Archived: Wednesday, February 17, 2021 10:56:46 AM
From: Martin, Joe
Sent: Friday, February 12, 2021 2:21:03 PM
To: Lester, Guy
Cc: Sears, Jessica; Leamons, Bryan
Subject: RE: [EXT] RE: BASF Corporation AR0037770 Temporary Pretreatment System
Importance: Normal

Thanks Guy. I am fine with them moving forward with this project. My only remaining question is if permitting is going to require effluent sampling while this treatment is being performed to ensure that the target effluent concentrations of 6:2 FTS are approximately what is in the effluent.

Thanks,
Joe

From: Lester, Guy
Sent: Thursday, February 11, 2021 10:55 AM
To: Martin, Joe
Cc: Sears, Jessica; Leamons, Bryan
Subject: FW: [EXT] RE: BASF Corporation AR0037770 Temporary Pretreatment System

Joe:

Here is the BASF response to your questions.

Guy Lester, P.E. | Permit Engineer
Division of Environmental Quality | Office of Water Quality
NPDES Permits Section
5301 Northshore Drive | North Little Rock, AR 72118
t: 501.519.0304 | e: lester@adeq.state.ar.us

ARKANSAS
ENERGY & ENVIRONMENT

Archived: Tuesday, March 9, 2021 2:44:02 PM
From: David W Sheaves
Sent: Friday, February 19, 2021 7:13:17 AM
To: Lester, Guy
Cc: Lee Bagby
Subject: BASF Corporation AR0037770 Temporary Pretreatment System
Importance: Normal

Good Morning Mr. Lester,

I am just following up on the email from the 12th regarding the request to discharge impacted storm water from the BASF Facility in West Memphis. I understand due to the weather in your area of the country work has been difficult. I'm just trying to get a sense as when BASF may receive feedback.

Thank you

Dave

Sincerely
David SHEAVES
Expert, Environmental Protection

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Postal Address: BASF Corporation, Global Digital Services, Main Admin, 1609 Biddle Avenue, 48192 Wyandotte, United States

Archived: Tuesday, March 9, 2021 2:43:23 PM
From: David W Sheaves
Sent: Thursday, February 11, 2021 10:15:31 AM
To: Lester, Guy
Cc: Lee Bagby; Michael J Gerdenich
Subject: RE: [EXT] RE: BASF Corporation AR0037770 Temporary Pretreatment System
Importance: Normal

Guy,

Attached are two studies demonstrating aquatic toxicology of the 6:2 Fluorotelomer. Conclusions reached from the papers, the fluorotelomer is deemed not aquatically toxic. LC50 values were typically greater than 100 mg/L for Daphia magna and Rainbow trout. The substance has been deemed to be not bioaccumulative in an aquatic environment via the same studies.

The carbon system has been designed with an Empty Bed Contact Time of 24.9 minutes at a flow rate of 100 gallons/minute.

We anticipate an 85% removal efficiency through the carbon treatment with a target of 150 ng/L in the effluent for the target compound 6:2 fluorotelomer. I have also attached the analytical report for the stormwater pond, the average of two samples for 6:2 fluorotelomer was 1,057 ng/L

Sincerely
David SHEAVES
Expert, Environmental Protection

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From: Lester, Guy <LESTER@adeq.state.ar.us>
Sent: Monday, February 8, 2021 4:10 PM
To: David W Sheaves <david.sheaves@basf.com>
Subject: [EXT] RE: BASF Corporation AR0037770 Temporary Pretreatment System

Mr. Sheaves:

The Planning Branch of the Office of Water Quality has the following questions concerning the chemicals in the proposed discharge:

1. What are the projected outfall concentrations of the chemicals?
2. What does the literature state is the efficacy of the treatment system towards PFAS in ambient water?
3. What is the toxicity of the chemicals being discharged, and if toxic, the half-life of each?

Please submit the Safety Data Sheets, also.

Guy Lester, P.E. | Permit Engineer
Division of Environmental Quality | **Office of Water Quality**
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From: Lester, Guy
Sent: Thursday, February 4, 2021 8:25 AM
To: 'David W Sheaves'
Subject: RE: BASF Corporation AR0037770 Temporary Pretreatment System

As of now, no additional information is needed. I am expecting any comments from other DEQ personnel by Monday. I will let you know if any additional information is required then.

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From: David W Sheaves [mailto:david.sheaves@basf.com]
Sent: Wednesday, February 3, 2021 6:01 PM
To: Lester, Guy
Cc: Lee Bagby
Subject: BASF Corporation AR0037770 Temporary Pretreatment System

Good evening Mr. Lester,

I was just following up to see if you had any questions or data needs regarding the submission I provided to your office on Monday.

As indicated BASF is eager to move forward with this process to relieve pressure on the accumulation of stormwater in our stormwater detention system.

Should you have any questions please don't hesitate to contact me via email or cell phone.

Sincerely
David SHEAVES
Expert, Environmental Protection

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Aquatic hazard, bioaccumulation and screening risk assessment for 6:2 fluorotelomer sulfonate

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HIGHLIGHTS

- 6:2 FTSA is a principal degradation product of short-chain fluorotelomer surfactants.
- 6:2 FTSA is not classified for aquatic hazard and presents little risk to aquatic organisms.
- 6:2 FTSA BCFs were <40 and the dietary BMF was 0.295.
- 6:2 FTSA is unlikely to bioaccumulate or biomagnify in aquatic systems.
- Studies are needed of the fate/effects of commercial AFFF surfactants.

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ABSTRACT

This study assessed the aquatic toxicity and bioaccumulation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA). Acute and chronic aquatic hazard endpoints indicate 6:2 FTSA is not classified for aquatic hazard according to GHS or European CLP legislation. The aqueous bioconcentration factors for 6:2 FTSA were <40 and the dietary assimilation efficiency, growth corrected half-life and dietary biomagnification factor (BMF) were 0.435, 23.1 d and 0.295, respectively. These data indicate that 6:2 FTSA is not bioaccumulative in aquatic organisms. Comparison of PNECs with the reported surface water concentrations (non-spill situations) suggests low risk to aquatic organisms from 6:2 FTSA. Future studies are needed to elucidate the biotic and abiotic fate of commercial AFFF surfactants in the environment.

1. Introduction

Perfluoroalkyl carboxylic (PFCA) and sulfonic acids (PFSA) such as perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) have been widely detected in the environment (Ahrens, 2011; Buck et al., 2011a; Houde et al., 2011). PFOA, PFHxS, PFOS and their longer carbon chain length homologues are persistent in the environment. The major historic global manufacturer of perfluoroalkyl sulfonyl chemistry using the electrochemical fluorination (ECF) process terminated manufacture of PFHxS, PFOS and higher homologues in 2002 (3M Company, 2000). In addition, a group of companies have recently committed to essentially eliminate the manufacture and use of perfluorooctanoic acid (PFOA), higher homologues and their potential precursors by 2015 (USEPA, 2006). Alternative short chain products based on perfluorobutane sulfonate (PFBS), per- and poly-fluorinated ethers and six-carbon fluorotelomer raw materials are commercially available (Ritter, 2010). While aquatic hazard data have been made available for fluorotelomer-based short chain products and related substances (ENVIRON, 2014), there is a need for additional hazard data on many alternatives (Wang et al., 2013).

Fluorinated organics have many unique and useful properties and have been broadly used (Kissa, 2001). Fluorinated surfactants have been used for decades as critical ingredients in aqueous film-forming foam (AFFF) products to help provide lower fire extinguishment times and extended burnback resistance because of their unparalleled low surface tension, wetting and spreading properties (Taylor, 1999; Pabon and Corpart, 1999, 2002; Buck

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0045-6535/

et al., 2011b). Historically, perfluoroalkyl sulfonates (PFSAs) such as PFOS and PFSA-based surfactant derivatives (e.g., $F(CF_2)_nSO_2N(R)R'$ where R = H, CH_3, C_2H_5, R' = additional functional group, n = 4, 6, 8) were the most widely used surfactants in AFFF (Place and Field, 2012; Clarke, 1992). Fluorinated surfactants based on fluorotelomer thiol ((Falk, 1984), e.g., $F(CF_2)_nCH_2CH_2SCH_2CH(OH)CH_2N^+H(CH_3)CH_2CO_2^-$) and sulfonyl chemistry ((Bertocchio and Foulletier, 1971) e.g., $F(CF_2)_nCH_2CH_2SO_2NHCH_2CH_2N^+(CH_3)_2CH_2CH_2CO_2^-$) have also been used in AFFF.

As a result of AFFF containing these fluorinated surfactants being used at fire training facilities to control and extinguish major Class B (i.e., flammable, combustible liquids and gases) fires, environmental degradation products such as PFSAs and fluorotelomer sulfonic acids (FTSAs) have been found in ground water and soil (Backe et al., 2013; Houtz et al., 2013). The perfluoroalkane sulfonyl surfactants degrade to the corresponding PFSA, e.g., PFOS, PFHxS, and PFBS (Moody and Field, 2000; Moody et al., 2003; Rhoads et al., 2008). Fluorotelomer thiol and sulfonyl surfactants degrade to form fluorotelomer sulfonates, e.g., $F(CF_2)_nCH_2CH_2SO_3^-$, n = 4, 6, 8 (Król et al., 2012; Moe et al., 2012; Schultz et al., 2004). Fluorotelomer-based products, such as 6:2 FTSA, do not degrade or metabolize to form PFSAs such as PFOS or PFHxS. The 6:2 FTSA has been shown to biodegrade in aerobic environments to form the 6:2 fluorotelomer alcohol (6:2 FTOH) which subsequently degrades to the expected terminal perfluoro- and polyfluoro-alkyl carboxylate degradation products (Wang et al., 2011).

There are extensive mammalian and aquatic toxicology data available for PFOS (Giesy et al., 2010; Beach et al., 2006) and perfluorobutane sulfonate, PFBS (Giesy et al., 2010, NICNAS, 2005). However, there are few published mammalian toxicology data (Viberg et al., 2013; Sundström et al., 2009) and no available open literature aquatic toxicity data for PFHxS. PFOS and PFHxS are persistent, bioaccumulative and toxic according to global regulatory criteria and are subject to bans and restrictions (UNEP, 2012).

Fluorinated surfactants based on six-carbon fluorotelomer raw materials have largely replaced PFOS and PFSA-based surfactants in AFFF products (Cortina and Korzeniowski, 2008a; Willson, 2010) with the exception of existing inventories of ECF-based surfactants and derivatives. Therefore, there is high interest in the physical, chemical and biological properties of the degradation product, 6:2 FTS ($F(CF_2)_6CH_2CH_2SO_3^-$), and its environmental profile compared to PFOS (Willson, 2010; Cortina and Korzeniowski, 2008b).

The objective of this study was to determine the acute and chronic aquatic toxicity, as well as the bioconcentration and bioaccumulation potential, of 6:2 FTSA. These data were used to derive a predicted-no-effect concentration (PNEC) for aquatic species and the PNEC used to evaluate reported concentrations in the aquatic environment to characterize aquatic risk. The aquatic hazard data from this study also are compared to data available for PFCAs and PFSAs with structurally similar numbers of total carbons and fluorinated carbons such as PFOS, PFHxS, PFOA and PFHxA.

2. Materials and methods

2.1. Test substances

The substances used for testing were potassium 1H,1H,2H,2H-tridecafluoro-1-octanesulfonate (K-6:2 FTS, CAS# 59587-38-1, $C_6F_{13}CH_2CH_2SO_3$-K+, purity: 97.9%; solid) and the protonated 1H,1H,2H,2H-tridecafluoro-1-octanesulfonic acid (6:2 FTSA, CAS# 27619-97-2, $C_6F_{13}CH_2CH_2SO_3H$, purity 30 wt% aqueous solution), both synthesized and characterized by DuPont.

2.2. Acute aquatic toxicity testing

The acute aquatic toxicity of K-6:2 FTS and 6:2 FTSA was evaluated in studies utilizing the rainbow trout (*Oncorhynchus mykiss*), the freshwater invertebrate, *Daphnia magna*, and the freshwater green alga, *Pseudokirchneriella subcapitata,* as the test species. These studies were conducted using Good Laboratory Practices (GLP) and in conformance with OECD test guidelines 203 (fish), 202 (*Daphnia*), and 201 (algae) and USEPA OPPTS test guidelines 850.1010 (*Daphnia*), 850.1075 (fish) and 850.5400 (algae), respectively. All study endpoints are reported based on measured test substance concentrations. Studies with 6:2 FTSA were conducted as limit studies with a single nominal test concentration of 120 mg L^{-1}. Relevant raw data for all studies are contained in the Supplemental Information.

2.3. Chronic aquatic toxicity testing

The chronic aquatic toxicity of K-6:2 FTS was evaluated in a 90-d early-life stage study utilizing the rainbow trout. The study was conducted using GLP and in conformance with OECD 210 and USEPA OPPTS 850.1400 test guidelines. Nominal test substance concentrations tested ranged from 0.625 to 10 mg L^{-1}.

A total of 80 embryos were exposed per concentration (20 embryos per embryo cup, 2 cups per replicate, 2 replicates per concentration) at test start. On day 40, after swim-up had begun in the control, the fingerlings were thinned to a total of 30 fish per concentration (15 fish per replicate, 2 replicates per concentration). Analytical verification of test substance concentrations was conducted on day −3, day 0, once weekly thereafter, and on day 90 at test end. Test solutions were supplied to each replicate test chamber at a rate of approximately 1.25 L of test solution per test chamber each hour, resulting in approximately 5 test solution volume additions of 6 L (30 L total) every 24 h. Embryos and alevins were held in relative darkness until day 39, and then held under a photoperiod of 16 h light and 8 h darkness (which included 30 min of transitional light between light and dark intervals) through test end. Test solutions were maintained between 11.9 and 12.9 °C (mean 12.2 °C). Daily observations were made for assessment of number of dead eggs, first and last day of hatching, first day of swim-up, and survival and abnormalities post-hatching. Standard length and blotted wet weight of surviving fingerlings were determined at test end.

2.4. In-vitro metabolism screening – trout hepatocytes

In vitro trout hepatocyte metabolism screening (Han et al., 2008) was performed with K-6:2 FTS prior to bioaccumulation testing. Two replicates were used per test with a positive control (4-nonylphenol), a dead cell control treatment and the test substance treatment evaluated in two experiments. The assay utilized a cell concentration of 2 × 106 cells mL^{-1} in L-15 medium at a pH of 7.8, an incubation temperature of 10–12 °C, and a test substance concentration of 5 μM (2.33 ppm). Acetonitrile was used as the dosing vehicle. Metabolism (i.e., loss) of parent test substance was evaluated at 5, 15, 30, 60, 120, and 240 min.

2.5. Bioconcentration and bioaccumulation testing

Bioconcentration and bioaccumulation of K-6:2 FTS were evaluated in aqueous and dietary exposures with rainbow trout to define the steady-state and kinetic bioconcentration factors (BCF) at two aqueous exposure concentrations and to determine the half-life, the assimilation efficiency, and the dietary biomagnification factor based on a single dietary exposure concentration. The study design complied with the following test guidelines and

guidance documents: USEPA, OPPTS, 850.1730, OECD TG 305 and Anonymous (2004).

Trout were exposed to K-6:2 FTS under flow-through conditions for 56 d during the uptake (exposure) phase of the study followed by a 28-d depuration phase in clean well water with no test substance. Two aqueous test substance concentrations (nominal 1 and 10 μg L^{-1}), a single spiked diet concentration (nominal 10 μg kg^{-1}) with no aqueous test substance exposure, and a dilution water control were used for testing with 60 fish per exposure treatment and control. Four replicate fish were sampled from each treatment at appropriate sampling intervals and processed as individual whole fish. Additional details for conduct of the in-life study are contained in the Supplemental Information.

2.5.1. Chemical analysis

Samples were analyzed using HPLC/MS/MS, typically with an Agilent Model 1100 HPLC with a Micromass Quattro Micro MS instrument and software. A Zorbax® RX-C8 LC column, 2.1 × 150 mm, 5 μm particle size, was used for analysis with a 0.400 mL min^{-1} flow rate at 40 °C and either a 55% Nanopure water:45% acetonitrile mobile phase (test solution samples) or a 70% Nanopure water:30% acetonitrile mobile phase (fish tissue and diet samples). The injection volume was 10 μL for whole fish tissue samples and diet samples and 50 μL for aqueous test solution samples. Additional details related to sample analyses are contained in the Supplemental Information.

The LOD and LOQ for aqueous samples from the bioconcentration/bioaccumulation study were determined to be 0.06 μg L^{-1} and 0.540 μg L^{-1}, respectively. The LOD and LOQ for fish and diet analyses were determined to be 0.08 μg kg^{-1} and 0.441 μg kg^{-1}, respectively. The calculated recovery of the QC samples for each sample matrix ranged from 84% to 107% for test solutions, from 77% to 113% for fish, and 102% for the spiked diet analysis.

2.6. Data analyses

Acute study endpoints were calculated using the probit procedure and SAS® software (2000). For algal studies, healthy cell counts were used to calculate the E_bC_{50} (biomass), E_yC_{50} (yield), E_rC_{50} (growth rate) and associated NOEC values. The statistical methods used for data analysis followed the approaches outlined in OECD statistical guidance for aquatic toxicity tests (OECD, 2006). All statistical tests were calculated at a significance level of $\alpha = 0.05$. Predicted No Effect Concentration (PNEC) calculation for evaluating aquatic risk followed REACH Chapter 10 (ECHA, 2008) guidance.

For the K-6:2 FTS bioconcentration/bioaccumulation study, steady state was defined as three consecutive measurement days over which there was no statistically significant increase in mean tissue residues in whole fish as recommended in the test guidelines. A 2-factor factorial ANOVA model with day and concentration as factors and fish as random effect was used together with simple, unadjusted *t*-tests to compare the mean tissue residues sampled on each fish sampling day. Once the steady state time period was determined, the mean tissue residue of fish sacrificed and tested on those three consecutive sampling dates was computed and used as the basis for calculation of the steady state aqueous bioconcentration factors as recommended in the test guidelines.

Individual fish wet weights at sacrifice for all time periods were tabulated separately for dietary test substance concentration and the control for all sampling days during the uptake and depuration phases. Wet weight data were converted to natural logs(ln) and plotted versus study day. A separate linear least squares correlation was calculated for the ln(fish weight) versus study day for both dietary and control treatments using data for individual fish and standard statistical procedures contained in Microsoft Office Excel 2003. There was no significant difference between the dietary and control treatment data as evidenced by the slopes (growth rates) from the regression equations; therefore, the data were pooled to calculate an overall fish growth rate for the study (k_{growth}).

Individual fish test substance concentration data (wet weight basis) were tabulated for the dietary exposure and control fish for individual sample times during the depuration phase. The individual fish tissue residue data were converted to their natural logarithms and plotted versus time (day). A linear least squares correlation was calculated for the ln(fish tissue residue) versus depuration day. The slope and intercept (day 0 of depuration) of the line are reported as the overall elimination rate ($k_{overall}$) and time zero concentration ($C_{0,depuration}$), respectively.

The growth corrected depuration rate was calculated by subtraction of the growth rate from the overall elimination rate ($k_{depuration} = k_{overall} - k_{growth}$). The chemical assimilation efficiency (α) was calculated using the equation:

$$\alpha = \frac{C_{0,depuration} \cdot k_{overall}}{I \cdot C_{food}} \cdot \left[1 - \exp\left(-k_{depuration} \cdot t\right)\right]$$

where I is the food ingestion rate ($g_{food}\ g_{fish}^{-1}\ d^{-1}$) and C_{food} is the chemical concentration in the food. The dietary biomagnification factor (BMF) was calculated as $BMF = I \cdot \alpha \cdot k_{depuration}^{-1}$ and the growth-corrected half-life was calculated as $t_{1/2} = 0.693 \cdot k_{depuration}^{-1}$ (Anonymous, 2004).

3. Results

During algae, daphnid and fish testing with K-6:2 FTS and 6:2 FTSA, the dilution water quality was acceptable, all chemical and physical parameters were within the expected ranges, and all relevant OECD test acceptance criteria were fulfilled.

3.1. Rainbow trout

The mean measured concentrations of K-6:2 FTS were not detected (ND, LOD = 0.0008 mg L^{-1}), 6.82, 14.6, 27.7, 53.4, and 107 mg L^{-1}. No mortality was observed at any test concentration. The 96-h LC_{50} was >107 mg L^{-1}.

The mean measured concentrations of 6:2 FTSA were ND (LOD = 0.0004 mg L^{-1}) and 108 mg L^{-1} in the control and limit test concentration, respectively. No mortality or sublethal effects were observed at the limit test concentration at test end. The 96-h LC_{50} was >108 mg L^{-1}.

3.2. D. magna

The mean measured concentrations of K-6:2 FTS were ND (LOD = 0.01 mg L^{-1}), 7.09, 14.3, 27.8, 53.4, and 109 mg L^{-1} and immobility at the end of 48 h was 0%, 25%, 15%, 25%, 30%, and 50%, respectively. The calculated 48-h EC_{50} was >109 mg L^{-1}.

The mean measured concentrations of 6:2 FTSA were ND (LOD = 0.0007 mg L^{-1}) and 112 mg L^{-1}. No immobility or sublethal effects were observed after 48 h. The 48-h EC_{50} was >112 mg L^{-1} for 6:2 FTSA.

3.3. Green algae – P. subcapitata

The mean measured concentrations of K-6:2 FTS were ND (LOD = 0.006 mg L^{-1}), 6.20, 12.4, 24.2, 47.6, 96, and 99.3 mg L^{-1} (abiotic control). Inhibition of growth relative to control after 72 h, expressed as biomass (cell number), was −8%, 2%, 14%, 3%, and 20%, respectively. Inhibition of growth expressed as area under the growth curve was −9%, 4%, 19%, −1%, and 14%, respectively.

Inhibition of growth expressed as the average specific growth rate was −1%, 0%, 2%, 0%, and 4%, respectively. The 72-h E_bC_{50} (biomass), E_rC_{50} (growth rate), and the associated NOECs were >96 mg L^{-1}.

The mean measured concentrations of 6:2 FTSA were ND (LOD = 0.0002 mg L^{-1}), 125, and 121 mg L^{-1} (abiotic control). Inhibition of growth expressed as cell number (biomass), area under the growth curve (biomass), and average specific growth rate after 72 h was 20, 22, and 4%, respectively. The 72-h limit test E_bC_{50}, E_rC_{50}, and associated NOECs were >125 mg L^{-1}.

3.4. Rainbow trout Early-life Stage (ELS) study

The mean measured concentrations of K-6:2 FTS were 0, 0.857, 1.66, 2.62, 4.85, and 8.70 mg L^{-1}. A summary of relevant biological observations from hatching to thinning and thinning to test end is presented in the Supplemental Information. The overall 90-d study NOEC was 2.62 mg L^{-1} based on mean, measured K-6:2 FTS concentrations and first day of hatching.

3.5. In-vitro trout metabolism assay

No apparent loss of K-6:2 FTS was observed after 4 h of incubation in the *in vitro* trout hepatocyte metabolism assay indicating that the bioaccumulation potential of the test substance may not be significantly affected by fish hepatic metabolism. However, this result is not a conclusive evaluation of potential metabolism in fish since it does not consider all relevant metabolic processes or routes of elimination.

3.6. Bioconcentration and dietary bioaccumulation test

Rainbow trout were exposed to K-6:2 FTS under flow-through conditions for 56 d (uptake phase) followed by a 28-d depuration phase. There was a problem with the initial low aqueous exposure concentration being incorrectly prepared during days 1–13 with the error corrected on day 13 and thereafter through the termination of the uptake phase on study day 56. The error was caused by a dilution error in the low test concentration stock solution preparation that resulted in the low test concentrations being ~10× greater than planned (approximately equal to the high test concentration) from test day 0–13. The error was not detected until day 13 due to analytical instrumentation availability issues that precluded analysis of collected test solution samples until day 13. Mean measured aqueous exposure concentrations for K-6:2 FTS from day 14 through day 56 were 1.08 μg L^{-1} and 9.32 μg L^{-1} and the measured dietary exposure concentration was 13.1 μg kg^{-1}. Full study analysis details appear in the Supplemental Information.

Figs. 1, 2 and 4 present the individual replicate fish ($N = 4$) and treatment mean whole body concentrations of 6:2 FTS for each sampling date of the uptake and depuration phases of the study. Based on the slopes of the regression lines for ln(wet weight) versus study day, no difference was apparent in the growth of control and dietary treatment fish and the data were pooled for subsequent analysis (Fig. 3). The calculated overall growth rate for the combined control and dietary treatment fish was 0.027 d^{-1}. For the purpose of the dietary biomagnification (BMF) calculations, the day 63 individual fish tissue residues were assumed to be equal to the limit of quantitation (LOQ) of 0.441 μg kg^{-1} instead of being equal to one-half of the LOD. The derived $k_{overall}$ and $C_{0,depuration}$, calculated depuration rate, growth-corrected half-life, assimilation efficiency (α) and biomagnification factor (BMF) from the dietary exposure as well as the steady state and kinetic BCF values from the aqueous exposures are presented in Table 1.

Uptake phase whole fish concentrations of 6:2 FTS reached steady-state equilibrium within 42 d or earlier in both aqueous and dietary exposures. After a maximum of 24 d of depuration, the measured concentrations in the whole fish were reduced approximately 95% or more from concentrations at the end of the uptake phase (day 56). However, measured concentrations in whole fish at the end of the uptake phase on day 56 were already less than 50% of the maximum uptake phase concentration in whole fish for both aqueous test concentrations and the dietary exposure. All bioconcentration factors (BCF) were <40 and the dietary assimilation efficiency, the growth-corrected half-life, and the dietary biomagnification factor (BMF) were 0.435 d, 23.5 d and 0.295, respectively (Table 1).

4. Discussion

When K-6:2 FTS and 6:2 FTSA data are compared to the aquatic toxicity data available for PFCAs and PFSAs with structurally similar numbers of total carbons and fluorinated carbons such as PFOS, PFHxS, PFOA and PFHxA, there is a clear difference between 6:2 FTSA and PFOS (Table 2). The 6:2 fluorotelomer sulfonate values are typically at least 10× less potent than PFOS. Acute endpoints for K-6:2 FTS, 6:2 FTSA and PFHxA for the fish, *Daphnia* and green algae were all greater than 96 mg L^{-1} (i.e., effectively >100 mg L^{-1} for classification and labeling purposes) while PFOS acute hazard endpoints range from 48 to 78 mg L^{-1} (Table 2). For PFHxS, which has six fluorinated carbons, no data were found for comparison of aquatic toxicity endpoints. Since no *D. magna* chronic data were available for the six carbon sulfonate substances, evaluation of chronic toxicity was based on the fish ELS and algae study NOECs for the 6:2 FTS. Algae have typically been among the most sensitive test organisms to perfluoroalkyl and polyfluoroalkyl compounds (Beach et al., 2006; Colombo et al., 2008; Hoke et al., 2012), however, the aquatic species most sensitive to PFOS was the chironomid, *Chironomus dilutus* (i.e., *Chironomus tentans*) (Phillips et al., 2007). Comparison of NOECs for the substances in Table 2 indicates that APFO was more toxic to green algae than 6:2 FTSA while PFOS was more toxic to fish. Thus, the eight carbon perfluoroalkyl substances are more toxic than the analogous six carbon polyfluoroalkyl and perfluoroalkyl substances as previously observed by multiple studies (Boudreau, 2002; Phillips et al., 2007; Latala et al., 2009; Hoke et al., 2012). Several studies also have reported that BCF and BAF values for PFCAs are directly related to perfluoroalkyl chain length (Martin et al., 2003a,b; Conder et al., 2008).

Yeung and Mabury (2013) investigated the bioconcentration of perfluorinated or polyfluorinated components in two commercially available firefighting foams. Their study consisted of an 11 d exposure phase followed by a 25 d depuration phase to mimic the study conducted by Martin et al. (2003a). The aqueous concentration of the 6:2 FTSA in one of the foam mixtures was maintained at 1.5 ± 0.9 μg L^{-1} during the uptake phase of the study. The analogous aqueous concentrations for PFHxA were 44 ± 16 ng L^{-1} during the uptake phase. The levels of 6:2 FTSA in fish (carcass and liver) were observed to increase for the first six days of the uptake phase but began to decrease after that and prior to the initiation of the depuration phase of the study. Concentrations of 6:2 FTSA were not detected in fish during the depuration phase of their study and PFHxA was not detected in fish tissues during either the uptake or depuration phase of their study. Based on these data, no BCF values for 6:2 FTSA or PFHxA could be reported from their study (Yeung and Mabury, 2013) and the authors suggested that biotransformation of the 6:2 FTSA occurred in fish. However, metabolites of 6:2 FTSA, such as PFHxA, were not detected in fish because these substances are not bioaccumulative in fish (Martin et al., 2003a,b; Conder et al., 2008).

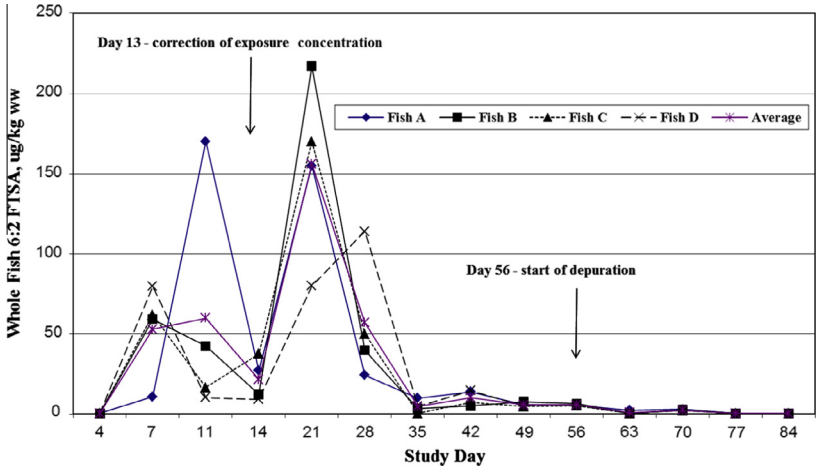

Fig. 1. Replicate (A–D) and mean whole fish K-6:2 FTS, μg kg^{-1}, (nominal 1 μg L^{-1} aqueous exposure).

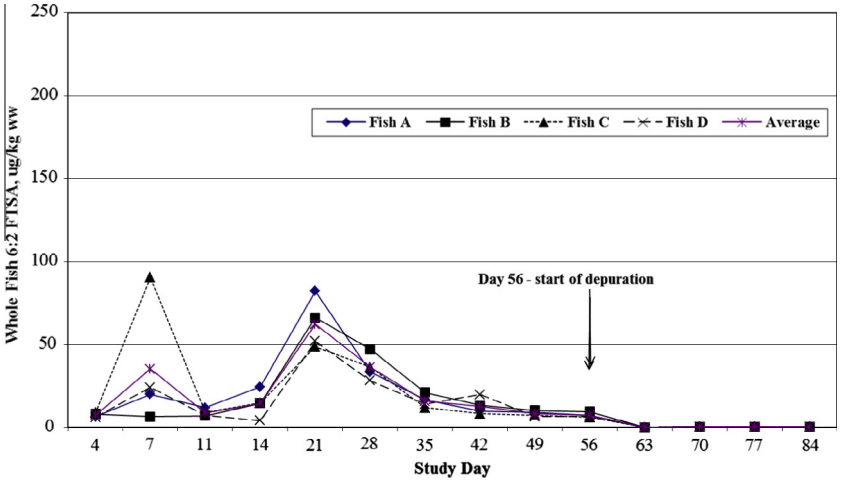

Fig. 2. Replicate (A–D) and mean whole fish K-6:2 FTS, μg kg^{-1}, (nominal 10 μg L^{-1} aqueous exposure).

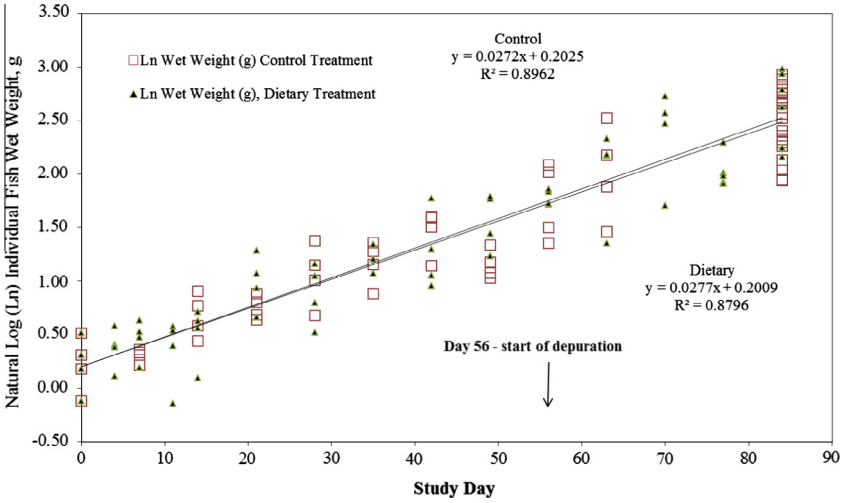

Fig. 3. Natural log(Ln) trout wet weight (g) vs. study day.

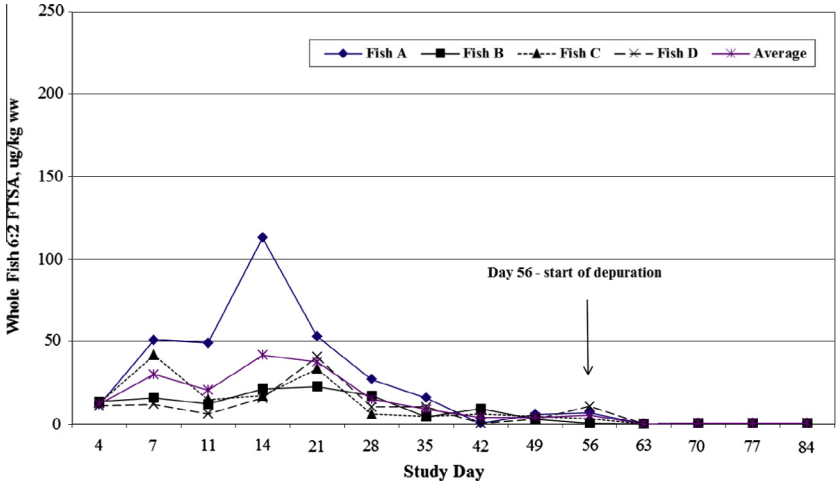

Fig. 4. Replicate (A–D) and mean whole fish K-6:2 FTS, μg kg^{-1}, (nominal 10 μg kg^{-1} dietary exposure).

Table 1
K-6:2 FTS dietary and aqueous bioaccumulation endpoints for rainbow trout.

Mean measured concentration	Mean steady state tissue residue (μg kg^{-1})	Steady-state BCF[a]	Kinetic BCF[b]
Aqueous exposure			
1.08 μg L^{-1}	24[c]	22	36
			<1[e]
9.32 μg L^{-1}	38.2[d]	4	3[f]
Dietary exposure			
13.1 μg kg^{-1}	k_{growth}		0.027 d^{-1}
	$k_{overall}$ (overall elimination rate)		0.057 d^{-1}
	$C_{0,depuration}$ (calculated tissue residue at start of depuration phase)		3.56 μg kg^{-1}
	$k_{depuration}$ (growth-corrected depuration rate)		0.030 d^{-1}
	α (assimilation efficiency)		0.435
	BMF (dietary biomagnification factor)		0.295
	Growth corrected half-life		23.1 d

[a] Mean steady state tissue residue divided by mean measured aqueous concentration.
[b] k_1 divided by k_2.
[c] Mean steady state residues from days 28 to 42.
[d] Mean steady state residues from days 21 to 35.
[e] Including day 63 depuration phase data.
[f] Excluding day 63 depuration phase data.

Table 2
Aquatic toxicity and bioaccumulation comparisons.

Substance	K-6:2 FTS (6:2 FTSA)	PFHxS	PFOS	PFHxA	PFOA/APFO
Anion	$C_6F_{13}CH_2CH_2SO_3^-$	$C_6F_{13}SO_3^-$	$C_8F_{17}SO_3^-$	$C_5F_{11}CO_2^-$	$C_7F_{15}CO_2^-$
No. of fluorinated (total) carbons	6 (8)	6 (6)	8 (8)	5 (6)	7 (8)
O. mykiss 96-h LC50	>107 mg L^{-1}[d] (>108 mg L^{-1})	No data	78 mg L^{-1}[a]	>99 mg L^{-1}[e]	>100 mg L^{-1}[c]
D. magna, 48-h EC_{50}	>109 mg L^{-1}[d] (>112 mg L^{-1})	No data	58 mg L^{-1}[a]	>96 mg L^{-1}[e]	>100 mg L^{-1}[c]
P. subcapitata, 72-h E_rC_{50}	>96 mg L^{-1}[d] (>125 mg L^{-1})	No data	48.2 mg L^{-1}[a]	>100 mg L^{-1}[e]	>100 mg L^{-1}[c]
P. subcapitata, 72-h $NOEC_r$	47.6 mg L^{-1}[d] (>125 mg L^{-1})	No data	42 mg L^{-1}[a]	>100 mg L^{-1}[a]	12.5 mg L^{-1}[a]
90-d *O. mykiss* ELS NOEC	2.62 mg L^{-1}[d] (No data)	No data	0.29 mg L^{-1}[a] (*P. promelas*)	9.96 mg L^{-1} (as PFHx anion)[f]	40 mg L^{-1}[c]
14-d *E. fetida* LC_{50}	500 mg kg^{-1}[g] (No data)	No data	500 mg kg^{-1}[g]	No data	No data
56-d *E. fetida* EC_{10}, Repro.	247 mg kg^{-1}[g] (No data)	No data	25 mg kg^{-1}[g]	No data	No data
Aquatic bioaccumulation	Not bioaccumulative[d]	Bioaccumulative[b]	Bioaccumulative[b]	Not bioaccumulative[b]	Not bioaccumulative[b]

[a] Beach et al. (2006).
[b] Martin et al. (2003a,b).
[c] Colombo et al. (2008).
[d] Present study.
[e] Hoke et al. (2012).
[f] Iwai (2012).
[g] Norwegian Pollution Control Authority (2006).

Table 3
$PNEC_{aquatic}$ ($\mu g\ L^{-1}$) values and PEC:PNEC ratios.

Substance	Range of reported non-spill; surface water concentrations (PEC, $\mu g\ L^{-1}$)	$PNEC_{aquatic,}$ ($\mu g\ L^{-1}$)	Maximum PEC:PNEC ratio
6:2 FTS	ND – 0.036[a]	52.4, K-6:2 FTS[b]	<0.001
		108, 6:2 FTSA[c]	<0.001
PFHxS	ND – 0.0328[d]	ND[e]	ND
PFOS	ND – 0.651[f]	1.2[g]	0.54
		0.61 – 6.66[h]	1.07
PFHxA	ND – 3.04[i]	199[b]	0.015
PFOA/APFO	ND – 87.1[i]	1250[j]	0.070

[a] Nguyen et al. (2011).
[b] Assessment factor (AF) of 50 applied to lowest chronic NOEC.
[c] AF of 1000 applied to lowest acute LC/EC50.
[d] Bossi et al. (2008).
[e] No aquatic toxicity data available to derive PNEC.
[f] Rostkowski et al. (2006).
[g] Beach et al. (2006), secondary chronic value.
[h] Qi et al. (2011).
[i] Hoke et al. (2012).
[j] Colombo et al. (2008).

The results of the aqueous bioconcentration study with K-6:2 FTS reported here provide definitive support for the 6:2 FTSA results of Yeung and Mabury (2013) without the potentially confounding effects of the other components of the commercial foam mixtures tested in their study. In addition, we extend those results by demonstrating that K-6:2 FTSA (and thus 6:2 FTS) is not bioaccumulated by fish from their diet. The low concentration in our aqueous bioconcentration study (i.e., $\sim$1 $\mu g\ L^{-1}$) mirrored the 6:2 FTSA exposure concentration from the study by Yeung and Mabury (2013). It is also noteworthy that the pattern of 6:2 FTSA uptake and elimination observed in the Yeung and Mabury study also was repeated in both the aqueous and dietary uptake phase exposures in the current investigation. Concentrations of 6:2 FTS in both aqueous and dietary exposures were observed to peak and then begin to decline during the exposure phase and prior to the initiation of the depuration phase of the study. This pattern of tissue residues peaking and then declining during the exposure phase of a dietary bioaccumulation study was also reported for PFBS by Falk et al. (2015) for adult rainbow trout. The Yeung and Mabury (2013) study and the current investigation support the conclusion that 6:2 FTSA is not bioaccumulative in fish and is unlikely to undergo aquatic foodchain biomagnification. In addition, the results from mammalian biopersistence screening in rats also provides support for the lack of bioaccumulation/persistence of 6:2 FTSA in mammals (Serex et al. 2008).

Comparison of the calculated PNECs with the available surface water concentrations (non-spill situations) presented in Table 3 for 6:2 fluorotelomer sulfonate and a suite of perfluorinated compounds evaluated in this study suggest the risk to aquatic organisms from 6:2 FTS is low based on reported surface water concentrations. Based on this evaluation, the risk quotients (i.e., PEC/PNEC ratios) for most substances in Table 3, with the exception of PFOS and PFHxS (for which no data are available), are multiple orders of magnitude below a level that would suggest potential risk to aquatic organisms. However, it is also important to note that the present evaluation of 6:2 FTS did not consider potential endocrine effects or effects on aquatic organism reproduction although an assessment factor (safety factor) of 50 was applied to the lowest chronic endpoint from the rainbow trout ELS study to develop the 6:2 FTS PNEC.

5. Conclusion

AFFF has unique properties and high societal value for the protection of people and property. This study provides important data regarding the aquatic toxicity properties of 6:2 FTS, a key potential degradation product from short-chain fluorotelomer-based AFFF surfactants. The available aquatic toxicity and bioaccumulation data for 6:2 FTS indicate that 6:2 FTS is not classified for aquatic hazard under either global GHS or European CLP legislation, that it is not bioaccumulative, and that it poses minimal risk to aquatic organisms. Future studies are needed to elucidate the biotic and abiotic fate of commercial AFFF surfactants in the environment. Finally, efforts should continue to develop commercial systems that can be used in the field that enable AFFF capture after deployment for either destruction or reuse (Baudequin et al., 2014).

Disclaimers

Animal Welfare Act compliance

This study complied with all applicable sections of the final rules of the Animal Welfare Act regulations (9 CFR) and the guidelines from the Guide for the Care and Use of Laboratory Animals (NRC, 1996) and the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia. DuPont Haskell Global Centers is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Acknowledgement

The authors thank Mark Russell and the anonymous reviewers for thoughtful input on the manuscript and dedicate this paper in memory of our colleague and friend, Mary A. Kaiser.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.01.033.

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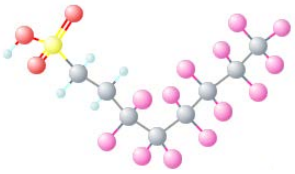
6:2 Fluorotelomer Sulfonate (6:2 FTS)
TOXICOLOGY AT A GLANCE



6:2 FTS

- In 2012-2015, the U.S. metal plating industry transitioned from PFOS to short-chain fluorotelomers (e.g., 6:2) FTS in their chromium electroplating processes.
- The standard suite of tests conducted for regulatory approval of industry manufacture and use is available for 6:2 FTS.
- Based on currently available data, 6:2 FTS is less toxic and less persistent in the environment compared to PFOS and does not bioaccumulate.

Mammalian Toxicology

- ✓ Several short duration studies show that 6:2 FTS can cause kidney and liver damage in rodent models.
- ✓ 6:2 FTS does not cause DNA damage.
- ✓ 6:2 FTS does not cause damage to the reproductive system or to the developing fetus in available rodent models.
- ✓ No chronic studies are currently available.
- ✓ No studies have been conducted to assess cancer, immune system toxicity, or endocrine disruption following 6:2 FTS exposure.

Ecological Toxicology

- ✓ 6:2 FTS is less toxic than PFOS in studies with fish, algae, water fleas (*Daphnia*), and earthworms.
- ✓ 6:2 FTS does not bioaccumulate in fish.
- ✓ A peer-reviewed aquatic hazard assessment concluded that 6:2 FTS presents little risk to aquatic organisms.

Human Exposure

- ✓ Human exposure is assessed by analyzing blood for the presence of 6:2 FTS; so far, only 4 studies have looked for 6:2 FTS in human blood.
- ✓ Available data indicate that the general population's exposure to 6:2 FTS is low and infrequent.
- ✓ 6:2 FTS has been detected at low levels in some consumer products, drinking water, air, and fish; human exposure may occur through any of these sources.

Environmental Occurrence

- ✓ 6:2 FTS has been found in air, snow, soil, groundwater, and surface water.
- ✓ 6:2 FTS has been detected at sites associated with fluorochemical manufacture or use of certain fire-fighting foams.
- ✓ 6:2 FTS is less persistent than PFOS.
- ✓ 6:2 FTS can degrade to short-chain perfluorinated compounds, but does not degrade to PFOS.
- ✓ Perfluorohexanoic and perfluoropentanoic acids are the primary degradation products, however, not all degradation products have been identified or well-studied.

Toxicology of 6:2 Fluorotelomer Sulfonate (6:2 FTS)

Historically, the metal plating industry has used, and continues to use, per- and polyfluoroalkyl substances (PFAS) in some metal plating applications. Most notably, beginning in the 1980s, perfluorooctanesulfonic acid (PFOS) was used as a mist suppressant in hard and decorative chromium plating, chromic acid anodizing, and chromium etch for plating on plastic processes. Due to concerns relating to toxicity and environmental persistence, the chrome plating industry transitioned from using PFOS to using a newer formulation, primarily using 6:2 fluorotelomer sulfonate (6:2 FTS), between 2012-2015. Compared to PFOS, 6:2 FTS has an improved toxicity profile in both rodent and ecological models, and is less persistent than PFOS in the environment and is not bioaccumulative. Further, available studies indicate that human exposure to 6:2 FTS is low and infrequent; comparatively, PFOS is detected in the blood of >99% of Americans. Collectively, available toxicology studies indicate that 6:2 FTS is a safer alternative to PFOS. However, significant data gaps do exist.

1. Mammalian Toxicology of 6:2 FTS

Hundreds of studies have been conducted on PFAS, such as PFOS, in laboratory animals including mice, rats, and primates. Furthermore, over 100 human epidemiology studies have been published on PFOS. Comparatively, the toxicology database for 6:2 FTS can be considered "limited", however, standard testing for regulatory approval of industry manufacture and use have been completed. Toxicology studies that have been completed for 6:2 FTS include (1) six DNA damage studies; (2) three skin irritation and/or sensitization studies; (3) two acute toxicity studies (i.e., single exposure studies); (4) two systemic toxicity studies (i.e., studies investigating multi-organ toxicity following repeated exposure); (5) one liver toxicity study; and (6) one reproductive and developmental toxicity study. Toxicity data following chronic exposure is currently not available. No human epidemiology studies investigating the relationship between exposure to 6:2 FTS and associated health effects have been conducted. 6:2 FTS has not been assessed for its ability to cause cancer, immune system effects, or endocrine (i.e., hormone) disruption, which are all health effects that have been associated with exposure to some PFAS.

6:2 FTS Toxicology Database

- DNA damage studies x 6
- Skin irritation/sensitization studies x 3
- Acute toxicity studies x 2
- Systemic toxicity studies x 2
- Liver toxicity study x 1
- Reproductive/developmental toxicity study x 1

Collectively, results from toxicology studies indicate that 6:2 FTS does not (1) cause damage to DNA; (2) does not act as a skin sensitizer (i.e., cause allergic skin reactions); and (3) does not cause toxicity to reproductive organs or to the developing fetus. In contrast, several studies have shown that PFOS can cause developmental toxicity. Furthermore, several acute toxicity studies (i.e., single dose studies testing for lethality) have demonstrated that 6:2 FTS is less acutely toxic than PFOS in laboratory animals. 6:2 FTS has been shown to cause skin irritation; however, this effect is unlikely to be relevant for the general population as it requires dermal contact with high concentrations of 6:2 FTS that is likely to only occur in settings with concentrated and specific use. Finally, rodent studies have demonstrated that exposure to 6:2 FTS can cause kidney and liver toxicity; comparatively, PFOS is not typically associated with kidney toxicity, but has been shown to

6:2 FTS does not cause:

- Skin sensitization
- DNA damage
- Reproductive or developmental toxicity

6:2 FTS does cause:

- Skin irritation
- Kidney and liver toxicity

cause adverse liver effects in numerous studies. Although results from these studies indicate that 6:2 FTS can cause kidney and liver toxicity, these results occur at higher exposure levels than typically seen in environmental settings. Laboratory studies of longer duration, multiple exposure levels, and with additional endpoints are still needed.

2. Human Exposure to 6:2 FTS

Human exposure to 6:2 FTS (and other PFAS) can be assessed by collecting and analyzing human blood samples for the presence of 6:2 FTS. Exposure of the general U.S. population to PFAS, such as PFOS, has been closely tracked via several large-scale studies, including the Center for Disease Control's National Health and Nutrition Examination Survey (NHANES); however, 6:2 FTS has not been included in NHANES. Four studies have measured human exposure to 6:2 FTS in populations from Australia, China, Hong Kong, and the United States. All four studies consistently demonstrate that human exposure to 6:2 FTS is low and infrequent, and that the average human blood level of 6:2 FTS is approximately 1000 times lower than that of PFOS. However, results from these studies are nearly a decade old, and it is unknown if trends in human exposure to 6:2 FTS have changed over time. Furthermore, no exposure data is available for workers exposed in occupational settings. More human exposure studies are needed.

Human Exposure to 6:2 FTS

- Human exposure to 6:2 FTS appears to be low and infrequent
- Humans may be exposed to 6:2 FTS through contaminated air, water, food products, or consumer products

Many PFAS, including PFOS, have been measured in both maternal and umbilical cord blood, which indicates that PFAS can cross the placenta, resulting in exposure to the developing fetus. One study has demonstrated that 6:2 FTS can also cross the placenta, although available toxicity data indicate that 6:2 FTS does not cause toxicity to the developing fetus in animal models.

Detection of 6:2 FTS in human blood demonstrates that human exposure occurs; however, the routes by which the general population may be exposed to 6:2 FTS are poorly understood. Exposure may occur through consumption of contaminated drinking water or food products, through inhalation of contaminated air, or through contact with certain consumer products. It is likely that human exposure to 6:2 FTS occurs through a combination of all of these routes, as 6:2 FTS has been detected in consumer products (i.e., polishes, carpets, circuit boards), drinking water, air, and in certain species of edible fish. Finally, there is evidence that certain PFAS used in food packaging products may breakdown to 6:2 FTS, and thus exposure to these "precursor" compounds may also indirectly contribute to human exposure.

3. Ecological Toxicology of 6:2 FTS

Several laboratory studies have been conducted to investigate the toxicity of 6:2 FTS to aquatic organisms such as rainbow trout, *Daphnia magna* (aquatic invertebrates), and green algae following both short-term (i.e., acute) and long-term (i.e., chronic) exposure. For both acute and chronic studies, 6:2 FTS was less toxic than PFOS to aquatic organisms. Further, a peer-reviewed aquatic hazard assessment concluded that 6:2 FTS "*presents little risk to aquatic organisms*[1]."

[1] Hoke et al. 2015. Aquatic hazard, bioaccumulation and screening risk assessment for 6: 2 fluorotelomer sulfonate. *Chemosphere*, *128*, pp.258-265.

The toxicity of 6:2 FTS to terrestrial organisms has been assessed in one species, the earthworm. In short-term acute studies, 6:2 FTS and PFOS caused toxicity at similar exposure levels. Alternatively, in long-term chronic studies, 6:2 FTS was less toxic than PFOS to earthworms. There is uncertainty surrounding the effects that 6:2 FTS may have on terrestrial organisms – more studies are needed.

Bioaccumulation is the accumulation of a chemical in an organism, and occurs when exposure exceeds the rate at which an organism can metabolize and/or excrete a chemical from the body. Bioaccumulation can be problematic, as it can result in the accumulation of a chemical in an organism to a level that may lead to adverse effect on organismal health. Both laboratory and field-based studies indicate that 6:2 FTS is unlikely to be bioaccumulative.

6:2 FTS is not bioaccumulative

- Available laboratory and field-based studies indicate that 6:2 FTS is unlikely to be bioaccumulative.

4. Environmental Occurrence and Fate of 6:2 FTS

PFAS can enter the environment through a variety of routes, including through waste streams (i.e., landfill leachate, wastewater treatment plant effluent), through production and manufacturing processes, or through consumer use. Environmental monitoring studies have reported detection of 6:2 FTS in a variety of environmental media, including air, snow, rain, groundwater, surface water (i.e., rivers, lakes, estuaries, oceans), sediment, and in biota such as amphipods, birds, bird eggs, earthworms, and fish. Environmental monitoring studies indicate that 6:2 FTS occurs at low-levels in the environment at most sites; however, higher levels of 6:2 FTS have been detected in environmental media at sites associated with point-sources of contamination such as fluorochemical manufacturing facilities or fire fighter training sites where PFAS-containing aqueous film forming foam (AFFF) has been used. For example, the highest level of 6:2 FTS reported in surface water not associated with point-sources of contamination is 36 ng/L (parts per trillion, ppt), whereas detection levels of up to 28,700 ng/L (ppt) 6:2 FTS have been detected in surface water in close proximity to sites where AFFF has been used.

Once in the environment, some PFAS such as PFOS are highly persistent and do not degrade. Alternatively, studies have demonstrated that 6:2 FTS can be degraded by bacteria in the environment under certain conditions. Studies have consistently demonstrated that degradation of 6:2 FTS leads to the formation of the short-chain perfluorocarboxylic acids (PFCAs) perfluorohexanoic acid (PFHxA) and perfluoropentanoic acid (PFPeA); however, over a dozen additional intermediate PFAS may also be formed during the degradation process. Although PFHxA has been shown to pose minimal risk to human health[2], very little information on the toxicity of the other PFAS formed during 6:2 FTS degradation is available, and the effects of these PFAS on ecological and human health need to be better understood.

Additional Detailed Information on 6:2 FTS Toxicology – See Appendix 1

[2] Luz et al. 2019. Perfluorohexanoic acid toxicity, part I: Development of a chronic human health toxicity value for use in risk assessment. *Regulatory Toxicology and Pharmacology*, *103*, pp.41-55.
[2] Anderson et al. 2019. Perfluorohexanoic acid toxicity, part II: Application of human health toxicity value for risk characterization. *Regulatory Toxicology and Pharmacology*, *103*, pp.10-20.

Appendix 1. Technical Support Document: 6:2 Fluorotelomer Sulfonate (6:2 FTS)

1. Overview of 6:2 FTS Toxicology

Compared to per- and polyfluoroalkyl substances (PFAS) such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), the toxicology database for 6:2 fluorotelomer sulfonate (6:2 FTS) may be considered "limited" even though the standard testing for regulatory approval for industry manufacture and use have been done. These data are available on the European Union (EU)'s REACH dossier and consists of industry-conducted guideline studies that do not appear to have undergone independent expert peer-review (ECHA 2018).

Between the EU dossier and available peer-reviewed publications, the toxicology database for 6:2 FTS consists of:

(1) six genotoxicity studies;

(2) three studies designed to test for skin sensitization and/or irritation;

(3) two acute toxicity studies;

(4) one reproductive/developmental toxicity study;

(5) two sub-chronic systemic toxicity studies; and

(6) one liver toxicity study.

Chronic (2-year) systemic toxicity or carcinogenicity studies, two-generational reproductive toxicity studies, or studies specifically designed to test for immunotoxicity, developmental neurotoxicity, or endocrine disruption have not yet been conducted for 6:2 FTS.

Additionally, several studies have been conducted to assess the ecological toxicity of 6:2 FTS, and include acute toxicity tests conducted with fish, invertebrates (*Daphnia magna*), algae, and earthworms; and chronic toxicity studies conducted with fish and earthworms.

A summary of toxicity studies and the main conclusions, as presented by the study authors, are provided in Table 1.

Appendix 1. Technical Support Document: 6:2 Fluorotelomer Sulfonate (6:2 FTS)

Table 1. Summary of 6:2 FTS Toxicology Studies.

Study	Main conclusion	Reference
Genotoxicity Studies		
OECD 471 - Bacterial reverse mutation assay	▪ 6:2 FTS is not mutagenic	ECHA
OECD 473 - In vitro mammalian chromosome aberration test in CHO cells	▪ 6:2 FTS-related chromosomal aberrations were not observed	ECHA
OECD 474 - Mammalian erythrocyte micronucleus test	▪ 6:2 FTS did not induce micronucleus formation	ECHA
OECD 475 - Mammalian bone marrow chromosome aberration test	▪ 6:2 FTS did not induce chromosomal aberrations	ECHA
OECD 486 - Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo	▪ 6:2 FTS did not induce unscheduled DNA synthesis	ECHA
OECD 489 - In vivo mammalian alkaline comet assay	▪ 6:2 FTS did not induce DNA damage in vivo	ECHA
Mammalian Toxicology Studies[1]		
OECD 429 - Skin sensitization (local lymph node assay) - conducted with mice	▪ 6:2 FTS is not a dermal sensitizer	ECHA
OECD 435 - In vitro membrane barrier test method for skin corrosion	▪ 6:2 FTS is irritating to skin ▪ 6:2 FTS is classified as skin corrosive Category 1A with the accompanying hazard statement (H314) "Causes severe skin burns and eye damage."	ECHA
In vivo (rabbit) skin irritation test	▪ 6:2 FTS is not irritating to the skin	Buck, 2015
OECD 402 - Acute (single dose) dermal toxicity - conducted with Sprague Dawley rats	▪ 6:2 FTS is not acutely toxic via the dermal exposure route	ECHA
OECD 420 - Acute (single dose) oral toxicity - fixed dose procedure - conducted with Wistar rats	▪ The oral LD50 is >300, but less than 2,000 mg/kg 6:2 FTS ▪ 6:2 FTS is classified as GHS Category 4, with the hazard statement (H302) "Harmful is swallowed."	ECHA
14-Day oral dose range finding study with Wistar rats	▪ Kidney toxicity observed (i.e., increased kidney weight, altered creatinine and urea levels)	ECHA
OECD 422 - Combined repeated dose (90-day) toxicity study with the reproduction/developmental toxicity screening test - conducted with Wistar rats	▪ 6:2 FTS is not a reproductive or developmental toxicant ▪ Kidney toxicity observed (i.e., elevated urea levels, increased incidence of multi-focal tubular dilation in male and female kidneys)	ECHA
28-Day subchronic study with CD1 mice (only 1 dose used; 5 mg/kg-day)	▪ Exposure to 6:2 FTS (ammonium salt) was associated with liver toxicity (i.e., increased liver weight, increased incidence of necrosis, hepatocellular hypertrophy)	Sheng et al. (2017)
Aquatic Toxicology Studies		
OECD 201 - Freshwater alga and cyanobacteria, growth inhibition test with green alga (*P. subcapitata*)	▪ The 72-hour EC50 ranges from >96 mg/L (potassium salt of 6:2 FTS) to >125 mg/L (6:2 FTS)	ECHA; Hoke et al., 2015
OECD 202 - Daphnia sp. acute immobilization test	▪ The 48-hour EC50 ranges from >109 mg/L (potassium salt of 6:2 FTS) to >112 mg/L (6:2 FTS)	ECHA; Hoke et al., 2015
OECD 203 - Acute toxicity test - conducted with rainbow trout	▪ The 96-hour LC50 ranges from >107 mg/L (potassium salt of 6:2 FTS) to >108 mg/L (6:2 FTS)	ECHA; Hoke et al., 2015
OECD 210 - Fish early-life stage toxicity test (90-day) - conducted with rainbow trout	▪ The 90-day NOEC is 2.62 mg/L 6:2 FTS (potassium salt)	ECHA; Hoke et al., 2015
Conclusions by Hoke et al. (2015)	▪ 6:2 FTS is not expected to pose a risk to aquatic organisms	Hoke et al., 2015

[1] Results from several unpublished toxicology and bioaccumulation studies for 6:2 FTS were presented at the Society of Environmental Toxicology and Chemistry Meeting (SETAC; Buck, 2018). Unpublished toxicology studies included 28-day oral and 4-hour inhalation studies, both conducted with rats. Further, a bioaccumulation study was conducted in rats in which 6:2 FTS was not found to be bioaccumulative.

Abbreviations:
EC50 = effective concentration that elicits 50% of the response; LC50 = lethal concentration that elicits 50% of the response; LD50 = lethal dose that elicits 50% of the response; NOEC = No observable effect concentration

2. Mammalian Toxicology

6:2 FTS is less acutely toxic than PFOS. Results from two acute toxicity studies are listed in the REACH registration dossier for 6:2 FTS (ECHA, 2018), and include oral and dermal exposure studies. 6:2 FTS was not found to be acutely toxic via the dermal route of exposure at doses of up to 2000 mg/kg in an Organization for Economic Cooperation and Development (OECD) Guideline 402 study (OECD 2017; Acute Dermal Toxicity). Alternatively, 6:2 FTS was found to have moderate acute toxicity via the oral exposure route in an OECD Guideline 420 study (OECD, 2002; Acute Oral Toxicity), with an LD50[1] value of between 300 to 2,000 mg/kg. Based on this result, 6:2 FTS was GHS (Globally Harmonized System) classified category 4 for acute oral toxicity, which has the accompanying hazard statement "H302: Harmful if swallowed." Additionally, Field & Seow (2017) report the acute oral LD50 for rats to be 1,871 mg/kg; however, the study authors did not provide a complete reference and this LD50 could not be verified. Comparatively, the acute oral LD50 value for PFOS has been reported to be 251 mg/kg in rats (Dean et al., 1978). These results demonstrate that 6:2 FTS is less acutely toxic than PFOS via the oral exposure route.

6:2 FTS is not genotoxic. No chronic toxicity studies investigating the carcinogenicity of 6:2 FTS have been conducted. However, a variety of *in vitro* and *in vivo* studies investigating the genotoxic and/or mutagenic potential of 6:2 FTS have been conducted and are reported in the REACH registration dossier for 6:2 FTS (ECHA, 2018). Results from these studies indicate that 6:2 FTS is:

- not mutagenic in the bacterial reverse mutation assay (Guideline 471; OECD, 1997a);
- does not cause DNA damage in an *in vivo* mammalian comet assay (Guideline 489; OECD, 2016a);
- does not induce micronuclei formation (indication of chromosomal damage) in a *in vivo* bone marrow assay (Guideline 474; OECD, 2016b);
- does not induce chromosomal aberrations *in vivo* (Guideline 475; OECD, 1997b) or *in vitro* (Guideline 473; OECD, 1997c) test systems;
- does not causes unscheduled DNA synthesis (Guideline 486; OECD, 1997d).

Collectively, these results indicate that 6:2 FTS is not genotoxic or mutagenic. Similarly, numerous studies have demonstrated that PFOS is not genotoxic [reviewed in USEPA, 2016].

6:2 FTS can cause kidney and liver toxicity. Three studies have been conducted that evaluate the toxicity of 6:2 FTS following repeat exposure. These studies include a 14-day dose-range finding study in rats (ECHA, 2018), a 28-day subchronic study in mice (Sheng et al., 2017), and a 90-day subchronic toxicity study in rats (ECHA, 2018). In the 14-day study, male and female

[1] The LD_{50} is the lethal dose that causes 50% mortality.

rats were orally exposed to 10, 50, or 100 mg/kg-day 6:2 FTS. No mortality or clinical signs of toxicity were reported in either sex at any dose. Signs of toxicity included reduced body weight gains and reduced food consumption in male (50 and 100 mg/kg-day treatment groups) and female (100 mg/kg-day group only) rats. Signs of kidney toxicity were observed, and included alterations in serum levels of creatinine and urea, and increased kidney weight in male (50 and 100 mg/kg-day treatment groups) and female (100 mg/kg-day group only) rats (ECHA, 2018).

In the 90-day OECD Guideline 422 study (OECD 1996; combined repeated dose toxicity study with the reproduction/developmental toxicity screening test) male and female rats were orally exposed to 5, 15, or 45 mg/kg-day 6:2 FTS. 6:2 FTS treatment had no effect on mortality, food consumption, or animal behavior, and no gross pathological or neoplastic histopathological findings were reported for either sex at any dose. Treatment-related reduced body weight gains were detected in both sexes in the highest treatment group (45 mg/kg-day). Several clinical signs of toxicity were observed, and included effects related to the skin (sparse hair, encrustations), eye (blepharospasm in one low-dose male rat), and respiratory system (sniffling respiration in two high-dose male rats); however, due to low-incidence and distribution, these effects were not considered treatment-related by the study authors. Hematological findings were reported, including reduced monocytes in male rats (5 mg/kg-day only); however, these effects were not considered treatment-related, as they did not occur in a dose-dependent manner. Mean total protein and albumin serum levels were slightly reduced in male rats (5 and 45 mg/kg-day, but not 15 mg/kg-day group); these effects did not occur in a dose-dependent manner and were not considered treatment-related. Alternatively, a dose-dependent increase in urea levels was reported for high-dose male rats, and was considered treatment related. Additional signs of kidney toxicity were also reported, and these effects were more pronounced in male rats. These effects included increased kidney weight in low- and high-dose male rats, and mild to moderate multifocal tubular dilation in the kidneys of high-dose male (5/12) and female (1/12) rats (ECHA, 2018).

In the 28-day study conducted by Sheng et al. (2017), male CD1 mice were orally exposed to 5.0 mg/kg-day 6:2 FTS, and liver toxicity was investigated. Exposure to 6:2 FTS resulted in increased liver weight, increased serum levels of aspartate transaminase and albumin, and signs of hepatocellular hypertrophy and necrosis. Collectively, these results indicate that 6:2 FTS is hepatotoxic. However, this study has limitations, including the small number of endpoints measured (kidney toxicity not investigated), and only a single exposure group was included in the study, which prevents any dose-response analysis.

Collectively, results from Sheng et al. (2017) and ECHA (2018) indicate that 6:2 FTS can cause kidney and liver toxicity in highly exposed rodent models. Comparatively, the majority of rodent toxicology studies conducted with PFOS do not report kidney toxicity [reviewed in USEPA, 2016], while subchronic and chronic toxicology studies have consistently demonstrated PFOS-induced liver toxicity [reviewed in USEPA, 2016].

6:2 FTS is not a reproductive or developmental toxicant. One study investigating the reproductive and developmental effects of 6:2 FTS has been conducted (ECHA, 2018). The study adhered to OECD Guideline 422 (OECD, 1996; Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) in which male and female rats were orally exposed to 6:2 FTS (5, 15, or 45 mg/kg-day) prior to mating, during mating, and throughout gestation and lactation for a total of approximately 90-days. For parental rats, no treatment-related changes were reported for any reproductive parameter, such as reproductive organ weight, estrous cyclicity, serum T4 hormone levels, or reproductive performance. Similarly, no clinical signs of toxicity, or treatment-related effects on survival, body weight, sexual maturation, organ weight, gross pathology or hormone levels were reported for pups. Collectively, these results indicate that 6:2 FTS is not a reproductive or developmental toxicant at doses of up to 45 mg/kg-day.

Alternatively, numerous studies have reported developmental effects associated with PFOS exposure in rats, mice, and rabbits [for a comprehensive review of PFOS-related developmental and reproductive effects see USEPA, 2016], and these effects have been reported as exposure levels as low as 1.6 – 10 mg/kg-day (Luebker et al., 2005a,b; Thibodeaux et al., 2003).

6:2 FTS can cause skin irritation, but not sensitization. One study has been conducted with mice to investigate the ability of 6:2 FTS to act as a skin sensitizer (i.e., cause allergic skin reactions). Results from the OECD Guideline 429 study (OECD 2010; Local Lymph Node Assay) indicate that 6:2 FTS is not a skin sensitizer (ECHA, 2018). Alternatively, 6:2 FTS is GHS classified category 1 for skin corrosivity, which has the following hazard statement "H314: Causes severe skin burns and eye damage." The GHS classification is based on results from an *in vitro* OECD Guideline 435 study (OECD, 2006; In Vitro Membrane Barrier Test Method for Skin Corrosion) that indicated 6:2 FTS is corrosive to skin (ECHA, 2018). 6:2 FTS was reported to be not irritating to the skin of rabbits in a non-peer-reviewed book chapter (Buck, 2015); however, no additional study details were provided. The reason for the discrepancies are unclear, but may related to the chemical form of 6:2 FTS administered. 6:2 FTS acid, which was used in the *in vitro* study, is expected to be more irritating/corrosive than non-acidic 6:2 FTS salts, which may have been used in the *in vivo* rabbit study; however, the form of 6:2 FTS used was not reported in Buck (2015).

6:2 FTS is classified as an eye irritant. No formal studies have been conducted to test if 6:2 FTS is an eye irritant. However, based on Regulation No. 1272/2008 skin corrosive chemicals are to be classified as serious eye damage category 1, which has the accompanying hazard statement H318: Causes serious eye damage" (ECHA, 2018). Comparatively, *in vivo* studies have demonstrated that PFOS can also cause severe eye irritation (Riker Laboratories, Inc., 1981).

3. Ecological Toxicology

The acute and chronic toxicity of 6:2 FTS has been investigated in several aquatic (fish, *Daphnia*, algae) and terrestrial (earthworms) species. In general, and as discussed in more detail below, 6:2 FTS is less toxic to aquatic and terrestrial organisms than PFOS (Table 2).

Table 2. Comparison of 6:2 FTS and PFOS ecotoxicity.

	6:2 FTS	PFOS
96-Hour Rainbow Trout LC_{50} (mg/L)	>107[a]	7.8 – 22[b]
48-Hour *Daphnia magna* Immobilization EC_{50} (mg/L)	>109[a]	67.2[b]
72-Hour *Pseudokirchneriella subcapitata* Growth Rate (E_rC_{50}; mg/L)	>96[a]	48.2[b]
72-Hour *Pseudokirchneriella subcapitata* $NOEC_r$ (mg/L)	47.6[a]	42[b]
90-Day Rainbow Trout NOEC (mg/L)	2.62[a]	0.29[b,c]
14-Day Earthworm LC_{50} (mg/kg)	373[d]	365[e]
56-Day Earthworm EC_{50} (mg/kg) - # of cocoons	566[f]	103[f]
56-Day Earthworm EC_{50} (mg/kg) - juvenile weight	253[f]	29[f]

[a] Hoke et al., 2015
[b] Beach et al., 2006
[C] NOEC is for a 47-d test with fathead minnows, not rainbow trout
[d] 3M, 2003
[e] Juong et al. (2010)
[f] Stubberud (2006)

Accronyms: EC_{50} = effective concentration that gives half-maximal response; E_rC_{50} =EC50 in terms of reduced growth rate; LC50 = lethal concentration that causes 50% mortality; NOEC = no observable effect concentration; NOECr = NOEC for growth rate

3.1 Aquatic Toxicology

6:2 FTS is less acutely toxic to aquatic organisms than PFOS. The acute toxicity of 6:2 FTS has been investigated in several species, including *Oncorhynchus mykiss* (rainbow trout), *Daphnia magna* (aquatic invertebrate), and *Pseudokirchneriella subcapitata (green algae).* Compared to PFOS, 6:2 FTS is less acutely toxic to rainbow trout, with a 96-hour LC50 value of >107 mg/L (Hoke et al., 2015), while the 96-hour LC50 value for PFOS ranges from 7.8 to 22 mg/L (Beach et al., 2006). Similarly, *Daphnia* are less sensitive to 6:2 FTS than to PFOS, with EC50 values of >109 mg/L 6:2 FTS (Hoke et al., 2015) and 67.2 mg/L PFOS (Beach et al., 2006). Alternatively, 6:2 FTS and PFOS inhibit green algae growth at similar concentrations, with no observable effect concentrations (NOECs) of 47.6 mg/L for 6:2 FTS (Hoke et al., 2015) and 42 mg/L for PFOS (Beach et al., 2006). However, the concentration at which 6:2 FTS inhibited green algae growth by 50% (EC50) is >96 mg/L (Hoke et al., 2015), while the EC50 for PFOS is 48.2 mg/L (Beach et al., 2006). These results indicate that the slope of the PFOS dose-response curve is steeper than that of 6:2 FTS, which indicates that 6:2 FTS is less toxic to green algae than PFOS.

6:2 FTS is less chronically toxic to fish than PFOS. One study investigating the chronic, 90-day, toxicity of 6:2 FTS in early-life stage rainbow trout has been conducted (Hoke et al., 2015). In this study, the most sensitive toxicological endpoint was embryo hatching, with a NOEC of 2.62 mg/L. No 90-day early-life stage studies with rainbow trout were identified for PFOS. However, results from a 47-day PFOS chronic toxicity study conducted with Fathead minnows is available (Beach et al., 2006). The lowest NOEC identified in this study was 0.29 mg/L. Although the studies were conducted in different species of fish, both were early-life stage chronic toxicity studies. Further, the PFOS NOEC is nearly 10-fold lower than the 6:2 FTS NOEC. This result indicates that fish are more sensitive to chronic PFOS exposure than to chronic 6:2 FTS exposure.

3.2 Terrestrial Toxicology

6:2 FTS and PFOS display similar acute toxicity to earthworms. One study investigating the acute toxicity of 6:2 FTS to terrestrial organisms has been conducted. In a study conducted by 3M (2003), the 14-day LC50 of 6:2 FTS for earthworms was determined to be 373 mg/kg soil (3M, 2003). Similarly, Juong et al. (2010) determined the 14-day LC50 value of PFOS to be 365 mg/kg soil. Both studies were conducted under similar experimental conditions. Thus, these results indicate 6:2 FTS and PFOS are similarly acutely toxic to earthworms.

6:2 FTS is less chronically toxic than PFOS to earthworms. A study by Stubberud (2006) reports results from an earthworm reproduction test in which the number of cocoons laid, hatching success, and juvenile worm weight was reported. The EC50 values for number of cocoons laid and juvenile weight were calculated to be 566 and 253 mg/kg soil, respectively, for 6:2 FTS. Alternatively, EC50 values for PFOS were 103 (cocoons laid) and 29 mg/kg soil (juvenile weight). These results indicate that 6:2 FTS is less chronically toxic to earthworms than PFOS.

More research on the potential terrestrial and mammalian toxicity of 6:2 FTS is needed.

4. Toxicokinetics and Bioaccumulation

6:2 FTS is eliminated more rapidly than PFOS. Toxicokinetics is the study of how the body handles a chemical, and includes analysis of a chemical's rate of adsorption, tissue distribution, metabolism, and excretion. Limited information is available on the toxicokinetics of 6:2 FTS. One *in vivo* toxicokinetics study and one *in vitro* metabolism study are reported in the REACH registration dossier for 6:2 FTS; however, minimal study details are provided (ECHA, 2018). For the toxicokinetic study, two single doses (dosing levels not provided) of 6:2 FTS were administered (species tested was not reported) and then fat, liver, plasma, and urine samples were collected to investigate the distribution and excretion of 6:2 FTS. Fat-to-plasma ratios could not be calculated for females, while ratios were <0.1 (low dose) to 0.1 (high dose) for

males indicating 6:2 FTS does not accumulate in fat. Similarly, liver-to-plasms ratios could not be determined for females, while ratios were 3.0 (low dose) to 3.1 (high dose) for males, indicating partitioning of 6:2 FTS to the liver. 65-68% of the administered dose of 6:2 FTS was recovered in urine 96-hours after dosing, and elimination half-lives ranged from 20.9 to 23.8 hours. Comparatively, the elimination half-life of PFOS following oral administration has been reported to range from 30.5 to 42.8 days for mice and 39.8 to 66.7 days for rats (Chang et al. 2012). These results indicate that 6:2 FTS will be eliminated more rapidly than PFOS.

In vitro studies indicate 6:2 FTS may not be readily metabolized. Two *in vitro* studies investigating the metabolism of 6:2 FTS have been conducted. In the first, 6:2 FTS was incubated with male rat liver S9 microsomes for 2-hours, which is a common *in vitro* method employed to estimate *in vivo* metabolism. No metabolism of 6:2 FTS was detected (ECHA, 2018). In a second study, 6:2 FTS was incubated with rainbow trout hepatocytes for 2-hours; no metabolism of 6:2 FTS was detected (Hoke et al., 2015). However, *in vitro* test systems do not completely replicate *in vivo* metabolism, thus, *in vivo* studies should be conducted to confirm *in vitro* results. Numerous studies have demonstrated that PFOS is recalcitrant to metabolism *in vitro* and *in vivo* [reviewed in USEPA, 2016].

6:2 FTS is not bioaccumulative. Bioaccumulation is the accumulation of a chemical in an organism, and occurs when exposure and adsorption of a chemical occurs at a faster rate than metabolism and/or excretion. Bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) are the ratio of a chemical in an organism to the concentration in the surrounding environment. According to the U.S. EPA, chemicals with BCFs >1000 are considered bioaccumulative, while other regulatory agencies in Canada, the European Union, and the United Nations consider chemicals with BAFs/BCFs of 2,000 to 5,000 to be bioaccumulative (Arnot and Gobas, 2006).

Laboratory- and field-based BCFs/BAFs for 6:2 FTS have been calculated for fish (rainbow trout, white sucker), earthworms, midge, and biofilm. As can be seen in Table 3, all estimated BCFs are below 1000 indicating that 6:2 FTS is not classifiable as bioaccumulative by the most conservative regulatory standard.

Table 3. Summary of 6:2 FTS BCFs and BAFs

Species	Exposure Details	BCF/BAF	Reference
Oncorhynchus mykiss (rainbow trout)	Aqueous exposure to 6:2 FTS	3 - 36	Hoke et al., 2015
	Dietary exposure to 6:2 FTS.[1]	0.3	
Oncorhynchus mykiss (rainbow trout)	Aqueous exposure to 6:2 FTS containing AFFF	ND	Yeung & Mabury, 2013
Chironomus riparius (midge)	Exposure to 6:2 FTS contaminated environmental sediments.	0.018	Bertin et al., 2014
Gammarus s pp. (Crustacea)	Exposure to 6:2 FTS contaminated environmental sediments.	0.88	Bertin et al., 2016
Catostomus commersonii (white sucker)	Field-based estimates	0.23 - 0.95	Munoz et al., 2017
Biofilm[2]	Field-based estimates	148	Munoz et al., 2018
Eisenia fetida (earthworms)	Exposure to 6:2 FTS contaminated soil from fire training facilities.	2.4	NPCA, 2008

[1] Dietary biomagnification factors are estimated for dietary exposures.

[2] Log BCF values were estimated to be 1.4, 1.5, and 3.6 from three different locations for a mean value of 2.17, which is equivalent to a BCF of 148.

ND = Not determined; accumulation was not sufficient for BCFs to be estimated.

Compared to PFOS, 6:2 FTS has reduced binding affinity to some proteins. In contrast to most bioaccummulative chemicals, which are lipophilic and accumulate in fat, PFOS bioaccumulation is associated with binding to proteins such as serum albumin, fatty acid binding protein in the liver, and organic anion transporters in the kidney. One study investigating 6:2 FTS protein binding has been published (Sheng et al. 2018). In this study, a fluorescent displacement assay was used to investigate PFOS and 6:2 FTS binding to human liver FABP (hL-FABP). The concentrations required to cause a 50% fluorescent displacement (IC50) were 1.34 and 78.97 µM for PFOS and 6:2 FTS, respectively, indicating that 6:2 FTS has reduced binding affinity to hL-FABP. Furthermore, Sheng et al. (2018) used molecular docking software to investigate the molecular interactions of PFAS with the hL-FABP binding pocket. Using this approach, no binding of 6:2 FTS to the hL-FABP was observed, whereas PFOS binding could be modelled.

5. Human Exposure to 6:2 FTS

Human exposure to 6:2 FTS appears to be low and infrequent. 6:2 FTS has been detected in human serum, thus demonstrating that exposure to 6:2 FTS can occur (Table 4). However, compared to PFOS, 6:2 FTS human biomonitoring data is limited, and only four studies reporting human serum levels of 6:2 FTS are available. In contrast, 100s of studies investigating

human exposure to PFOS have been conducted. Additionally, results from all 6:2 FTS biomonitoring studies are based on small sample sizes, and were conducted nearly a decade ago. Thus, it is unclear how human exposure to 6:2 FTS has changed over the past decade, or how occupational exposure levels compares to that of the general population. Additional research on the potential sources of 6:2 FTS and extent of human exposure are needed.

Table 4. Summary of 6:2 FTS Biomonitoring Data

Location	Sampling Year	Sample/ gender/ age	N	FOD	LOD (ng/L)	Min (ng/L)	Median (ng/L)	Arithmetic Mean (ng/L)	Max (ng/L)	Reference
U.S.	2009	Serum, M	20	42%	5	<LOD	--	5.91	18.39	Lee & Mabury (2011)[1]
		Serum, F	20	65%	5	<LOD	--	9.28	29.54	
China	2009	Matenal serum	50	64%	3	<LOD	11.55	13.39	48.24	Yang et al. (2016)[2]
		Cord serum	50	68%	3	<LOD	13.53	20.99	90.21	
Hong Kong	--	Blood, M + F	20	100%	--	0.34	1.17	2.19	7.89	Loi et al. (2013)[4]
Australia	2011	Serum, Age: 0-4	4	50%	--	<LOD	--	10	20	Eriksson et al. (2017)[1,3]
		Serum, Age: 5-15	4	0%	--	--	--	--	--	
		Serum, Age: 16-30	4	25%	--	<LOD	--	10	50	
		Serum, Age: 31-45	4	25%	--	<LOD	--	10	30	
		Serum, Age: 46-60	4	0%	--	--	--	--	--	
		Serum, Age: >60	4	0%	--	--	--	--	--	

[1] For purposes for calculating the mean, samples with 6:2 FTS levels below the LOD were substituted with a value of zero.

[2] For puposes of calculating the mean, samples with 6:2 FTS levels below the LOD were substituted with a value of the LOD divided by the square root of two.

[3] N = 4 pooled serum samples; each sample consisted of 100 individual pooled serum samples.

[4] Serum data is reported in units of pg/g, which is equivalent to parts per trillion (ppt); ng/L is also equivalent to ppt

"--" = Value not provided; "FOD" = frequency of detection; "LOD" = limit of detection

One biomonitoring study was conducted in the United States in 2009 (Lee & Mabury, 2011). In this study, 6:2 FTS was detected in the serum of 42% of male participants and 65% of female participants with arithmetic mean serum levels ranging from 5.9 ng/L (males) to 9.3 ng/L (females). These serum levels are significantly lower than the arithmetic mean serum levels of PFOS reported in the general U.S. population. During the 2009-2010 NHANES survey period, PFOS was detected in the serum of >99% of participants with median levels ranging from 7,800 ng/L (females) to 11,800 ng/L (males) (CDC, 2018). These findings indicate that human exposure to 6:2 FTS is lower than that of PFOS.

6:2 FTS can cross the placenta resulting in fetal exposure. One study has demonstrated that 6:2 FTS can cross the placental resulting in exposure to the developing fetus (Yang et al., 2016). In this study, 6:2 FTS was measured in paired maternal and cord serum from 50 Chinese mothers. The frequency of detection and mean serum levels of 6:2 FTS were similar for maternal and cord serum, and the median placental transfer ratio was 1.2:1 indicating that 6:2 FTS can freely cross the placenta. This finding is similar to PFOS, which has also been shown to cross the placenta.

6. Exposure Routes

Human exposure to 6:2 FTS may occur via a variety of routes, including consumption of contaminated drinking water or food products, through inhaling 6:2 FTS in the air, or through contact with consumer products that may contain 6:2 FTS. Field and Seow (2017) recently reviewed available monitoring data, and demonstrated that exposure to 6:2 FTS through these routes is possible, as 6:2 FTS has been detected in (1) consumer products including circuit boards, carpets, and polishes; (2) in drinking water in Spain, Germany, and France; (3) in air; and (4) in certain species of fish (see Field and Seow (2017) for a complete review).

Although, it is apparent that human exposure to 6:2 FTS occurs, it remains unknown whether humans are exposed directly to 6:2 FTS or to PFAS precursors that can degrade to 6:2 FTS. Lee & Mabury (2011) suggest that indirect exposure to 6:2 FTS may occur first through exposure to disubstituted thioether phophates (S-diPAPS; also known as fluorotelomer mercaptoalkyl phosphate esters or FTMAPs), which are used in food packaging products (Trier et al., 2011), and can be metabolized to 6:2 FTS. A wide variety of additional 6:2 FTS precursors have been proposed based on chemical structures (See Table 12 of Field & Seow, 2017), many of which are constituents of firefighting foams (i.e., AFFF); however, little empirical data exists to directly link degradation of these compounds to 6:2 FTS. It is likely that direct exposure to 6:2 FTS and precursors both contribute to human exposure.

7. Environmental Occurrence & Persistence

7.1 Environmental Occurrence

6:2 FTS is detected at low levels and frequencies at sites associated with non-point-sources of contamination. Several studies have monitored the environmental occurrence of 6:2 FTS at both sites associated with non-point sources and with point sources (i.e., firefighting training facilities or fluoropolymer facilities) of contamination [reviewed in Field and Seow, 2017]. 6:2 FTS has been detected in (1) landfill leachate and sediment in Sweden, Norway, Germany, and the U.S.; (2) in wastewater treatment plant influent, effluent, and sludge; (3) in rain and snow; (4) in surface waters including rivers, lakes, estuaries, and oceans; and (5) in organisms including amphipods, birds, bird eggs, worms, and fish. In general, 6:2 FTS is detected at low levels and frequencies in environmental samples collected from sites with non-point sources of contamination, while higher detection frequencies and levels are often reported at sites with known point sources of contamination (Field and Seow, 2017). For example, the highest level of 6:2 FTS reported in surface waters not associated with point sources of contamination is 36 ng/L (Nguyen et al, 2011), while the highest level of 6:2 FTS reported in surface water with known point sources of contaminate (fire-fighting foam) is 28,700 ng/L (Boiteux et al., 2016).

7.2 Environmental Persistence

Biodegradation of 6:2 FTS occurs under aerobic, sulfur-limiting, conditions. Several studies have been conducted to investigate biodegradation of 6:2 FTS under varying conditions. An OECD Guideline 301B (CO_2 Evolution Test; OECD 1992) study was conducted to test whether 6:2 FTS could be utilized as a carbon source by bacteria. Over 28-days a maximum loss of 42% was observed; based on test guidelines this result indicates that 6:2 FTS is not readily biodegradable (ECHA, 2018). Similarly, several studies have demonstrated that 6:2 FTS is not readily biodegradable under anaerobic conditions (Zhang et al. 2016), or in activated sludge from WWTPs under anaerobic or aerobic conditions (Ochoa-Herrera et al., 2016; Wang et al., 2011). Alternatively, several studies have demonstrated that 6:2 FTS can be utilized as a sulfur source by bacteria under aerobic, sulfur-limiting conditions (Key et al., 1998; Shaw et al., 2019; Van Hamme et al., 2013; Zhang et al., 2016). Zhang et al. (2016) report that 6:2 FTS undergoes rapid biodegradation in aerobic sediment, with a biodegradation half-life of less than five days. Biodegradation pathways of 6:2 FTS have also been proposed in several publications, and PFHxA (PFCA with 5 fluorinated carbons), PFPeA (PFCA with 4 fluorinated carbons) and the 5:3 fluorotelomer acid are consistently reported to be the primary terminal degradation products (Shaw et al., 2019; Van Hamme et al., 2013; Wang et al.. 2011; Zhang et al., 2016). However, numerous intermediary PFAS are formed throughout the biodegradation process. Shaw et al. (2019) identified 16 intermediary metabolites, many of which are shown in the proposed major degradation pathway for 6:2 FTS (Figure 1). Major metabolites identified by Shaw et al. (2019) include: 6:2 fluorotelomer (6:2 FT) alcohol (6:2

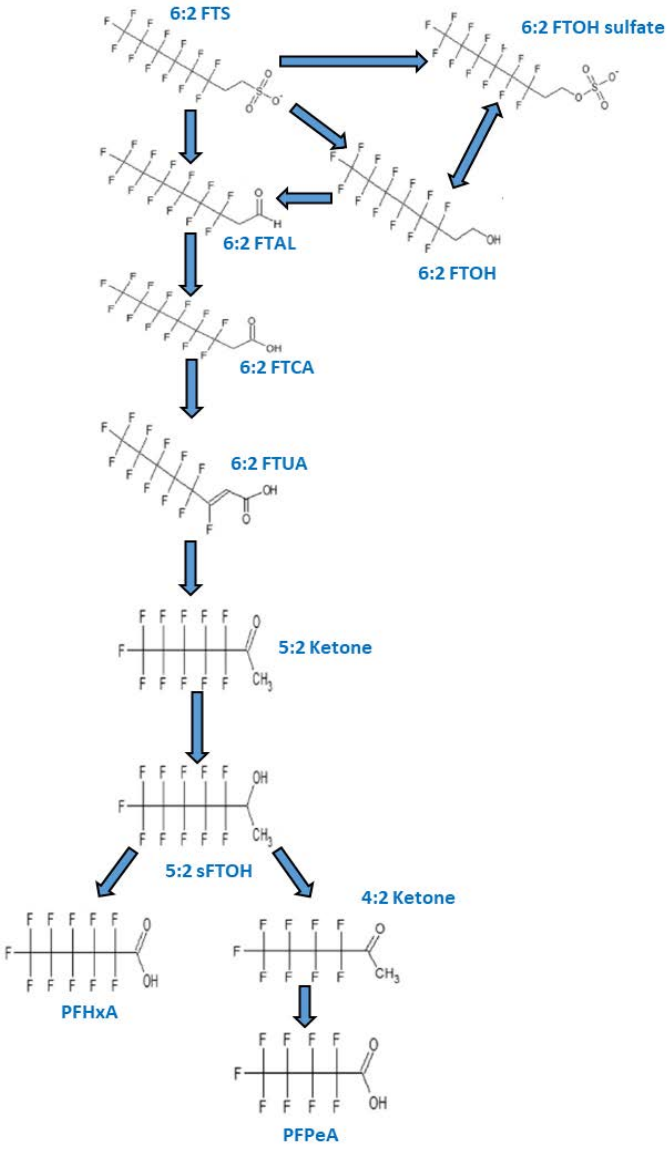


Figure 1. Proposed major degradation pathway for 6:2 FTS. Adapted from Shaw et al. (2019).

FTOH), 6:2 FT alcohol sulfate (6:2 FTOH sulfate), 6:2 FT aldehyde (6:2 FTAL), 6:2 FT acid (6:2 FTAC), 6:2 FT unsaturated acid (6:2 FTUA), 5:2 FT ketone, 5:2 FT secondary alcohol (5:2 sFTOH), 4:2 FT ketone, and the short-chain PFCAs PFHxA and PFPeA. Although, the terminal degradation product PFHxA has been shown to pose minimal risk to human health (Anderson et al., 2019), very little toxicological information is available for the other metabolites formed during 6:2 FTS biodegradation, or the proposed terminal degradation products formed through proposed minor degradation pathways, such as PFBA and 4:2 FTUA (Shaw et al., 2019).

Significant and important data gaps related to the environmental and *in vivo* degradation of 6:2 FTS remain.

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