two pups per litter, as per protocol. Because only two pups per litter were examined, the statistical power to detect changes compared to control pups is diminished compared to a protocol that could have measured T4 levels in all pups.

The summary stated that there were no effects on the litter data in the number of pups, pup survival, growth, sex ratio, and developmental parameters (incidence of runts, external malformations, pup weights). The study was performed according to protocol; however, because of the importance of developmental endpoints observed in similar PFAS[3] studies, shortcomings of the developmental screening study for 6:2 FTS are notable:

- No examination of skeletal malformations in pups
- No examination of visceral malformations in pups
- No neurobehavioral examinations in pups
- Limited sample size (2 pups per litter) chosen for examination of thyroid T4

The NOAEL for reproductive and developmental toxicity was reported as ≥ 45 mg/kgBW/day. However, the higher T4 levels in male pups in low- and mid-dose groups could be indicative of an adverse effect at the 5 mg/kg (low-dose) group. The authors stated that the standard deviations of mean T4 levels measured in the groups of this study were large, lowering the ability for a statistical test to find differences between dose and control groups.

28-Day Repeated Dose Study

In a peer reviewed study groups of 20 male CD-1 mice were dosed for 28 days consecutively by gavage to 0 or 5 mg/kgBW/day ammonium 6:2 FTS (6:2 FTSA; purity >99%), CAS No. 59587-39-2, (Sheng et al., 2017). Hepatotoxicity was the focus of this study which measured the following: liver weight and liver to body weight ratio, and serum and liver concentrations of 6:2 FTSA, lipids, cytokines, enzymes, and mRNA.

After 28 days of exposure, body weight was not significantly different than control mice (Sheng et al., 2017). The absolute and relative liver weights were significantly increased by 19 and 22%, respectively. The concentrations of 6:2 FTSA in serum and liver were 18.52 µg/mL and 194.44 µg/g, respectively, which were three orders of magnitude higher than that in the control group. Serum aspartate aminotransferase (AST) concentrations were increased significantly ($p<0.05$; roughly 31% increase) compared to control mice (128 ±

[3] Health-Based Drinking Water Value Recommendations For PFAS In Michigan (June 17, 2019) Michigan Science Advisory Workgroup. Authors: Dr. Jamie Dewitt, Mr. Kevin Cox, Dr. David Savitz. https://www.michigan.gov/documents/pfasresponse/Health-Based_Drinking_Water_Value_Recommendations_for_PFAS_in_Michigan_Report_659258_7.pdf

6.44 IU vs 167.8 ± 16.73 IU, control group vs dose group mean ± SE, respectively). Serum total cholesterol (T-CHO) and triglycerides (TG) were not affected by administration of 6:2 FTSA. Sheng et al. (2017) reported:

> Except for the increased AST level, no significant changes in other liver function indexes were observed, indicating that the injury was not as serious as that induced by PFOA or PFOS. In addition, the lack of lipid accumulation in the mouse liver after exposure to 6:2 FTS confirmed the moderate hepatic injury.

The authors confirmed the occurrence of liver injury in mice exposed to 6:2 FTSA by finding increased liver weight, significant decrease in liver cell numbers, hepatocellular hypertrophy and small areas of necrosis as observed in histopathologic examination of the liver by light microscopy. The authors reported that levels of Peroxisome Proliferation Activated Receptor Alpha (PPARα) and its downstream genes (such as Cyp4a10, FABP1, Acox1, and Cpt1a) did not change significantly. It was stated that this finding implies non-activation of the PPARα pathway, which differs from findings reported for other per- and polyfluoroalkyl substances (PFAS) such as perfluorooctanoic acid (PFOA) and PFOS (Sheng et al., 2017).

Differing from PPARα, the Peroxisome Proliferation Activated Receptor Gamma (PPARγ) expression was significantly upregulated in the livers of the 6:2 FTSA exposure group compared with that of the control group. The authors also reported biomarkers of liver inflammation by finding increases in the cytokines Tumor Necrosis Factor Alpha (TNFα) and Interleukin (IL)-10 in both serum and liver, IL-1β in serum, and IL-6 in liver.

Considering that areas of necrosis were found in the liver of CD-1 mice, the dose level of 5 mg/kgBW/day 6:2 FTSA is a frank effect level. Since this was the lowest dose (and only dose) used in the study by Sheng et al. (2017), a LOAEL was not identified in this study.

Derivation of Chronic Reference Dose and ITSLs

There were no inhalation studies available to derive an inhalation screening level for 6:2 FTS. The appropriateness of the oral studies to derive an inhalation screening level was evaluated. Based on the water solubility and surfactant properties of 6:2 FTS it was assumed that 6:2 FTS would be fully absorbed via the respiratory tract. At low concentrations 6:2 FTS is not expected to have critical portal of entry effects on the lung; however, at higher concentrations it is expected to be irritating to the skin and eyes because of the sulfonic acid portion of the molecule. Since 6:2 FTS is not likely to be metabolized, the first-pass effect of metabolism by the liver is not expected. These assumptions should be re-evaluated periodically as additional information about this substance is likely to be added to the toxicological database.

A Reference Dose (RfD) was derived from the 90-day (subchronic) study reported by ECHA (2019). Because the summaries provided by ECHA did not report incidences of lesions for each dose group benchmark dose methodology could not be used (EPA, 2012). Therefore, the NOAEL/LOAEL method was used to derive the point of departure (POD) dose. The RfD was then used to derive ITSL.

Derivation of Chronic ITSL based on 90-Day Study

In the subchronic study summarized by ECHA (2019) the experimental NOAEL ($NOAEL_{EXP}$) in Wistar female rats was identified as 5 mg/kgBW/day. The human equivalent dose ($NOAEL_{HED}$) is calculated from the rats using a dosimetric adjustment factor (DAF) as follows:

$$NOAEL_{HED} = NOAEL_{EXP} \times DAF$$

The DAF is based on the human to animal body weight ratio raised to the ¾ power (EPA, 2011a). The mathematical equivalent of body weight ratio raised to the ¾ power is:

$$DAF = (W_a/W_h)^{0.25}$$

Where, W_a is 0.25 kg which is the estimated post-parturition body weight of female Wistar rats (Hayakawa, et al. 2013), and W_h is 80 kg which is the average adult body weight in the U.S. (EPA, 2011b).

The 90-day $NOAEL_{HED}$ is calculated as:

$$NOAEL_{HED} = 5 \text{ mg/kgBW/day} \times (0.25\text{kg}/80\text{kg})^{0.25}$$
$$NOAEL_{HED} = 5 \text{ mg/kgBW/day} \times 0.236$$
$$NOAEL_{HED} = 1.18 \text{ mg/ kgBW/day}$$

The RfD is calculated as:

$$RfD = (NOAEL_{HED})/(UF_A \times UF_H \times UF_S \times UF_D)$$

Where,
UF_A is an uncertainty factor of 3 to account for the differences between animals and humans (decreased from 10 to 3 because using the interspecies DAF decreases the uncertainty)
UF_H is 10 to extrapolate from average humans to sensitive humans in the population
UF_S is 10 to extrapolate from subchronic duration to chronic duration
UF_D is 10 to extrapolate from an incomplete database to complete database (see note).

The total uncertainty factor is 3000.

Note: The database uncertainty factor (UF_D) of 10 was deemed appropriate based on concerns for immunotoxicological effects of PFAS (MSAP, 2018 and ATSDR[4], 2018), poor reporting of findings of the heart (especially no histopathological detail), and other organs, no reporting of individual rat and group biological values, and small sample size used to evaluate developmental endpoints. If an appropriately performed and reported 6:2 FTS immunotoxicology study was made available for review, a reduction or removal of the UF_D should be considered.

$$RfD = (1.18)/(3 \times 10 \times 10 \times 10)$$
$$RfD = 0.00039 \text{ mg/kgBW/day}$$

An ITSL can be derived from the RfD, using the equation in Rule 232(1)(b)[5]. This rule uses the ratio of body weight to inhalation rate of 3.5 (from 70 kg/20m³) and is not likely to

[4] ATSDR uses a factor of 10 called a “modifying factor” when the toxicological database and other data deficiencies warrant its usage. For the purposes of this assessment the two terms are considered analogous.
[5] Air Pollution Control Rules. Rule 336.1232(1)(b) et seq. of the Michigan Administrative Code promulgated pursuant to Part 55, Air Pollution Control, of the Natural Resources and Environmental Protection Act (NREPA), 1994 PA 451, as amended.

change significantly if updated body weight and inhalation rate values were used. Table 1 shows the summary of the ITSL derivation.

Chronic ITSL = RfD × body weight/daily inhalation rate × unit conversion
Chronic ITSL = 0.00039 mg/kgBW/day × 70kg/20m³ × 1000µg/mg
Chronic ITSL = 1.37 µg/m³
Chronic ITSL = 1 µg/m³; rounded to 1 significant figure

Pursuant to Rule 232(2)(b), annual averaging time is applied to the ITSL.

Table 1. Summary of Candidate ITSL

Screening Level Type	**Chronic**
Study Duration, type,	90-day, oral
Species	Rats
Study Reference	ECHA, 2020
NOAEL	5 mg/kg
LOAEL	15 mg/kg
Effect	↓ rel. heart wt.
Point of Departure (POD) Type	NOAEL
DAF	0.238
POD Human Equivalent Dose	1.18 mg/kg
Uncertainty Factors (type)	
UF_A (animal to human)	3
UF_H (sensitive individuals)	10
UF_L (LOAEL to NOAEL)	-
UF_S (subchronic to chronic)	10
UF_D (database)	10
UF_{Total}	3000
Oral Chronic RfD (mg/kg)	0.00039
Oral-to-Inhalation Dosimetry	70kg/20m³
Chronic ITSL (µg/m³)	1
Chronic ITSL Averaging Time	Annual

Discussion

There is limited quality and quantity of toxicological data on 6:2 FTS, and additional information could result in a change in the RfD. Confidence in the RfD and the subsequently derived ITSL is low because the basis of the RfD and ITSL is an ECHA summary of a study, rather than review of the study itself. There is the possibility that EGLE's interpretation of the study may differ from that of ECHA.

Species differences in sensitivity add to the low confidence RfD and ITSL. In contrast to the 90-day NOAEL in rats of 5 mg/kgBW/day identified by ECHA (2020), the same dose of 5 mg/kgBW/day in mice exposed for 28 days was found to cause necrosis of the liver. Mice are obviously more sensitive to liver toxicity effects of 6:2 FTS. Robust testing in mice should be performed to determine a NOAEL for hepatotoxicity and other endpoints, including developmental and reproductive effects.

The report summarized by ECHA (2020) did not find adverse effects of 6:2 FTS on the rat liver but did find possible cardiac effects at 15 mg/kg/day. The same study found kidney lesions, which are common in male rats. In the study summarized by ECHA (2020) it was stated that other histopathological changes observed were about equally distributed

amongst the different treatment groups or occurred in one or a few animals only; however, only textual summaries were provided and no individual or group data values were presented as verification of these statements.

The Sheng et al (2017) study evaluated only one dose level, did not identify a NOAEL or LOAEL, and focused solely on liver effects and serum biomarkers, most of which were indicative of inflammation. Sheng et al. (2017) stated that these biomarkers are like those used to evaluate the effects of other PFAS such as PFOS and PFOA. Sheng et al. (2017) measured serum concentrations of 6:2 FTS at 18.5 µg/ml. This can be compared to the average PFOS (potassium salt) serum concentration of 1040 µg/ml that was reported in mice exposed to 2.1 mg/kgBW/day PFOS for 60 days by Dong, et al. (2009). The Dong, et al. (2009) study is the shortest duration rat study using PFOS that is available for comparing PFOS and 6:2 FTS serum concentrations. Sheng et al (2017) also reported high levels of 6:2 FTS in the liver (194.44 µg/g) which could indicate that 6:2 FTS has a potential to bioaccumulate in the body. The 90-day study reported by ECHA (2019) did not report on serum 6:2 FTS levels in rats. Table 2 shows the serum levels of 6:2 FTS and PFOS in male mice at similar doses and durations of exposure.

Table 2. Comparison of 6:2 FTS and PFOS Serum Levels in Male Mice

Chemical (salt)	**6:2 FTS** ammonium salt	**PFOS** potassium salt	**PFOS** potassium salt
Author	Sheng et al. (2017)	Zheng et al. (2008)	Dong et al. (2009)
Dose (duration)	5 mg/kg/day (28 days)	5 mg/kg/day (7 days)	2.1 mg/kg/day (60 days)
Species	CD1	C57BL/6	C57BL/6
Serum Conc.	18.5 µg/ml	110 µg/ml	1040 µg/ml

Conclusion

The ITSL for 6:2 FTS is 1 µg/m³, with annual averaging time. The critical effect is relative cardiac weight decrease in female rats at 15 mg/kgBW/day, with a NOAEL of 5 mg/kgBW/day.

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Appendix

Necrosis of liver tissue as observed by Sheng et al. (2017) at 5 mg/kgBW is considered a Frank Effect Level (FEL). FELs are typically not used directly to derive reference doses (RfDs). However, a candidate acute ITSL can be derived from the 28-day study by Shang et al (2000) if one assumes the 5 mg/kgBW is a lowest-observed-adverse-effect-level (LOAEL). The human equivalent dose $LOAEL_{HED}$ of 0.719 mg/kg was used. The acute oral screening level is calculated as follows.

Acute Oral Screening Level = $LOAEL_{HED}/(UF_A \times UF_H \times UF_L \times UF_D)$

Where

UF_A is an uncertainty factor of 3 to account for the differences between animals and humans (decreased from 10 to 3 because using the interspecies DAF decreases the uncertainty)

UF_H is 10 to extrapolate from average humans to sensitive humans in the population

UF_L is 10 to extrapolate from a LOAEL to a NOAEL

UF_D is 10 to extrapolate from an incomplete database to complete database. (See Note at the bottom of page 7.)

Acute Oral Screening Level = (0.719 mg/kg)/(3 × 10 × 10 × 10)

Acute Oral Screening Level = 0.000239 mg/kg

An acute ITSL is derived from the candidate acute oral screening level, using the default oral-to-inhalation route conversion.

Candidate Acute ITSL = acute RfD × (adult body weight)/ (daily inhalation rate)

Candidate Acute ITSL = 0.00239 mg/kg × 70kg/20m^3 × 1000µg/mg

Candidate Acute ITSL = 8.392 µg/m^3

Candidate Acute ITSL = 10 µg/m^3; rounded to 1 significant figure. (1E+1 µg/m^3)