Technical Support Document:
Reference Dose and Fish Consumption Triggers for Perfluoroundecanoic Acid (PFUnDA)

Interagency Toxics in Biota Committee (TIBC) Risk Subcommittee

October 26, 2022

Members of the TIBC Risk Subcommittee:

Gloria Post, Ph.D., D.A.B.T., Chair and Primary Author
Gary Buchanan, Ph.D. (retired)
Josephine Bonventre, Ph.D.
Jessie Gleason, M.S.P.H.
Daniel Millemann, Ph.D.

[Please cite as: Toxics in Biota Risk Subcommittee (2022)]

Executive Summary

The interagency Toxics in Biota Committee (TIBC) requested that the Risk Subcommittee determine fish consumption triggers for perfluoroundecanoic acid (PFUnDA; CAS # 2058-94-8) because this contaminant is highly bioaccumulative in fish and has been detected at high levels in recreationally caught fish in New Jersey. A literature search was performed for relevant peer reviewed publications, and risk assessments from other states were identified. PFUnDA is found in the blood serum and breast milk of the general population, and it has a long human half-life, estimated as 12 years. It has also been detected in human umbilical cord blood and fetal organs. In several studies, fish consumption has been associated with increased blood serum levels of PFUnDA.

Like other per- and polyfluoroalkyl substances (PFAS), PFUnDA causes hepatic and developmental toxicity in laboratory animals. The critical endpoint selected as the basis for the Reference Dose (RfD) was increased relative liver weight in male rats exposed to 0.3 mg/kg/day PFUnDA for 42 days (Takahashi et al., 2014); the NOAEL for this effect was 0.1 mg/kg/day. Histopathological changes indicative of liver damage and increased blood serum levels of liver enzymes at a higher dose (1.0 mg/kg/day) indicate that the hepatic effects of PFUnDA are adverse, and mode of action (MOA) evaluation indicates that they are relevant to humans. PFUnDA also caused decreased body weight in offspring of maternally-exposed dams on postnatal day (PND) 0 and PND 4 at 1.0 mg/kg/day in this study.

Other toxicological studies reported toxicity to testicular Leydig cells in male rats and pancreatic effects related to the onset of diabetes in female mice from a strain susceptible to diabetes at doses lower than the LOAEL for increased relative liver weight in male rats. While these effects were not appropriate as the primary basis for the RfD, they indicate the potential for PFUnDA to cause toxicology effects that are more sensitive than increased relative liver weight.

To develop the RfD, Benchmark Dose modeling was performed on the dose-response data for increased relative liver weight in male rats, and a BMDL (lower confidence level on the Benchmark Dose for a 10% change) of 0.19 mg/kg/day was identified. An interspecies dosimetric adjustment factor based on the ratio of half-lives of PFUnDA in humans and rats is needed to account for the much slower excretion of PFUnDA in humans than in rats to convert the BMDL to a Human Equivalent Dose (HED). Because the half-life for PFUnDA in rats has not been reported, a half-life of 30 days in male rats was estimated from available toxicokinetic data. A dosimetric adjustment factor of 146 (the ratio of the human half-life, 12 years [4380 days], and rat half-life, 30 days) was applied to derive the HED of 0.0013 mg/kg/day (1300 ng/kg/day). Uncertainty factors (UFs) of 10 for intra-human variability, 3 for interspecies extrapolation, 10 for use of a study with less than chronic duration, and 3 for potentially more sensitive effects, resulting in a total UF of 1000, were applied to the HED of 1300 ng/kg/day. The resulting RfD is 1.3 ng/kg/day.

Fish consumption triggers were developed from the RfD of 1.3 ng/kg/day, using default exposure assumptions of 70 kg body weight and 227 g fish meal size[1]. The trigger value for unlimited consumption is 0.40 ng/g (ppb), and triggers for less frequent consumption are 2.8 ng/g for weekly, 12.0 ng/g for monthly, 36.5 ng/g for once every 3 months, and 146 ng/g for yearly consumption, with no consumption advised at levels >146 ng/g.

PFUnDA reaches the developing fetus and is present in breast milk; these exposures are of concern because the fetus and infant are susceptible to the developmental effects of PFUnDA. For these reasons, it is not advisable for subgroups susceptible to developmental effects (i.e., pregnant and nursing women, young children, women of childbearing age) to receive large doses of PFUnDA, even if infrequent. Therefore, consistent with the approach used for other PFAS, consumption of fish with PFUnDA levels higher than the advisory trigger for monthly consumption is not recommended for individuals in these high-risk groups.

Introduction

The purpose of this document is to develop a Reference Dose (RfD) and fish consumption triggers for perfluoroundecanoic acid (PFUnDA; C11) in response to a request from the Interagency Toxics in Biota Committee (TIBC).

On January 19, 2022, Dr. Gary Buchanan, Chair of the TIBC, requested in an email that the TIBC Risk Subcommittee evaluate whether an RfD for PFUnDA for use as the basis for fish consumption advisories can be developed. It was noted that high levels of PFUnDA have been

[1] While the USEPA Office of Water and Superfund programs have updated the default adult body weight assumption to 80.0 kg and increased the default drinking water ingestion rate accordingly, USEPA has not updated its exposure assumptions for body weight and fish consumption rate for the development of fish consumption triggers.

found in fish tissue from locations in different parts of New Jersey and that PFUnDA is bioaccumulative in fish, even more so than perfluorooctane sulfonate (PFOS).

The Risk Subcommittee reviewed the available scientific literature on PFUnDA and determined that there is sufficient information to develop an RfD for use as the basis for fish consumption triggers for PFUnDA. The TIBC can use these triggers together with fish tissue monitoring data to develop fish consumption advisories, which provide non-regulatory advice on how the public can reduce their risk by avoiding or limiting consumption of certain recreationally caught fish.

The RfD presented in this document is based on the information that is currently available and is intended to be used only for fish consumption advisory triggers. These triggers are used to protect public health by reducing exposure to fish contaminated with PFUnDA. The Risk Subcommittee recommends that if NJDEP considers development of regulatory standards for PFUnDA in the future, the basis for the RfD developed in this document be reevaluated to determine whether it is of sufficient certainty for regulatory use. Such a reevaluation should consider any more recent scientific information that has become available.

Document Development Process and Scope of Document

A PubMed literature search for the keywords (perfluoroundecanoic acid OR PFUnDA OR PFUnA or PFUDa) AND (toxicity OR toxicology) conducted on March 15, 2022 identified 109 citations. These publications were screened by title (and abstract, when necessary) to identify publications potentially relevant to development of an RfD. Relevant publications included those reporting data on toxicity or toxicokinetics of PFUnDA in mammalian species, as well as additional publications judged to provide relevant supporting information for RfD development including selected publications on human exposure to PFUnDA, toxicity of PFUnDA in non-mammalian species, and *in vitro* studies of PFUnDA. Additional relevant studies that were cited in the publications from the PubMed search (i.e., through backward searching) were also reviewed.

The literature search also identified numerous human studies of associations of blood serum PFAS with a variety of health endpoints. Such studies typically evaluate potential associations of the health effect of interest with the multiple PFAS detected in the blood serum of the subjects. As discussed in the section on *Human Epidemiological Studies* below, information on those studies that specifically noted associations of health endpoints with PFUnDA are included in Appendix 1 of this document.

Guidance and standards developed by other agencies

USEPA has not conducted a toxicity assessment or developed standards or guidance values for PFUnDA. Information on toxicity assessments of PFUnDA from three other states (Hawaii, Texas, Wisconsin) was identified in the Interstate Technology and Regulatory Council PFAS Water and Soil Values Table (ITRC, 2022) and the Environmental Council of the States (ECOS)

White Paper on Processes & Considerations for Setting State PFAS Standards (Longsworth, 2022).

Hawaii

The Hawaii Department of Health (HIDOH, 2021, https://health.hawaii.gov/heer/files/2021/11/PFASActionLevelsWAttachmentHIDOHApril-2021.pdf) developed RfDs for 18 PFAS including PFUnDA. These RfDs were used to develop Action Levels for drinking water, ground water, surface water, and soil. The RfD for PFUnDA of 5 ng/kg/day was developed by applying the relative potency factor (RPF) of 4 for PFUnDA that was provided by Netherlands National Institute for Public Health (RIVM) (2018; later published by Bil et al., 2021) to the USEPA (2016a) RfD for perfluorooctanoic acid (PFOA) of 20 ng/kg/day. The RIVM (2018) RPFs are based on relative potency for causing increased liver weights in male rats as compared to PFOA, which is the index compound and is assigned a RPF of 1. To apply the RPF of 4, the USEPA (2016a) PFOA RfD of 20 ng/kg/day was divided by 4 to arrive at the PFUnDA RfD of 5 ng/kg/day. A major uncertainty in the HIDOH approach is that the relative potencies of PFUnDA and other PFAS as compared to PFOA are estimated by applying the RIVM (2018) RPFs to the USEPA (2016a) RfD for perfluorooctanoic acid (PFOA), which is based on developmental delays in mouse offspring. However, the RIVM (2018) RPFs are based on relative potency for increased liver weight in male rats and are not intended for use in estimation of the relative potencies of PFAS for other toxicological effects such as the developmental effects that are the basis of the USEPA (2016a) RfD.

Texas

The Texas Council for Environmental Quality (TCEQ, 2016) developed Reference Doses for 16 PFAS including PFUnDA. These RfDs are used as the basis for Protective Concentration Levels for groundwater and soil. TCEQ did not identify any toxicity data for PFUnDA. Based on the assumption that PFAS toxicity increases with carbon chain length, RfDs for PFAS for which no toxicity data was identified were based on the RfD for another PFAS with the same or longer carbon chain. (Note: Although not discussed by TCEQ, the assumption that toxicity increases with chain length is not necessarily true for PFAS of very long chain lengths such as perfluorododecanoic acid [PFDoDA, C12].) TCEQ used its Reference Dose of 12 ng/kg/day for PFDoDA as the RfD for PFUnDA.

The PFDoDA RfD is based on a 14-day rat study (Shi et al., 2007) in which the NOAEL and LOAEL for decreased body weight were stated to be 1 and 5 mg/kg/day, respectively; it was stated that serum testosterone and estradiol were also reduced at 5 mg/kg/day. Because no half-life data were available to develop a rat-to-human interspecies toxicokinetic extrapolation factor, the mouse-to-human toxicokinetic factor of 81 from the USEPA (2009) Provisional Drinking Water Health Advisory was used for the toxicokinetic adjustment. It should be noted that the scientific basis for this factor appears to be uncertain since it is based on the half-life of mice, while the RfD is based on a rat study. A total uncertainty factor of 1000 was applied to the

NOAEL of 1 mg/kg/day, including an intra-human UF of 10, an interspecies UF of 1 (i.e., no adjustment), a LOAEL-to-NOAEL UF of 1 (i.e., no adjustment), a subacute-to-chronic UF of 10, and a database UF of 10 for "significant insufficiencies (e.g., no chronic or subchronic studies)." It should be noted that it is standard practice in the derivation of RfDs for PFAS by New Jersey and USEPA, as well as numerous other states, to use an interspecies UF of 3, not 1 as was used by Texas, to account for toxicodynamic differences when a chemical-specific interspecies toxicokinetic factor is used.

Wisconsin

The Wisconsin Department of Health Services (WIDHS, 2020) developed RfDs for 12 PFAS including PFUnDA for use in developing recommended groundwater standards. The WIDHS RfD of 300 ng/kg/day is based on the NOAEL of 0.3 mg/kg/day for decreased offspring body weight in the rat repeated dose and reproductive-developmental study (Takahashi et al., 2014). This study is summarized in detail in the *Animal Toxicology* section below. A total UF including an intraspecies UF of 10, an interspecies UF of 10, and a database limitation UF of 10 were applied. No adjustment was made (i.e., UFs of 1) for less than chronic exposure since the critical effect results from developmental exposures over a short time period, or for LOAEL-to-NOAEL extrapolation, since a NOAEL was used.

WIDHS also developed an RfD of 10 ng/kg/day for the more sensitive hepatic effects (NOAEL of 0.1 mg/kg/day) in parental males in Takahashi et al. (2014) that included a total UF of 10,000 intraspecies UF of 10, interspecies UF of 10, less-than-chronic duration UF of 10, database limitation UF of 10). However, this lower RfD was dismissed by stating that rodent hepatic effects of PFAS in general are not relevant to humans. As discussed in detail in the *Mode of Action* section, New Jersey and USEPA have determined this conclusion is not supported by the available scientific information.

Additionally, WIDHS used the default interspecies UF of 10 in development of the RfD for PFUnDA. This default UF is composed of two UFs equal to the square root of 10 each. These UFs are rounded to 3, and include 3 for interspecies toxicodynamic differences and 3 for interspecies toxicokinetic differences. As discussed in the *Reference Dose Development* section below, it is generally accepted that the default UF of 3 for toxicokinetic differences does not sufficiently account for the much slower excretion of PFAS in humans than in laboratory animals since the difference in PFAS excretion rate between humans and laboratory animals is much greater than 3-fold. The much slower excretion of PFAS in humans than in laboratory animals results in a much higher internal dose in humans than in animals given the same administered dose, and this difference should be accounted for with chemical-specific data on interspecies toxicokinetic differences.

Biomonitoring and human exposure data

Data on serum PFUnDA from the U.S. general population are available for the NHANES monitoring cycles from 1999-2000 through 2017-18 (CDC, 2022a). The 2017-18 data are the most recent data that are available, and the NHANES website (CDC, 2022b) states that "the NHANES program suspended field operations in March 2020 due to the coronavirus disease 2019 (COVID-19) pandemic. As a result, data collection for the NHANES 2019-2020 cycle was not completed and the collected data are not nationally representative."

PFUnDA was detected in the U.S. general population, although not as frequently as some other long chain PFAS such as PFOA, PFOS, PFNA, and perfluorohexanoic acid (PFHxS). The 50th percentile (median) serum PFUnDA level was below the limit of detection (LOD; 0.2 - 0.3 ng/L) in the three NHANES cycles from 1999 to 2008. In 2009-10, the median value was 0.172, which was above the LOD of 0.1 ng/ml in this NHANES cycle. In the four more recent NHANES cycles from 2011 to 2018 (Table 2), all with an LOD of 0.1 ng/ml, the median serum PFUnDA value was at or above the LOD in 2011-12 (0.120 ng/ml) and 2017-18 (0.100 ng/ml), but below the LOD in 2013-14 and 2015-16.

In general, serum PFUnDA levels (Tables 1 and 2, below) in NHANES are much lower than for PFOA and PFOS. For comparison to the PFUnDA data in Tables 1 and 2, the 50th percentile (median) NHANES serum levels for PFOA were approximately 5.2 ng/ml in 1999-2000 and 1.4 ng/ml in 2017-18, and approximately 30 ng/ml in 1999-2000 and 4.3 ng/ml in 2017-18 for PFOS.

The four most recent NHANES cycles from 2011 to 2018 included data for Asians, while the earlier cycles shown in Table 1 did not. It is notable that the serum PFUnDA levels in Asians were two- to several-fold higher than in the other racial/ethnic groups (Mexican Americans, Non-Hispanic Blacks, Non-Hispanic Whites, all Hispanics) at all percentiles (50th, 75th, 90th, 95th) in all four cycles. Review of all NHANES serum PFAS data indicates that serum levels of several other long-chain PFAS also appear to be higher in Asians than other groups, but this difference is most notable for PFUnDA.

Yu et al. (2021) present serum PFAS data from residents of two NJ communities whose drinking water was contaminated with perfluorononanoic acid (PFNA, C9) from a nearby industrial source. The industrial facility used and discharged a mixture of PFAS consisting primarily of PFNA (~70%) and also containing PFUnDA (~24%), as discussed in DWQI (2015). In this vicinity, PFUnDA that was concluded to arise from this industrial source was detected in soil, surface water, and fish (Washington et al., 2020; Goodrow et al., 2020), but it was not detected groundwater (which is the drinking water source) in this vicinity, likely due retention in the soil from binding to soil particles (McCord et al., 2020).

The study population in Yu et al. (2021) included 120 adults (age 20-74; 38 males, 82 females) who had lived in the impacted communities for at least two years before drinking water exposure stopped in 2014. While the focus of the study was PFNA, serum levels for 12 PFAS including PFUnDA are presented. Serum PFUnDA levels from 2017-18 in the study population can be compared with 2017-18 NHANES data (Table 2) and 2016-18 New Jersey general population data (Yu et al., 2020) for adults age 20 and older. Comparison with NHANES and the New Jersey general population data suggests higher PFUnDA exposure in the residents of the impacted communities. Specifically, the geometric mean, median, and 95th percentile of serum PFUnDA concentrations were 0.215, 0.201, and 1.193 ng/ml in residents of the impacted communities (Yu et al., 2021), as compared with 0.129, 0.100, and 0.400 ng/ml in 2017-18 NHANES (Table 2) and 0.10, 0.11, and 0.43 ng/ml in the NJ general population in 2016-18 (Yu et al., 2020).

Table 1 (from CDC, 2022a)

Serum Perfluoroundecanoic acid (PFUA or PFUnDA) (1999 - 2010)

CAS Number 2058-94-8

Geometric mean and selected percentiles of serum concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

Demographic Categories	Survey (Years)	Geometric Mean (95% CI)	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total population	99-00	*	<LOD	<LOD	.300 (<LOD-.400)	.400 (.300-.700)	1562
Total population	03-04	*	<LOD	<LOD	<LOD	.600 (<LOD-1.30)	2094
Total population	05-06	*	<LOD	.300 (.200-.400)	.500 (.400-.800)	.700 (.600-1.20)	2120
Total population	07-08	*	<LOD	.200 (<LOD-.300)	.400 (.300-.500)	.600 (.500-.800)	2100
Total population	09-10	.172 (.151-.196)	.200 (.100-.200)	.300 (.200-.400)	.600 (.400-.800)	.900 (.600-1.10)	2233

Limit of detection (LOD, see Data Analysis section) for Survey years 99-00, 03-04, 05-06, 07-08, and 09-10 are 0.2, 0.3, 0.2, 0.2, an

<LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Table 2 (from CDC, 2022a)

Serum Perfluoroundecanoic acid (PUFA or PFUnDA) (2011 - 2018)

CAS Number 2058-94-8

Geometric mean and selected percentiles of serum concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

Demographic Categories	Survey (Years)	Geometric Mean (95% CI)	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total population	11-12	*	.120 (.100-.140)	.220 (.180-.250)	.380 (.310-.520)	.620 (.440-.790)	1904
Total population	13-14	*	<LOD	.200 (.100-.200)	.300 (.200-.400)	.500 (.400-.600)	2168
Total population	15-16	*	<LOD	.100 (.100-.200)	.200 (.200-.500)	.400 (.300-.500)	1993
Total population	17-18	.125 (.115-.135)	.100 (.100-.100)	.200 (.200-.200)	.300 (.200-.300)	.400 (.300-.500)	1929
Age 12-19 years	11-12	*	<LOD	.110 (<LOD-.140)	.180 (.140-.220)	.250 (.190-.390)	344
Age 12-19 years	13-14	*	<LOD	<LOD	.200 (.100-.200)	.200 (.200-.300)	402
Age 12-19 years	15-16	*	<LOD	<LOD	.100 (<LOD-.200)	.200 (.100-.400)	353
Age 12-19 years	17-18	*	.100 (<LOD-.100)	.100 (.100-.200)	.200 (.100-.200)	.200 (.100-.500)	313
Age 20+ years	11-12	.146 (.129-.165)	.130 (.110-.150)	.230 (.190-.280)	.420 (.330-.530)	.660 (.490-.840)	1560
Age 20+ years	13-14	*	<LOD	.200 (.100-.200)	.300 (.300-.500)	.500 (.400-.700)	1766
Age 20+ years	15-16	*	<LOD	.100 (.100-.200)	.300 (.200-.300)	.400 (.300-.500)	1640
Age 20+ years	17-18	.129 (.119-.140)	.100 (.100-.100)	.200 (.200-.200)	.300 (.200-.400)	.400 (.300-.600)	1616
Males	11-12	*	.120 (.100-.150)	.220 (.180-.260)	.340 (.290-.480)	.590 (.390-.820)	966
Males	13-14	*	<LOD	.200 (.100-.200)	.300 (.200-.500)	.500 (.400-.700)	1032
Males	15-16	*	<LOD	.100 (.100-.200)	.200 (.200-.500)	.400 (.300-.500)	964
Males	17-18	.121 (.114-.129)	.100 (.100-.100)	.200 (.200-.200)	.300 (.200-.300)	.300 (.300-.400)	952
Females	11-12	*	.120 (.100-.140)	.210 (.180-.270)	.420 (.320-.540)	.650 (.520-.740)	938
Females	13-14	*	<LOD	.200 (.100-.200)	.300 (.200-.400)	.500 (.400-.600)	1136
Females	15-16	*	<LOD	.100 (.100-.200)	.200 (.200-.500)	.400 (.300-.500)	1029
Females	17-18	.128 (.115-.143)	.100 (.100-.100)	.200 (.200-.200)	.300 (.200-.400)	.400 (.300-.800)	977
Mexican Americans	11-12	*	<LOD	.150 (.130-.180)	.230 (.190-.300)	.310 (.230-.330)	211
Mexican Americans	13-14	*	<LOD	<LOD	.200 (.100-.300)	.200 (.100-.600)	332
Mexican Americans	15-16	*	<LOD	<LOD	.100 (<LOD-.200)	.200 (.100-.400)	370
Mexican Americans	17-18	*	.100 (<LOD-.100)	.100 (.100-.200)	.200 (.100-.300)	.200 (.100-.400)	297
Non-Hispanic Blacks	11-12	.164 (.135-.199)	.140 (.110-.190)	.260 (.200-.390)	.530 (.350-.840)	.840 (.520-1.26)	485
Non-Hispanic Blacks	13-14	*	.100 (<LOD-.200)	.200 (.100-.400)	.500 (.300-.700)	.700 (.500-.900)	455
Non-Hispanic Blacks	15-16	*	<LOD	.200 (.100-.200)	.300 (.200-.400)	.400 (.300-.700)	439
Non-Hispanic Blacks	17-18	.144 (.124-.168)	.100 (.100-.200)	.200 (.200-.300)	.400 (.300-.500)	.600 (.400-.900)	430
Non-Hispanic Whites	11-12	*	.110 (<LOD-.140)	.200 (.160-.240)	.330 (.280-.410)	.450 (.330-.690)	666
Non-Hispanic Whites	13-14	*	<LOD	.200 (.100-.200)	.300 (.200-.400)	.400 (.300-.600)	862
Non-Hispanic Whites	15-16	*	<LOD	.100 (.100-.200)	.200 (.100-.300)	.300 (.200-.500)	619
Non-Hispanic Whites	17-18	.120 (.111-.130)	.100 (.100-.100)	.200 (.200-.200)	.300 (.200-.300)	.400 (.300-.400)	667
All Hispanics	11-12	*	.110 (<LOD-.130)	.180 (.160-.210)	.310 (.230-.390)	.420 (.300-.630)	406
All Hispanics	13-14	*	<LOD	.100 (<LOD-.100)	.200 (.100-.200)	.200 (.100-.600)	537
All Hispanics	15-16	*	<LOD	<LOD	.200 (.100-.200)	.300 (.200-.300)	629
All Hispanics	17-18	.111 (.100-.123)	.100 (.100-.100)	.200 (.100-.200)	.200 (.200-.300)	.300 (.200-.300)	473
Asians	11-12	.338 (.277-.412)	.330 (.220-.510)	.840 (.630-1.01)	1.38 (1.02-2.33)	2.40 (1.33-3.41)	291
Asians	13-14	.274 (.231-.326)	.300 (.200-.400)	.600 (.400-.800)	1.00 (.900-1.20)	1.50 (1.00-2.40)	236
Asians	15-16	.233 (.189-.287)	.300 (.200-.300)	.500 (.400-.600)	1.00 (.600-1.40)	1.50 (.800-2.20)	220
Asians	17-18	.210 (.162-.273)	.200 (.100-.300)	.400 (.300-.600)	.800 (.500-1.60)	1.10 (.600-2.50)	257

Limit of detection (LOD, see Data Analysis section) for Survey years 11-12, 13-14, 15-16, and 17-18 are 0.100, 0.100, 0.100, and 0.100 respectively.

<LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Biomonitoring Summary: https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html

Factsheet: https://www.cdc.gov/biomonitoring/PFAS_FactSheet.html

A study of pregnant women from the San Francisco, CA area found that women who reported eating fish at least once a week had higher serum PFUnDA levels than those who ate fish less frequently. Serum PFUnDA levels were similar in those who ate red meat at least once a week compared with those who ate it with less frequently, and serum PFUnDA levels were lower in those who consumed poultry at least once a week than in those who consumed poultry less often (Eick et al., 2021). Studies of European populations have reported that higher levels of serum PFUnDA and other long chain PFAS that are highly bioaccumulative in aquatic organisms are associated with consumption of fish and shellfish (Haug et al., 2010; Klenow et al., 2013; Thépaut et al., 2021), while consumption of fruits and vegetables was associated with higher levels of PFAS that are not highly bioaccumulative in fish (PFOA, PFHxA, PFHxS; Klenow et al., 2013).

PFUnDA exposures to the fetus and infant are of potential concern because PFUnDA, like other PFAS, causes developmental toxicity in laboratory animals (Takahashi et al., 2014; discussed in detail below). Like other PFAS, PFUnDA has been detected in human breast milk (So et al., 2006; Fujii et al., 2012; Motas Guzman et al., 2016; Nyberg et al., 2018; Macheka et al., 2022).

These studies evaluated breast milk from women in Africa, Asia, and Europe; no breast milk data from North America were identified. PFUnDA was also found in human cord blood (Kung et al., 2021), placenta (Mamsen et al., 2017; Bangma et al., 2020), and fetal organs (Mamsen et al., 2017; Mamsen et al., 2019).

PFUnA was also detected in all 12 liver samples that were evaluated in a study of residents of Catalonia, Spain (Karrman et al., 2010).

Toxicokinetics

Summary

In general, PFAS are excreted much more slowly in humans than in laboratory animals. For this reason, the same administered dose results in a much higher internal dose (e.g., blood serum level) in humans than in laboratory animals. Toxicokinetic data from humans and laboratory animals are used to develop chemical-specific adjustment factors that account for this interspecies toxicokinetic difference in RfDs (and cancer slope factors, when applicable).

Information on toxicokinetics of PFUnDA is relatively limited. Sources of toxicokinetic data include a study in mice after intravenous and oral administration (Fujii et al., 2015); a study in microminipigs after oral administration (Guruge et al., 2016); limited data on toxicokinetics in rats (Goecke-Flora et al., 1996; Mertens et al., 2010), and data on human urinary clearance (Zhang et al., 2013; Fujii et al., 2015), human biliary clearance (Fujii et al., 2015), and estimated human half-life (Zhang et al., 2013). More details on each of these studies are provided below. All of the studies reviewed in this section evaluated other PFAS in addition to PFUnDA. Data on other PFAS, particularly PFOA, PFNA, and perfluorodecanoic acid (PFDA; C10), are discussed below along with the PFUnDA data for comparison purposes.

Available data indicate that PFUnDA is well absorbed after oral exposure. Like other perfluoroalkyl acids (PFAAs; carboxylates and sulfonates with a totally fluorinated carbon chain), PFUnDA is not metabolized. PFUnDA accumulated in liver to a greater extent than both shorter chain and longer chain perfluoroalkyl carboxylates (PFCAs) in mice, and to a greater extent than shorter chain PFCAs in microminipigs. In both mice and humans, urinary clearance of PFUnDA was much slower than for shorter-chain PFCAs and fecal clearance was similar as in shorter-chain PFCAs, resulting in fecal clearance contributing a much greater proportion of total clearance for PFUnDA than for shorter chain PFCAs. The limited available data indicates that most PFUnDA is also excreted in the bile, rather than the urine, in rats. The rate of PFUnDA excretion appears to be generally similar in males and females in both mice and rats, in contrast to some other PFCAs (e.g., PFOA, PFNA) that are much more rapidly excreted in female rats than male rats. PFUnDA is excreted much more slowly in humans than in rodents.

The elimination half-lives of PFUnDA in male rats and humans are of interest because they are used for the interspecies toxicokinetic adjustment in the RfD developed herein, which is based on toxicity in male rats (see below). Although the half-life of PFUnDA in rats is not reported in

the literature, the half-life in male rats was estimated (see below) as 30 days based on available toxicokinetic data. Based on the available information, the estimated mean half-life of PFUnDA in humans is 12 years.

Animal studies

Mouse study (Fujii et al., 2015)

Study description

Fujii et al. (2015) provides information on absorption, distribution, and excretion of PFUnDA in male and female mice. A single dose of PFUnDA dissolved in ethanol:water:dimethyl sulfoxide (5:4:1; dosing volume - 10 ml/kg) was administered by intravenous (IV) injection (9 per sex per group) or oral gavage (9 per sex per group). The IV dose was 0.31 µmol/kg, calculated to be 0.175 mg/kg, and the oral dose was 3.13 µmol/kg, calculated to be 1.77 mg/kg. Other PFCAs included in the study using the same protocol and doses (µmol/kg) were perfluorohexanoic acid (PFHxA; C6), perfluoroheptanoic acid (PFHpA; C7), PFOA, PFNA, PFDA, PFDoDA, perfluorotridecanoic acid (PFTrDA; C13), and perfluorotetradecanoic acid (PFTeDA; C14). Blood samples were collected for PFUnDA analysis at 0, 0.5, 1, 3, 6, 12, and 24 hours after IV administration, and at the same timepoints except 0.5 hours after gavage administration. Urine and feces were collected for PFUnDA analysis for 24 hours after dosing in metabolic cages. Mice were sacrificed 24 hours after dosing, and liver, kidney, brain, and adipose tissue were collected for PFUnDA analysis. The human component of Fujii et al. (2015) is discussed below.

Absorption

Data from Fujii et al. (2015) indicates that PFUnDA is virtually completely absorbed after oral administration to male and female mice. PFUnDA detected in the feces after oral administration arises both from PFUnDA that is not absorbed and passes through the gastrointestinal tract and PFUnDA eliminated via biliary excretion, while PFUnDA in the feces after IV dosing comes only from biliary excretion. The percent of the PFUnDA that was absorbed after oral dosing to mice was estimated by Fujii et al. (2015) by comparing the fecal clearance after IV and gavage administration. Fecal clearance was 2.4$\pm$0.9 ml/day/kg after IV dosing and 5.5$\pm$2.6 ml/day/kg after gavage in males and 3.0$\pm$1.4 ml/day/kg after IV dosing and 2.9$\pm$1.7 ml/day/kg after gavage in females; the average for both sexes was 2.7$\pm$1.2 ml/day/kg after IV dosing and 4.2$\pm$2.5 ml/day/kg after gavage. These data were used to estimate the percentage of the dose absorbed after oral (gavage) dosing as 99.3% in males, 100.0% in females, and an average of both sexes of 99.6%.

Distribution

Fujii et al. (2015) provides information on tissue distribution in male and female mice for PFUnDA and eight other PFCAs. The percentage of total dose distributed to blood serum, liver, brain and adipose tissue in mice 24 hours after dosing is shown for PFUnDA and the other PFCAs in Table 3 for IV dosing and Table 4 for gavage dosing. By far the greatest percentage of the PFUnDA dose was found in the liver (90.1% in males and 69.2% in females after gavage;

78.4% in males and 53.3% in females after IV). About 10% of the dose was present in blood serum after gavage dosing, with slightly higher percentages after IV administration, and the percentages of the dose in kidney, brain, and adipose tissue were much lower (0.04 to 1.7%).

Comparison of data for PFUnDA and other PFCAs indicates that after gavage administration, the percentage of the dose distributed to the liver was higher for PFUnDA than for any of the other shorter chain and longer chain PFCAs included in the study; this was also true after IV administration except that the percentage in liver was almost identical for PFUnDA and PFDA (C10). Additionally, the percentage of the PFCA dose in blood serum decreased with chain length in both sexes with both IV and gavage dosing.

Excretion

In male and female mice, the percentage of PFUnDA excreted in urine 24 hours after dosing was much lower than for shorter chain PFCAs (Fujii et al., 2015). For example, it was 100-fold lower than for PFOA and 10 to 15-fold lower than for PFNA after gavage administration. In contrast, the percentage of PFUnDA in feces (0.6 to 0.9% for IV; 0.9% to 1.2% for oral) was generally similar as for other PFCAs with 8 or more carbons (Tables 3 and 4).

Clearance values

Based on the data from their mouse toxicokinetic studies, Fujii et al. (2015) provide values for the urinary, fecal, and total (urinary plus fecal) clearance of PFUnDA and other PFCAs in males, females, and the average of both sexes in the first 24 hours after IV and oral dosing. IV data are more meaningful than oral data for fecal clearance because oral clearance includes PFAS that was not absorbed in the gastrointestinal tract. The average values for males and females for IV clearance are discussed herein since the clearance data for males and females were generally similar.

Urinary clearance in mice decreased with increasing chain length from 8 to 11 carbons (11.4, 3.3, 0.9, 0.4 ml/day/kg for PFOA, PFNA, PFDA, and PFUnDA, respectively) while fecal clearance was slightly higher for PFUnDA (2.7 ml/day/kg) than for PFOA, PFNA, and PFDA (1.5, 1.2, and 1.6 ml/day/kg, respectively). Total clearance was highest for PFOA (13.0 ml/day/kg), primarily due to the higher urinary clearance, while total clearance for PFNA, PFDA, and PFUnDA was generally similar (4.5, 2.5, and 3.1 ml/day/kg, respectively). The percent of total clearance through urine decreased and percent clearance through feces correspondingly increased with increasing chain length from 8 to 11 carbons. Specifically, the percent of clearance through urine was 88%, 73%, 36%, and 13% for PFOA, PFNA, PFDA, and PFUnDA, while the percent clearance through feces was 12%, 27%, 64%, and 87%. These data indicate that fecal clearance of PFUnDA was approximately seven-fold higher than urinary clearance.

Elimination half-life

Elimination half-life values are not provided in the mouse toxicokinetic study. This study evaluated distribution and excretion for only 24 hours (Fujii et al., 2015).

Table 3 (from Fujii et al., 2015).

Table S5. Distribution and excretion of PFCAs 24 h after IV administration (0.313 μmol/kg)

		PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)
		Male (N=9)								
Average body weight of mice	g					25.9 (1.5)				
Average of administrated dose	nmol					8.2 (0.5)				
						100%				
Serum[a]	nmol	<0.03	<0.03	2.65(0.5)	2.23(0.4)	1.61(0.3)	1.13(0.2)	0.62(0.1)	0.50(0.1)	0.47(0.2)
	%[b]	-	-	32.3%(4.5%)	27.2%(6.0%)	19.6%(4.1%)	13.7%(2.4%)	7.5%(1.5%)	6.1%(1.2%)	5.7%(0.9%)
Liver	nmol	<0.03	0.10(0.2)	3.88(0.4)	5.62(0.9)	6.55(1.3)	6.43(1.4)	5.46(1.3)	5.97(1.4)	5.26(1.5)
	%[b]	-	1.3%(2.6%)	47.4%(4.8%)	68.5%(12.3%)	79.9%(17.5%)	78.4%(19.2%)	66.6%(18.3%)	72.8%(18.7%)	64.2%(18.9%)
Kidney	nmol	<0.01	0.02(0.04)	0.11(0.02)	0.09(0.02)	0.09(0.01)	0.10(0.02)	0.09(0.02)	0.16(0.03)	0.21(0.03)
	%[b]	-	0.2%(0.5%)	1.3%(0.2%)	1.1%(0.1%)	1.1%(0.2%)	1.2%(0.2%)	1.2%(0.2%)	1.9%(0.4%)	2.6%(0.5%)
Brain	nmol	<0.01	<0.01	0.01(0.003)	0.01(0.01)	0.02(0.01)	0.03(0.01)	0.02(0.01)	0.03(0.01)	0.03(0.01)
	%[b]	-	-	0.1%(0.0%)	0.1%(0.1%)	0.2%(0.1%)	0.3%(0.1%)	0.3%(0.1%)	0.4%(0.1%)	0.4%(0.1%)
Adipose tissue[c]	nmol	<0.01	0.01(0.01)	0.13(0.20)	0.05(0.03)	0.04(0.03)	0.05(0.04)	0.05(0.04)	0.09(0.06)	0.12(0.08)
	%[b]	-	0.1%(0.1%)	1.5%(2.3%)	0.6%(0.4%)	0.5%(0.4%)	0.7%(0.4%)	0.6%(0.4%)	1.1%(0.7%)	1.5%(0.9%)
Urine	nmol	8.31(5.1)	8.11(4.2)	0.61(0.4)	0.11(0.1)	0.021(0.027)	0.008(0.007)	0.004(0.004)	0.004(0.004)	0.003(0.003)
	%[b]	101.3%(27.5%)	99.0%(27.3%)	7.4%(4.5%)	1.3%(0.7%)	0.3%(0.1%)	0.1%(0.03%)	0.0%(0.02%)	0.1%(0.03%)	0.04%(0.01%)
Feces	nmol	0.38(0.4)	0.26(0.4)	0.05(0.04)	0.04(0.02)	0.04(0.02)	0.05(0.02)	0.04(0.02)	0.06(0.03)	0.09(0.05)
	%[b]	4.7%(5.4%)	3.2%(5.3%)	0.6%(0.5%)	0.5%(0.2%)	0.5%(0.2%)	0.6%(0.2%)	0.5%(0.2%)	0.8%(0.3%)	1.1%(0.6%)
Total[d]	nmol	8.72(1.9)	8.51(2.1)	7.44(0.8)	8.14(1.1)	8.37(1.4)	7.80(1.5)	6.30(1.4)	6.81(1.4)	6.19(1.5)
Total recovery [d]	%[b]	106.3%(48.2%)	103.7%(42.9%)	90.7%(9.8%)	99.2%(11.9%)	102.1%(17.3%)	95.1%(19.2%)	76.8%(18.3%)	83.1%(18.4%)	75.5%(18.6%)
		Female (N=9)								
Average body weight of mice	g					20.1 (1.2)				
Average of administrated dose	nmol					6.4 (0.4)				
						100%				
Serum[a]	nmol	<0.02	<0.02	2.01(0.8)	2.06(0.8)	1.45(0.5)	0.93(0.3)	0.48(0.2)	0.36(0.2)	0.27(0.3)
	%[b]	-	-	31.5%(7.1%)	32.2%(9.7%)	22.7%(5.9%)	14.6%(3.5%)	7.5%(1.6%)	5.7%(1.6%)	4.2%(1.8%)
Liver	nmol	<0.03	0.03(0.1)	1.93(0.3)	2.93(0.4)	3.41(0.5)	3.41(0.5)	3.00(0.5)	3.49(0.5)	3.01(0.4)
	%[b]	-	0.5%(1.0%)	30.2%(4.1%)	45.8%(7.2%)	53.3%(8.9%)	53.3%(8.2%)	46.9%(6.7%)	54.6%(7.5%)	47.1%(6.4%)
Kidney	nmol	<0.01	<0.01	0.09(0.01)	0.10(0.01)	0.10(0.02)	0.11(0.02)	0.10(0.02)	0.14(0.03)	0.16(0.03)
	%[b]	-	-	1.4%(0.2%)	1.6%(0.2%)	1.6%(0.4%)	1.7%(0.4%)	1.6%(0.4%)	2.2%(0.5%)	2.5%(0.6%)
Brain	nmol	<0.01	<0.01	0.01(0.002)	0.01(0.003)	0.02(0.01)	0.03(0.01)	0.03(0.01)	0.03(0.01)	0.03(0.01)
	%[b]	-	-	0.1%(0.03%)	0.2%(0.06%)	0.4%(0.1%)	0.5%(0.1%)	0.4%(0.1%)	0.5%(0.2%)	0.5%(0.2%)
Adipose tissue[c]	nmol	<0.01	0.01(0.01)	0.06(0.02)	0.07(0.05)	0.08(0.07)	0.09(0.08)	0.09(0.07)	0.15(0.09)	0.19(0.10)
	%[b]	-	0.1%(0.2%)	0.9%(0.3%)	1.1%(0.7%)	1.3%(1.0%)	1.5%(1.2%)	1.4%(1.0%)	2.3%(1.2%)	2.9%(1.4%)
Urine	nmol	5.05(1.7)	4.23(2.3)	0.41(0.3)	0.14(0.1)	0.03(0.01)	0.01(0.002)	0.004(0.003)	0.004(0.002)	0.003(0.003)
	%[b]	79.0%(29.1%)	66.1%(37.6%)	6.4%(3.8%)	2.2%(1.2%)	0.4%(0.2%)	0.1%(0.04%)	0.1%(0.05%)	0.1%(0.03%)	0.1%(0.04%)
Feces	nmol	1.00(0.85)	0.84(0.85)	0.08(0.06)	0.06(0.03)	0.05(0.03)	0.06(0.03)	0.04(0.02)	0.05(0.02)	0.06(0.04)
	%[b]	15.6%(13.5%)	13.1%(13.5%)	1.3%(1.0%)	0.9%(0.4%)	0.8%(0.4%)	0.9%(0.4%)	0.7%(0.3%)	0.8%(0.4%)	1.0%(0.6%)
Total[d]	nmol	6.13(1.8)	5.32(2.4)	4.09(0.5)	4.43(0.5)	4.61(0.6)	4.35(0.5)	3.68(0.5)	4.26(0.5)	3.83(0.5)
Total recovery [d]	%[b]	94.9%(43.4%)	79.9%(48.9%)	71.7%(8.2%)	84.1%(11.2%)	80.5%(10.3%)	72.5%(8.6%)	58.6%(6.6%)	66.2%(7.9%)	58.3%(7.2%)

Values are means (SD). Lower values in parentheses are the mean percentages.
a. Calculated by assuming 56 ml/kg mouse body weight for the male mice blood volume and 65 mg/kg mouse body weight for the female mice blood volume (Richers et al., 1972)
b. Percentage of administrated dose
c. Calculated by assuming a mice body fat percentage of 2.3% (Richers et al., 1972)

Table 4 (from Fujii et al., 2015).

Table S6. Distribution and excretion of PFCAs 24 hr after gavage administration (3.13 μmol/kg)

		PFHxA (C6)	PFHpA (C7)	PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)
		Male (N=9)								
Average body weight of mice	g					24.3 (1.6)				
Average of administrated dose	nmol					77.1 (5.0)				
						100%				
Serum[a]	nmol	<0.32	<0.32	20.85(5.9)	23.74(3.8)	19.05(3.0)	10.96(2.0)	5.48(1.4)	3.88(1.3)	2.57(1.1)
	%[b]	-	-	27.0%(6.4%)	30.8%(3.9%)	24.7%(3.1%)	14.2%(2.3%)	7.1%(1.7%)	5.0%(1.7%)	3.3%(1.5%)
Liver	nmol	<0.36	<0.36	30.30(2.9)	54.11(7.1)	63.08(11.8)	69.48(10.0)	54.81(10.4)	51.24(9.7)	34.53(7.6)
	%[b]	-	-	39.3%(3.1%)	70.2%(7.0%)	81.8%(12.3%)	90.1%(11.9%)	71.1%(10.9%)	66.5%(10.5%)	44.8%(8.8%)
Kidney	nmol	<0.07	<0.07	1.02(0.3)	0.74(0.2)	0.73(0.2)	0.76(0.2)	0.74(0.2)	0.99(0.3)	1.12(0.3)
	%[b]	-	-	1.3%(0.1%)	1.0%(0.1%)	0.9%(0.05%)	1.0%(0.1%)	1.0%(0.05%)	1.3%(0.1%)	1.5%(0.1%)
Brain	nmol	<0.07	<0.07	0.10(0.1)	0.11(0.1)	0.18(0.1)	0.29(0.2)	0.21(0.1)	0.22(0.1)	0.16(0.1)
	%[b]	-	-	0.1%(0.0%)	0.1%(0.1%)	0.2%(0.1%)	0.4%(0.1%)	0.3%(0.05%)	0.3%(0.04%)	0.2%(0.03%)
Adipose tissue[c]	nmol	<0.11	<0.11	0.07(0.07)	0.06(0.06)	0.04(0.03)	0.03(0.02)	0.02(0.01)	0.02(0.01)	0.02(0.01)
	%[b]	-	-	0.1%(0.1%)	0.1%(0.1%)	0.05%(0.04%)	0.04%(0.03%)	0.03%(0.02%)	0.03%(0.02%)	0.02%(0.01%)
Urine	nmol	47.01(9.5)	36.42(26.2)	3.26(2.3)	0.32(0.2)	0.08(0.05)	0.03(0.02)	0.02(0.01)	0.02(0.01)	0.01(0.01)
	%[b]	61.0%(10.3%)	47%(31%)	4%(3%)	0.4%(0.2%)	0.1%(0.1%)	0.04%(0.02%)	0.02%(0.01%)	0.03%(0.01%)	0.02%(0.01%)
Feces	nmol	5.90(5.6)	6.06(5.5)	1.38(0.9)	1.05(0.6)	0.99(0.5)	0.94(0.4)	0.83(0.4)	2.36(1.2)	4.73(2.0)
	%[b]	7.7%(7.6%)	7.9%(7.5%)	1.8%(1.2%)	1.4%(0.8%)	1.3%(0.7%)	1.2%(0.6%)	1.1%(0.5%)	3.1%(1.6%)	6.1%(2.7%)
Total[d]	nmol	52.92(8.6)	43.25(23.9)	54.31(5.6)	67.74(8.3)	73.95(12.1)	78.06(10.0)	61.01(10.2)	59.16(9.4)	44.45(7.2)
Total recovery[d]	%[b]	68.6%(10.0%)	55.5%(28.5%)	73.9%(7.1%)	103.9%(8.6%)	109.1%(12.3%)	107.0%(11.3%)	80.6%(10.4%)	76.2%(9.7%)	56.0%(7.6%)
		Female (N=9)								
Average body weight of mice	g					20.6 (2.2)				
Average of administrated dose	nmol					65.3 (7.1)				
						100%				
Serum[a]	nmol	<0.22	<0.22	24.56(4.4)	20.08(4.5)	16.70(4.2)	10.20(2.9)	5.41(1.5)	4.05(1.1)	2.79(0.7)
	%[b]	-	-	37.6%(4.8%)	30.8%(4.8%)	25.6%(4.9%)	15.6%(3.5%)	8.3%(1.9%)	6.2%(1.4%)	4.3%(0.8%)
Liver	nmol	<0.30	1.18(1.7)	17.68(3.2)	33.10(6.3)	41.48(8.0)	45.17(9.4)	37.32(8.4)	35.02(8.6)	23.24(7.4)
	%[b]	-	1.8%(2.7%)	27.1%(5.0%)	50.7%(10.6%)	63.5%(13.8%)	69.2%(16.4%)	57.1%(14.8%)	53.6%(15.1%)	35.6%(12.6%)
Kidney	nmol	<0.05	0.10(0.2)	0.90(0.3)	0.83(0.2)	0.81(0.2)	0.86(0.2)	0.81(0.1)	1.07(0.2)	1.16(0.2)
	%[b]	-	0.2%(0.1%)	1.4%(0.1%)	1.3%(0.1%)	1.2%(0.05%)	1.3%(0.04%)	1.2%(0.04%)	1.6%(0.04%)	1.8%(0.04%)
Brain	nmol	<0.06	<0.06	0.07(0.02)	0.08(0.02)	0.15(0.04)	0.21(0.06)	0.17(0.05)	0.18(0.05)	0.13(0.04)
	%[b]	-	-	0.1%(0.01%)	0.1%(0.01%)	0.2%(0.02%)	0.3%(0.04%)	0.3%(0.04%)	0.3%(0.04%)	0.2%(0.03%)
Adipose tissue[c]	nmol	<0.11	<0.11	0.04(0.01)	0.03(0.01)	0.04(0.01)	0.03(0.01)	0.03(0.02)	0.03(0.02)	0.02(0.02)
	%[b]	-	-	0.1%(0.03%)	0.1%(0.02%)	0.1%(0.03%)	0.05%(0.03%)	0.05%(0.05%)	0.05%(0.04%)	0.04%(0.03%)
Urine	nmol	43.10(13.3)	29.95(15.6)	2.62(1.5)	0.38(0.2)	0.10(0.1)	0.03(0.01)	0.01(0.005)	0.01(0.007)	0.01(0.004)
	%[b]	66.0%(17.3%)	45.9%(21.5%)	4.0%(2.5%)	0.6%(0.3%)	0.1%(0.1%)	0.04%(0.02%)	0.02%(0.01%)	0.02%(0.01%)	0.01%(0.01%)
Feces	nmol	3.68(4.3)	3.98(3.5)	0.93(0.6)	0.65(0.5)	0.62(0.4)	0.58(0.3)	0.47(0.3)	1.10(0.7)	1.95(1.5)
	%[b]	5.6%(7.9%)	6.1%(6.1%)	1.4%(1.0%)	1.0%(0.7%)	1.0%(0.6%)	0.9%(0.6%)	0.7%(0.5%)	1.7%(1.3%)	3.0%(2.6%)
Total[d]	nmol	46.78(13.4)	38.05(14.0)	39.06(6.5)	47.85(7.5)	52.54(8.2)	53.53(9.4)	43.14(8.2)	41.41(8.4)	30.04(7.1)
Total recovery[d]	%[b]	71.6%(17.0%)	53.9%(21.1%)	71.7%(7.7%)	84.5%(12.6%)	91.7%(13.9%)	87.4%(15.6%)	67.7%(14.3%)	63.5%(14.8%)	44.9%(12.4%)

Values are means (SD). Lower values in parentheses are the mean percentages.
a. Calculated by assuming 56 ml/kg mouse body weight for the male mice blood volume and 65 mg/kg mouse body weight for the female mice blood volume (Richers et al., 1972)
b. Percentage of administrated dose
c. Calculated by assuming a mice body fat percentage of 2.3% (Richers et al., 1972)
d. Total for the blood, liver, kidney, brain, adipose tissue, urine and feces.

Microminipig study – Guruge et al. (2016)

Study design

Guruge et al. (2016) evaluated the time course of blood levels and tissue distribution of PFUnDA and nine other perfluoroalkyl acids (PFAAs) in female microminipigs. Female microminipigs (n=3) were administered a gelatin capsule containing doses of 3 mg/kg each of PFUnDA and nine other PFAAs (PFOS, perfluorobutanoic acid [PFBA, C4], perfluoropentanoic acid [PFPeA, C5], PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFDoDA); two control female microminipigs were given a capsule that did not contain PFAAs. Blood was collected for PFAA analysis prior to dosing and at 3, 6, 9, 12, and 24 hours and 2, 4, 8, 11, 15, and 21 days after dosing. Animals

were sacrificed 21 days after dosing, and PFAAs were analyzed in liver, kidney, spleen, and muscle.

Absorption

Absorption was not evaluated in Guruge et al. (2016).

Distribution

Guruge et al. (2016) reported that 27% of the administered dose of PFUnDA was recovered from blood, liver, kidney, spleen and muscle in female microminipigs at sacrifice 21 days after dosing. PFUnDA concentrations in liver were 5.9 times higher than in blood (noting that data are reported for whole blood, not blood serum). The liver:blood ratio for PFUnDA was higher than for shorter-chain PFCAs (e.g., PFOA-0.3; PFNA-1.2; PFDA-3.3) and PFOS (3.4), but lower than for PFDoDA (C12; 10.3). The PFUnDA tissue:blood ratios were lower for other tissues (kidney -1.0; muscle - 0.3; and spleen -1.0). As was the case for the liver:blood ratios, tissue:blood ratios for the other tissues for shorter chain PFCAs and PFOS were lower than for PFUnDA, and they were higher for PFDoDA than for PFUnDA. Additionally, the percentage of the administered PFUnDA dose in liver was higher than in blood for the other tissues at 21 days after dosing. In general, the percent of the administered dose in liver increased, and the percent in blood decreased, with increasing chain length for the PFCAs.

Excretion

Urinary and fecal excretion was not evaluated in the microminipig toxicokinetic study (Guruge et al., 2016).

Elimination half-life

Elimination half-lives in microminipigs were estimated based on decline in PFAA blood levels for 21 days after a single oral dose. Half-lives for PFOA, PFNA, PFDA, and PFUnDA were estimated as 63.0, 49.5, 40.8, and 38.5 days, respectively. This pattern of shorter half-life with increasing chain length from 8-11 carbons differs from the pattern in rodents, in which half-lives generally increase with increasing chain length in this range.

Rat studies – (Mertens et al., 2010; Goecke-Flora et al., 1996)

Mertens et al. (2010) is a rat subchronic toxicity study of S-111-S-WB (i.e, Surflon; discussed in DWQI, 2015), a technical mixture of perfluorocarboxylates (PFCAs) consisting primarily of PFNA, with smaller percentages of PFOA, PFUnDA, and perfluorotridecanoic acid (PFTrDA, C13). The toxicokinetic component of this study included single dose (route not stated, assumed to be oral) and repeated dose evaluations. A single dose of 0.025, 0.125, or 0.6 mg/kg Surflon was administered to rats (5 per sex). Urine and feces were collected at 0-6, 6-12, and 12-24 hours, and then daily through 7 days, and analyzed for PFOA, PFNA, PFUnDA, and PFTrDA. The total amount of each PFCA excreted in urine and feces was calculated. In the repeated dose study, rats were dosed orally with 0.025 or 0.125 mg/kg/day (5 per sex per dose level) or 0.6 mg/kg/day (6 per sex) for 92 days. Blood samples were taken during weeks 2, 4, 7, 9, 10, 11, and

12 of dosing. In the mid-dose (0.125 mg/kg/day) group, urine and feces were collected at 0-6, 6-12, and 12-24 hours and daily through 7 days after the final dose on day 91.

Some aspects of the reporting of the results of the toxicokinetic studies in Mertens et al. (2010) lack detail, including numerical data. After repeated dosing, serum concentrations of PFUnDA were similar in males and females given the same dose, indicating that the excretion rate of PFUnDA is similar in both sexes of rats; this was also the case for PFTrDA. In contrast, serum levels of PFOA and PFNA were much higher in males than females given the same dose; both of these PFCAs are known to be much more rapidly excreted in female rats than male rats. The authors state that steady-state serum PFUnDA concentrations had not yet been attained in male or female rats after 90 days of daily dosing, and the figure depicting serum PFUnDA over time shows that serum levels were still increasing at the end of the study. Since steady-state is attained after 4 to 5 half-lives with repeated dosing (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3223885/pdf/pch16535.pdf), these data suggest a PFUnDA half-life in rats of at least 2.5 weeks (18 days).

Mertens et al. (2010) did not estimate elimination half-lives of PFUnDA or the other PFAS present in the PFAS mixture used in their study. Urine PFUnDA data were not reported because of lack of accuracy due to low recovery in the analytical method. The authors state that about 15% of a single dose of PFUnDA was eliminated in the feces in the 7 days after dosing in both male and female rats, and time to excrete half of the amount excreted in 7 days was about 40-50 hours. In contrast, about 250 – 370% of the daily dose was excreted in the 7 days following 90 days of daily dosing, indicating that accumulation had occurred over the 90-day dosing period.

An earlier study (Goecke-Flora et al., 1996) used fluorine-19 NMR spectra to evaluate whether PFHpA, PFNA, and PFUnDA were present in urine and/or bile of male rats after a single IV dose (PFHpA -150 mg/kg; PFNA and PFUnDA – 50 mg/kg). PFUnDA was detected only in bile, while PFNA was detected in both urine and bile and PFHpA was detected only in urine. It should be noted that PFAS (and other compounds) that are excreted in bile undergo enterohepatic circulation, in which bile containing PFAS is released into the small intestine followed by reabsorption of the PFAS from the small intestine (with the non-absorbed portion excreted in the feces). This process slows the rate of excretion of compounds that are primarily excreted in the bile.

A preliminary estimate of the half-life of PFUnDA in male rats can be made using information for rats from Mertens et al. (2010) and Goecke-Flora et al. (1996), as well as the information for mice from Fujii et al. (2015). Mertens et al. (2010) report that 15% of a single dose was excreted in the feces in 7 days, and urinary excretion is assumed to be negligible compared to fecal excretion based on Goecke-Flora et al. (1996) and further supported by the seven-fold lower clearance in urine than in feces in mice in Fujii et al. (2015). Based on this information, it is assumed that 85% of the dose remains in the body of the male rat after 7 days, based solely on fecal elimination. An online half-life calculator (https://www.calculator.net/half-life-

calculator.html?type=1&nt=85&n0=100&t=7&t12=&x=46&y=15) provides a half-life estimate of 30 days if 85% of the dose remains in the body after 7 days. It should be noted that this is an upper bound estimate, since consideration of urinary excretion would result in a lower half-life value. A lower half-life value would result in a higher interspecies toxicokinetic extrapolation factor (see *Interspecies dosimetric adjustment* section below), which would result in a lower RfD.

Human studies

Fujii et al. (2015)

Fujii et al. (2015) estimated human urinary and fecal clearance from paired urine-serum and bile-serum samples from Japanese subjects. Bile (drainage for 24 hours) and paired serum samples came from 3 male and 2 female patients (age 68-90) from whom bile was drained for a medical condition. Urine (collected for 24 hours) and paired serum samples came from healthy adult volunteers (5 per sex; age 21-81). Fecal clearance was not measured directly but was estimated by assuming 98% reabsorption from bile for each of the PFCAs evaluated, based on estimated 98% biliary reabsorption of PFOA. The estimate of 98% biliary reabsorption of PFOA was based on an assumed human volume of distribution for PFOA of 200 ml/kg (from previously reported mouse data) and a human half-life for PFOA of 3.8 years from a study of occupationally exposed retired workers (Olsen et al., 2007), and a body weight of 50 kg was assumed for estimating both urinary and fecal clearance. Because of the assumptions used to estimate human fecal clearance, these values are more uncertain than the human urinary clearance values and the mouse urinary and fecal clearance values from the same study.

Relative urinary, fecal, and total clearance of the PFCAs in humans followed a generally similar pattern as was observed in mice (Figure 1). Specifically, urinary clearance declined with chain length from 8 to 11 carbons (0.044, 0.038, 0.015, and 0.005 ml/day/kg for PFOA, PFNA, PFDA, and PFUnDA, respectively), while estimated fecal clearance was relatively constant (0.052, 0.024, 0.050, and 0.060 ml/day/kg for PFOA, PFNA, PFDA, and PFUnDA, respectively). However, estimated clearance values were much lower in humans than in mice (Figure 1). The estimated animal:human total clearance ratios based on mouse oral clearance data are 115:1, 52:1, 45:1, and 68:1 for PFOA, PFNA, PFDA, and PFUnDA, respectively. It should be noted that these animal:human ratios are uncertain because of the uncertainty in the human fecal clearance, which is the major portion of the estimated total human clearance for most of these PFAS, including PFUnDA.

Figure 1 (from Fujii et al., 2015).

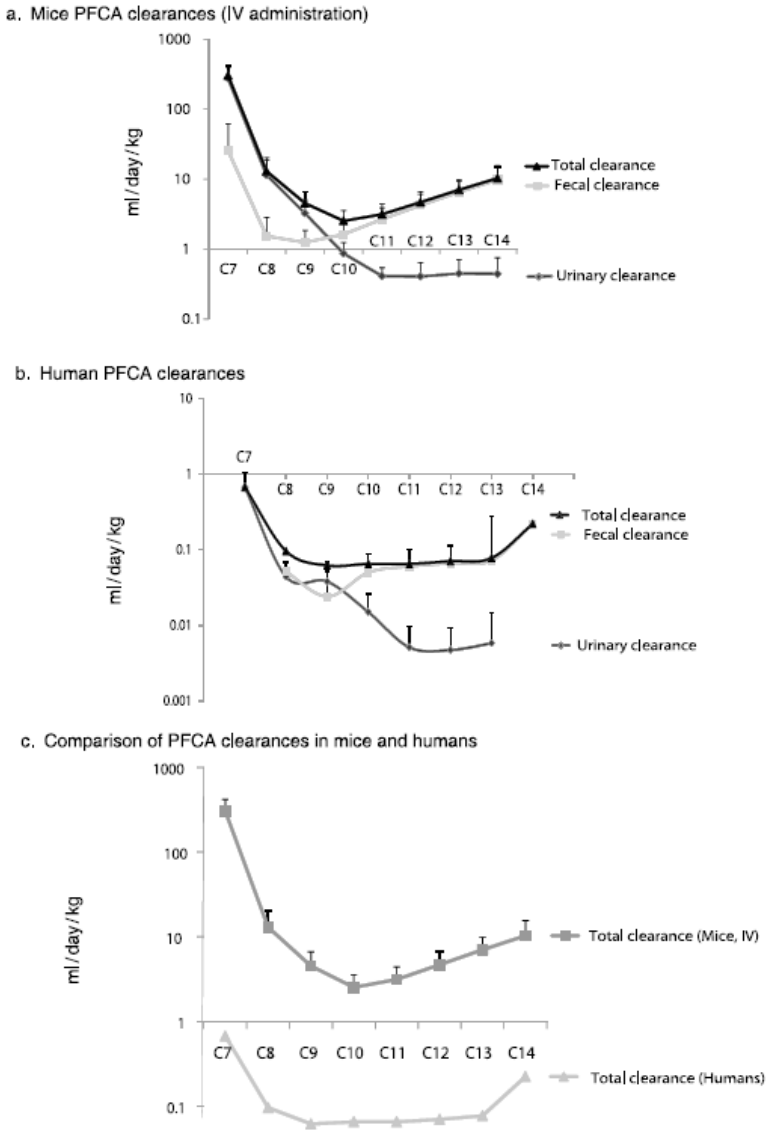


Fig. 3. PFCA (perfluoroalkyl carboxylic acid) clearances in mice and humans (values are means ± SD).

Zhang et al. (2013)

Zhang et al. (2013) also provides human urinary clearance values and elimination half-life estimates for PFUnDA and other PFAAs from a study of the Chinese general population. These estimates are based on paired urine and serum concentrations from 47 males (age 20-88 years), 19 females older than 50 years (maximum age 78 years), and 20 females age 50 years or younger (i.e., of childbearing age; minimum age 21 years). In this study, a morning urine sample was collected, and total daily urinary excretion was estimated by assuming a total daily urine volume

of 1.2 L for females and 1.4 L for males. In estimating clearance values, body weights of 55 kg for females and 65 kg for males were assumed.

Mean urinary clearance values were determined separately for women $\leq$ 50 years of age and older women and men combined. Urinary clearance estimates from Zhang et al. (2013) are much higher than those reported by Fujii et al. (2015). In younger women, the urinary clearance of PFOA, PFNA, PFDA, and PFUnDA was estimated as 0.30, 0.25, 0.066, and 0.064 ml/day/kg, respectively. In older women and men, the values for PFOA, PFNA, PFDA, and PFUnDA were 0.77, 0.15, 0.096, and 0.065 ml/day/kg, respectively. Coincidentally, the urinary clearance values for PFUnDA in Zhang et al. (2013) are essentially identical to the total clearance value of 0.065 ml/day/kg (0.060 ml/day/kg fecal clearance plus 0.005 ml/day/kg urinary clearance) estimated by Fujii et al. (2015).

Zhang et al. (2013) provide estimated elimination half-lives for PFAS in women age years 50 or younger and in men and older women. The estimates are based on the urinary clearance values discussed above, with elimination through menstrual blood loss also considered for younger women. However, fecal elimination is not considered in these half-life estimates, and, all other factors being equal, half-live values that consider fecal elimination would be lower. Mean half-life values in men and older women for PFOA, PFNA, PFDA, and PFUnDA were 2.6, 4.3, 12, and 12 years, respectively. In younger women, the estimated half-lives for PFOA, PFNA, PFDA, and PFUnDA were 2.1, 2.5, 4.5, and 4.5 years, respectively. In all cases, geometric mean and median values were somewhat lower than mean values.

Health Effects

Human epidemiology

Numerous studies have evaluated associations of serum PFAS with a wide variety of health outcomes, and long-chain PFAS that have a large epidemiological database, particularly PFOA and PFOS, are associated with health effects such as increased serum cholesterol, increased liver enzymes, decreased birth weight, and decreased antibody response to vaccines, and, for PFOA, certain types of cancer. These human epidemiology studies typically evaluate multiple PFAS, including PFUnDA in some cases, and the literature search conducted for this document identified studies that report associations of PFUnDA with a number of health effects; these studies are listed in Appendix 1. These health effects include decreased birth weight and other measures of fetal growth in one or both sexes, increased serum cholesterol, increased carotid artery atherosclerosis in the elderly, decreased lipids in neonatal cord blood, decreased eczema and asthma in children, increases or no effect in different studies of childhood infections, changes in parameters related to thyroid function, decreased estradiol and testosterone in men, and other endpoints.

A more thorough evaluation of the epidemiological literature would be needed to determine the strength and consistency of the evidence for associations of PFUnDA with health endpoint(s).

However, only one or a small number of studies report associations with serum PFUnDA for each health endpoint, and other studies of these health endpoints that included PFUnDA among the PFAS that were measured in blood serum did not find associations with PFUnDA.

Additionally, many of the studies that report associations of health effects with PFUnDA also report associations of other PFAS with these health effects. As discussed in the New Jersey Drinking Water Quality Institute Health Effects Subcommittee evaluations of PFOA and PFOS (DWQI, 2017; DWQI, 2018), serum levels of multiple PFAS are often correlated. The relative contribution of individual PFAS to observed associations with health effects cannot be determined without special modeling approaches that were not performed in the studies reporting associations with PFUnDA. Therefore, evaluation of the specific impact of PFUnDA on the observed associations is not possible as other PFAS that co-occur with PFUnDA may have driven these associations. For the reasons discussed above, the human studies are not appropriate as the basis for a toxicity factor (i.e., RfD) for PFUnDA.

Animal Toxicology

Toxicity studies of PFUnDA in mammalian species include: a 14-day oral range finding study in rats (Takahashi et al., 2014); an oral repeated dose (males – 42 days of dosing; females – 41-46 days of dosing) and reproductive- developmental study in rats (Takahashi et al., 2014); two oral rat studies of effects on Leydig cells (Yan et al., 2021; Xin et al., 2022); a study of development of insulitis and diabetes in a mouse strain that is a model of Type 1 diabetes that used much lower doses than the other mammalian studies (Bodin et al., 2016); and an oral study of effects on mast-cell mediated allergic inflammation in mice that used much higher doses than the other mammalian studies (Lee and Kim, 2018). An additional study of hepatic biochemical effects in rats exposed via IV injection (Goecke-Flora and Reo, 1996) is discussed under mode of action, below. Important gaps in the toxicity database for PFUnDA include a lack of chronic and carcinogenicity data, lack of mouse studies at relevant doses, and lack of studies of specific endpoints such as immune system function that are known to be sensitive endpoints for other PFAAs.

14-day oral range finding study in rats (Takahashi et al., 2014)

A 14-day oral range finding study in rats is briefly reported in the *Materials and Methods* section of the publication reporting an oral repeated dose and reproductive-developmental study in rats (Takahashi et al., 2014). Male and female Crl:CD (SD) rats were dosed with 2, 6, 20, 60, 200, or 600 mg/kg/day (presumably by gavage, although not stated) for 14 days. The number of animals per group is not stated, but is assumed to be 5 per sex based on the statement that deaths occurred in 5/5 males and 4/5 females at 20 mg/kg/day, and in all animals at 60, 200, and 600 mg/kg/day. Liver weight was increased in all males and females at 2 and 6 mg/kg/day, and serum levels of the liver enzyme ALP and blood urea nitrogen were increased in males at these same two doses.

Oral repeated dose and reproductive- developmental study in rats (Takahashi et al., 2014)
Crl:CD (SD) rats, age 10 weeks, were dosed by oral gavage with 0, 0.1, 0.3, or 1.0 mg/kg/day PFUnDA (CAS RN 2058-94-8; 98.5% pure) in corn oil; controls were dosed with the corn oil vehicle. Males (12 per dose group) were dosed daily for 42 days, starting 14 days before mating. Five of the 12 males in each dose group were used as a recovery group and evaluated 14 days after the end of dosing. Females (12 per dose group) were dosed daily for 41-46 days, from 14 days before mating and throughout mating and gestation, until lactation day 4. Additional groups of control and high dose females (5 per group) that were not mated were dosed daily for 42 days followed by a 14-day recovery period.

Parameters observed in all animals during the study included clinical signs (daily); body weight (twice per week in main group males, females prior to mating, and recovery group males and females; gestation day [GD] 0, 4, 7, 11, 14, 17, and 20, and postnatal day [PND] 2 and 4 in pregnant females); functional observation battery in cage, in the hand, and open field (once per week during dosing period). Parameters including sensory reactions, grip strength, and spontaneous motor activity observed in 5 animals per group in main group males and recovery group males and females on dosing day 37, in recovery group males and females on day 8 of recovery, and main group females on PND 4.

Urinalysis was performed during the last week of dosing, and blood was collected for hematology and clinical chemistry evaluations at sacrifice. At sacrifice, the following organs were weighed from all animals: brain, thyroid, thymus, heart, liver, spleen, kidney, adrenal gland, testis, and epididymis. Histopathological examination was performed for 5 control and high dose (1.0 mg/kg/day) males and females, and for all animals if histopathological changes were found in high dose animals, for the following organs: cerebrum, cerebellum, pituitary gland, spinal cord, sciatic nerve, thyroid, parathyroid, adrenal glands, thymus, spleen, lymph nodes, heart, lung, trachea, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, bladder, testis, epididymis, uterus, seminal vesicle, sternum and femur.

Main group females were evaluated for estrous cyclicity with vaginal lavage prior to mating, which began after 14 days of dosing. Pregnant females delivered spontaneously and nursed until sacrifice on PND 4. Parameters evaluated include litter size, number of live and dead pups of each sex, and pup body weight on PND 0 and 4. Pups were evaluated for malformations on PND 0 and gross internal examination was performed at sacrifice on PND 4.

No mortality occurred during the study. There were no clinical signs, effects on the functional observation battery, or changes in sensory reactivity or spontaneous motor activity other than decreased forefoot grip strength in both males and females in the 1.0 mg/kg/day recovery group.

Body weight gain (p-value<0.05) was decreased in high dose (1.0 mg/kg/day) males and non-mated females during the dosing and recovery periods, and in main group females during the lactation period. Additionally, body weight was decreased (p<0.05) in non-mated females at the end (days 38 and 41) of the dosing period and throughout (days 0-13) the recovery period. Since food consumption was decreased only on PND 4 in high dose females, the body weight changes at 1.0 mg/kg/day in males and in females at other timepoints were considered a direct effect of PFUnDA, rather than secondary to decreased food consumption.

Hematology changes considered to be treatment-related at the high dose (1.0 mg/kg/day) were decreased fibrinogen and decreased activated partial prothrombin time (p<0.05) in main group and recovery group males, and decreased fibrinogen (p<0.05) in main group females. Clinical chemistry changes considered to be treatment-related at the high dose (1.0 mg/kg/day) were increased blood urea nitrogen in main group (p<0.01) and recovery group (p<0.05) males and females, increased ALP (p<0.01) in main group males and recovery group males and females, decreased total protein (p<0.01) in main group males and females and recovery group females, and decreased albumin in main group males. There were no treatment related effects on urinalysis parameters.

Organ weight changes noted by the authors were as follows: Relative liver weight was increased (p<0.01) at 0.3 mg/kg/day in main group males, and absolute liver weight (p<0.01, in all dosed groups except for p<0.05 in recovery group females) and relative liver weight (p<0.01) were increased at 1.0 mg/kg/day in main group and recovery group males and females. Additionally, absolute and relative spleen weight were decreased (p<0.05) at 1.0 mg/kg/day in main group males.

The only gross pathological changes that were noted were enlarged livers in 2 of 7 males and dark red foci in the stomach in 3 of 7 males at 1.0 mg/kg/day in the main group.

Histopathological changes were observed in the liver as listed below. These changes did not occur in the control groups:

- Hepatocellular hypertrophy at 0.3 mg/kg/day in 3 of 12 males (25%) and 1 of 12 females (8%) in the main group, and at 1.0 mg/kg/day in 7 of 7 males (100%) and 11 of 12 females (92%) in the main group and 5 of 5 males and females (100%) in the recovery group. In males, the effect was more severe at 1.0 mg/kg/day in both the main and recovery groups than at 0.3 mg/kg/day in the main group. In females, the effect was more severe at 1.0 mg/kg/day in the recovery group than in the main group.
- Focal necrosis (minimal) at 1.0 mg/kg/day in 2 of 7 males (26%) and 2 of 12 females (17%) in the main group.

- Hepatocyte degeneration at 1.0 mg/kg/day in 3 of 5 males and females (60%) in the recovery group.
- Diffuse hepatocyte vacuolation at 1.0 mg/kg/day in 3 of 7 main dose males (43%) and 1 of 5 recovery group males (20%).
- Cell infiltration of Glisson's sheath (connective tissue surrounding the liver) at 1.0 mg/kg/day in 2 of 5 females (40%) in the recovery group.

Additionally, erosion of the glandular stomach was observed at 1.0 mg/kg/day in 3 of 7 males (43%) in the main dose group.

In the reproductive-developmental component of the study, PFUnDA did not cause effects on the estrous cycle, reproductive performance, delivery, nursing, sex ratio of live pups, or viability on PND 4, and there were no visible abnormalities in the pups at birth. Thymic remnants noted during the gross pathology examination at sacrifice on PND 4 in the necks of two pups at 0.3 mg/kg/day and two pups at 1.0 mg/kg/day were not considered to be treatment related.

Body weights of both male and female pups was decreased ($p<0.01$) at 1.0 mg/kg/day on PND 0 and PND 4. Since maternal body weight of pregnant dams was not decreased in the dosed groups, the authors concluded that the decreased pup body weight on PND 0 was a direct effect of *in utero* exposure to PFUnDA and that the decrease on PND 4 resulted from *in utero* and/or lactational exposure.

Based on the results of the study, the authors identified the liver as a sensitive target organ for PFUnDA. They noted that the hematological, clinical chemistry, and liver histopathological effects, including increased severity of hepatocellular hypertrophy in males, persisted until the end of the 14-day recovery period, suggesting that PFUnDA is slowly excreted. They also noted that males appeared to be more sensitive to hepatic effects of PFUnDA than females, since liver weight was significantly ($p<0.01$) increased in males but not females at 0.3 mg/kg/day, and the hepatic histopathological changes were more severe in males.

The authors identified a NOAEL for systemic effects of 0.1 mg/kg/day in males and females, based on hepatocellular hypertrophy in both sexes at 0.3 mg/kg/day; relative liver weight was also significantly increased in males at this dose. The NOAEL for reproductive-developmental effects was identified as 0.3 mg/kg/day, based on decreased pup body weight at 1.0 mg/kg/day.

<u>Studies of effects of oral exposure to PFUnDA on Leydig cells in rats (Yan et al., 2021; Xin et al., 2022)</u>

Two studies from the same research group evaluated effects of oral exposure to PFUnDA on testicular Leydig cells in rats (Yan et al., 2021; Xin et al., 2022).

In Yan et al. (2021), male Sprague-Dawley rats (9 per dose group) were dosed by gavage with 0, 1, 5, or 10 mg/kg/day PFUnDA in corn oil for 21 days from age 35 days to 56 days; controls

were dosed with the corn oil vehicle, and animals were sacrificed at the end of the dosing period. This dosing period was selected to include the period of pubertal Leydig cell development. In the low dose group (1 mg/kg/day), body weight gain during the dosing period did not differ from controls, while body weight was significantly decreased at the end of the dosing period in the two higher dose groups (5 and 10 mg/kg/day). Absolute testis and epididymis weights were also decreased compared to controls at 5 and 10 mg/kg/day, but relative testis weight was increased; relative epididymis weight data were not provided.

Serum testosterone and luteinizing hormone levels were significantly decreased at all doses, while serum follicle stimulating hormones was decreased only at the two higher doses, and serum estradiol was not affected; these parameters were evaluated at sacrifice at the end of the dosing period. Immunohistochemical staining for proteins specific to Leydig cells in general, mature Leydig cells, and Sertoli cells indicated that the number of Leydig cells and mature Leydig cells in the testes were decreased at 5 and 10 mg/kg/day while Sertoli cell numbers were not affected. Histopathological evaluation of several sections of the epididymis indicated that the number of sperm in the epididymal tubules was decreased in PFUnDA-treated rats. Photographs of the microscopic histology at each dose are included in the publication, but quantitative information on the dose-response for this effect is not provided.

Additional mechanistic studies reported in Yan et al. (2021) evaluated effects of PFUnDA on testicular gene expression, protein levels, lipids, oxidative stress, and phosphorylation pathways related to regulation of autophagy. In summary, several testicular genes were downregulated at all doses of PFUnDA, while others were affected only at higher doses. Similarly, levels of proteins associated with affected genes were decreased, some at all doses and others only at higher doses. Testicular triglyceride levels were increased at 5 and 10 mg/kg/day, while testicular cholesterol levels were unaffected. PFUnDA also induced oxidative stress and decreased phosphorylation of proteins related to autophagy; these effects were stated by the authors to potentially affect the differentiation of Leydig cells.

Based on the results of Yan et al. (2021), Xin et al. (2022) evaluated the effects of lower doses of PFUnDA on Leydig cell function in adult male rats. Eight-week-old male Sprague-Dawley rats (10 per dose group) were dosed by gavage with 0, 0.1, 0.5, 1, or 5 mg/kg/day PFUnDA for 28 days; controls were dosed with the corn oil vehicle, and animals were sacrificed at the end of the dosing period. One animal each in the 0.1 and 1 mg/kg/day groups and two animals in the 0.5 mg/kg/day group died from dosing errors. At the end of the dosing period, body weight was reduced by 15% and 55% in the 1 and 5 mg/kg/day groups, respectively. Absolute and relative testis weights were reduced at 5 mg/kg/day, and absolute epididymis weight (relative weight not shown) was reduced at 1 and 5 mg/kg/day. The authors state that data for the specific endpoints related to Leydig cell function evaluated in this study are not shown for the 5 mg/kg/day dose group because of the large magnitude of the body weight decrease at this dose.

At the end of the dosing period, serum testosterone levels were decreased at all doses, and the decrease was significant ($p<0.05$) at 0.5 and 1 mg/kg/day, but not at 0.1 mg/kg/day. Serum luteinizing hormone and follicle stimulating hormone levels were not affected. Immunohistochemical staining of proteins that are biomarkers for Leydig cells in general and mature Leydig cells indicated that the total Leydig cell number was reduced at all doses with significance ($p<0.01$) at 1 mg/kg/day, and the number of mature Leydig cells was significantly reduced in a dose-related manner at all doses (0.1 mg/kg/day and higher). Studies of Leydig cell gene expression and protein levels indicated that PFUnDA down regulated genes and decreased levels of proteins related to testicular function, with some genes and proteins affected at the low dose (0.1 mg/kg/day) and others affected at higher doses. Similarly, PFUnDA decreased phosphorylation of proteins related to Leydig cell function, with phosphorylation of some proteins affected at the low dose (0.1 mg/kg/day) and others affected at higher doses.

Study of development of insulitis and diabetes in a mouse strain that is a model of Type 1 diabetes that used much lower doses than the other mammalian toxicology studies (Bodin et al., 2016)

Bodin et al. (2016) studied the effects of exposure to PFUnDA beginning *in utero* and continuing through adulthood in female non-obese diabetic (NOD) mice, a model strain for Type 1 diabetes. Dams (approximately 23-24 per dose group) were exposed to 0, 3, 30, and 300 µg/L PFUnDA in drinking water prior to mating and throughout gestation and lactation. As such, offspring were potentially exposed *in utero* and through breast milk, and female offspring (8-10 per litter) were further exposed through drinking water to these same concentrations from weaning to up to 30 weeks of age. Female offspring were evaluated for development of insulitis (inflammation of the islets of Langerhans in the pancreas) and diabetes; females were selected because they are more susceptible than males to these effects in this mouse strain.

Based on body weight and measured drinking water consumption at 10 weeks of age, the doses were calculated as about 0.417, 41.7, and 41.7 µg/kg/day (0.000417, 0.00417, and 0.0417 mg/kg/day). These doses are much lower than in the other animal studies described above.

There were no effects on litter size or the sex ratio of offspring in the PFUnDA-treated groups. Early signs of diabetes including alterations in pancreatic histology were evaluated in female offspring at 7 and 11 weeks of age. The average grade of severity of insulitis was significantly increased ($p<0.05$) at the high dose (300 µg/L) at 11 weeks, and the authors stated that there was a trend toward increased severity at 7 weeks, although effects were not statistically significant. In pancreatic islets where insulitis was not present, the number of tissue resident macrophages was decreased with increased PFUnDA dose, although this effect was not statistically significant, and the number of apoptotic cells was significantly increased at the high dose at age 11 weeks. The incidence of diabetes increased over time in all groups starting at age 15 weeks and did not

significantly differ between dose groups, but development of diabetes appeared to be delayed at the two lower doses (3 and 30 µg/L).

Study of effects of oral exposure to PFUnDA on mast cell-mediated allergic inflammation in mice (Lee and Kim, 2018)

Lee and Kim (2018) state that ovalbumin is used as a sensitizer in a model of active systemic anaphylaxis, which is relevant for evaluation of mast cell function and allergic inflammation. The Materials and Methods section of Lee and Kim (2018) state that male ICR mice (5 per dose group) were dosed by intraperitoneal injection with ovalbumin on days 0 and 7, followed by oral dosing of PFUnDA, PFHpA, PFNA, or PFDA in phosphate buffered saline (100 mg/kg) on days 9, 11, and 13. Control groups not dosed with ovalbumin and/or PFAAs are not mentioned. It is stated that on day 14, another dose of ovalbumin was injected intraperitoneally, and rectal temperature was measured every 10 minutes until sacrifice at 70 minutes, and that at sacrifice, blood was collected for measurement of serum histamine, TNF-alpha, IgE, and IgG_1. However, the experimental protocol presented in the Materials and Methods section is inconsistent with the data shown in the Results section, which include data from a group of mice not dosed with ovalbumin or PFAAs and groups of mice dosed with PFAAs but not ovalbumin. Additionally, other aspects of the experimental protocol are also not clearly described. As such, it was not possible to evaluate the results and conclusions of this study. Additionally, the dose of PFUnDA used in this study (100 mg/kg) is much higher than the doses at which effects occurred in the rat toxicity studies described above (Takahashi et al., 2014 – 0.3 mg/kg/day; Xin et al., 2022 – 0.1 mg/kg/day). For these reasons, this study was not considered relevant to RfD development, and it was not further summarized or considered.

Mode of action

The discussion in this section focuses on the mode of action (MOA) for hepatic toxicity of PFUnDA, since this effect was selected as the critical endpoint for the RfD (see *Reference Dose Development* section, below). Additional data relevant to the potential MOAs for other effects of PFUnDA (for example, thyroid disruption in zebrafish; Kim et al., 2021) are not discussed herein.

Detailed evaluations of the MOA for hepatic effects in rodents of other PFAS were performed by the New Jersey Drinking Water Quality Institute (PFNA - DWQI, 2015; PFOA – DWQI, 2017; PFOS – DWQI, 2018) and NJDEP (chloroperfluoropolyether carboxylates, ClPFPECAs – NJDEP, 2021). These evaluations concluded that the hepatic effects of these PFAS in rodents are adverse and relevant to humans. Specifically, it was concluded that increased liver weight and hepatocellular hypertrophy caused by these PFAS are accompanied by and/or progress to other more severe effects including necrosis and other adverse histopathological changes, and increased serum levels of liver enzymes. The human relevance of hepatic effects of PFAS in laboratory animals is further supported by human epidemiological studies that report associations

of PFOA, PFNA, and ClPFPECAs with increased serum levels of liver enzymes. The New Jersey RfDs for PFOA, PFNA, and ClPFPECAs are based on increased relative liver weight in rodents. For PFOS, a candidate RfD based on hepatic effects in rats was developed, but the final RfD was based on immune system suppression in mice, which was a more sensitive endpoint.

The conclusions of recent USEPA evaluations of several PFAS regarding adversity and human relevance of hepatic effects in rodents are consistent with those of NJ DWQI and NJDEP. USEPA selected rodent hepatic toxicity as the critical effect for the RfDs for PFBA (draft USEPA, 2021a) and GenX (USEPA, 2021b), and USEPA also considered rodent hepatotoxicity as a potential critical effect for the updated RfDs for PFOA (draft USEPA 2021c), PFOS (draft USEPA 2021d), and the RfD for PFHxA (draft USEPA, 2022).

Additionally, scientists from The Netherlands National Institute for Public Health (RIVM) reviewed data relevant to the MOA of rodent hepatic effects of PFAS and concluded that the rodent hepatic effects of PFAS in general should be considered adverse and relevant to humans for the purposes of risk assessment (Bil et al., 2021). Bil et al. (2021) developed relative potency factors (RPFs) for 23 PFAS including PFUnDA based on relative potency (via the oral exposure route) for increased relative liver weight in male rats. PFOA was designated as the index compound and assigned an RPF of 1, and the RPFs for the other 22 PFAS were based on comparison to PFOA.

The New Jersey, USEPA, and RIVM documents cited above conclude that increased liver weight and other hepatic effects of PFAS occur through both peroxisome proliferator activated receptor-alpha (PPAR-alpha) dependent and PPAR-alpha independent processes. The relative importance of PPAR-alpha dependent and independent processes for rodent hepatic effects differs among PFAS (Post et al., 2017). For example, hepatic effects of PFOS in rats appear to be almost totally PPAR-alpha independent, while both PPAR-alpha dependent and independent processes contribute to the rodent hepatic effects of PFOA and PFNA.

Several studies provide information on the MOA for hepatic effects of PFUnDA. These data indicate that, like other PFAS, PFUnDA can activate both PPAR-alpha and other receptors unrelated to PPAR-alpha (i.e., the estrogen receptor) in the liver.

Goecke-Flora and Reo (1996) reported that the activity of fatty acyl CoA-oxidase, a marker for PPAR-alpha activation, in male rats injected intraperitoneally with a single 50 mg/kg dose of PFUnDA was about four times higher than in control rats 3 and 5 days after dosing.

Wolf et al. (2012) evaluated PFCAs with 4-12 carbons and perfluoroalkyl sulfonates (PFSAs) with 4, 6, and 8 carbons for *in vitro* transactivation of mouse and human PPAR-alpha in transiently infected COS-1 cells. Because this is an *in vitro* study, differences in the receptor

transactivation dose-response data among PFAAs reflect differences in intrinsic potency for receptor transactivation but do not reflect differences in toxicokinetics (i.e., generally higher internal doses from the same administered dose with increasing chain length). PFUnDA activated both mouse and human PPAR-alpha. PFUnDA, as well as PFOA and PFNA, were among the more potent activators of mouse PPAR-alpha, while the three PFSAs were less potent than the 9 PFCAs. In contrast, the potency for PFOA and PFNA activation of human PPAR-alpha was similar as for mouse PPAR-alpha, while PFUnDA was about 10-fold less potent as an activator of human PPAR-alpha than mouse PPAR-alpha.

Additionally, PFUnDA activated the hepatic estrogen receptor with potency similar to PFOA and PFNA in rainbow trout (Benninghoff et al., 2011), a species which is insensitive to PPAR-alpha activation (Tilton et al., 2008). In a subsequent study that did not include PFUnDA (Benninghoff et al., 2012), PFOA, PFNA, and PFDA were tested for liver tumor promoting activity in rainbow trout. All three PFCAs increased the incidence and number of liver tumors in rainbow trout that had been initiated with aflatoxin, and they also induced a genomic signature similar to that induced by 17-beta-estradiol. In contrast, clofibrate, a model PPAR-alpha activating compound, did not promote liver tumors and did not regulate genes in common with 17-beta-estradiol (Tilton et al., 2008).

Development of Reference Dose

There are no human or animal data on the potential carcinogenicity of PFUnDA. Therefore, the fish consumption triggers for PFUnDA are based on a RfD for non-carcinogenic effects.

Selection of critical study and endpoint

Non-carcinogenic toxicological effects that are sensitive, well established, adverse or a precursor to adverse effect(s) and considered relevant to humans are appropriate for consideration as the basis for RfD development.

In the limited toxicological database for PFUnDA, the studies at which effects were reported at relatively low doses include the rat repeated dose and reproductive-developmental study in which parental males were exposed for 42 days and parental females were exposed for 41-46 days (Takahashi et al., 2014), the study of Leydig cell function in adult male rats exposed for 28 days (Xin et al., 2022), and the study of insulitis and diabetes development in female NOD mice (Bodin et al., 2016).

In Takahashi et al. (2014), the LOAEL for systemic effects was 0.3 mg/kg/day. At this dose, relative liver weight was significantly increased ($p<0.01$) compared to controls in males, and hepatocellular hypertrophy occurred in 3 of 12 males (25%) and 1 of 12 females (8%) but not in any controls. The LOAEL for developmental effects in this study was 1.0 mg/kg/day. At this dose, pup body weight was significantly decreased ($p<0.01$) on PND 0 and PND 4 in the absence of decreased maternal body weight.

In Xin et al. (2022), the LOAEL was 0.1 mg/kg/day, the lowest dose included in the study. The number of mature Leydig cells, as assessed by immunohistochemical staining of a protein biomarker, was significantly decreased at all doses in a dose-related fashion, including at 0.1 mg/kg/day ($p<0.05$). Statistically significant decreases in related parameters, including total number of Leydig cells and serum testosterone levels, occurred at higher doses. Additionally, some biochemical changes related to Leydig cell function including changes in expression of some genes, levels of some proteins, and phosphorylation of some proteins were statistically significant at 0.1 mg/kg/day, while other changes were statistically significant at higher doses.

In Bodin et al. (2016), the grade of severity of insulitis and the number of apoptotic cells in the pancreatic islets in NOD mice, a model strain for Type 1 diabetes, were significantly increased ($p<0.05$) at the high dose (approximately 0.042 mg/kg/day) at 11 weeks. The incidence of diabetes increased over time in all groups starting at age 15 weeks and did not significantly differ between dose groups, but development of diabetes appeared to be delayed at the two lower doses (approximately 0.00042 and 0.0042 mg/kg/day).

From the studies and toxicological endpoints described above, increased relative liver weight in male rats exposed for 42 days (Takahashi et al., 2014) was selected as the primary basis for the RfD, and dose-response evaluation was performed for this dataset. In Takahashi et al. (2014), relative liver weight was increased in males at all doses in a dose-related fashion, with statistically significant increases at the two higher doses (0.3 and 1.0 mg/kg/day). Therefore, the NOAEL and LOAEL for increased relative liver weight in males in this study were identified as 0.1 mg/kg/day and 0.3 mg/kg/day, respectively. Increased relative liver weight in females and decreased pup body weight were less sensitive endpoints than increased relative liver weight in males in this study. Although Bodin et al. (2016) and Xin et al. (2022) reported toxicological effects at lower doses than Takahashi et al. (2014), they were not elected as the primary basis for the RfD for reasons discussed below.

As discussed in the *Mode of Action* section above, increased relative liver weight is a well-established and sensitive endpoint that is considered relevant to humans for PFAS in general and has been used as the critical endpoint for RfDs for several other PFAS. There is no information to suggest that the increased relative liver weight caused by PFUnDA in rats is not relevant to humans, and the selection of increased relative liver weight as the critical endpoint for PFUnDA is supported by other effects of PFUnDA that are indicative of liver damage in Takahashi et al. (2014). Specifically, serum levels of the liver enzyme ALP were increased at 1.0 mg/kg/day in males at the end of the dosing period and in both sexes after a 14 day recovery period; severity of hepatocellular hypertrophy increased with increasing dose in males both at the end of dosing and after a 14 day recovery period; focal necrosis at 1.0 mg/kg/day was reported in both sexes at the end of the dosing period; hepatocyte degeneration was reported at 1.0 mg/kg/day in males and females at the end of the recovery period; and hepatocyte vacuolation, potentially associated with steatosis (NJDEP, 2021) was reported at 1.0 mg/kg/day in males at the end of dosing and after the recovery period.

Although the LOAEL of 0.1 mg/kg/day for decreased number of mature Leydig cells in Xin et al. (2022) is lower than the LOAEL of 0.3 mg/kg/day for hepatic effects in Takahashi et al. (2014), the Leydig cell effects were not selected as the critical effect for RfD development for several reasons. First, this effect is not as well established as hepatic effects for PFAS in general and, to our knowledge, it has not been used as the basis for RfD development by USEPA or other agencies. Additionally, data for this effect are presented in a bar graph by Xin et al. (2022) and numerical data are not presented. Benchmark Dose modeling could not be performed for this effect since numerical data are needed and they were not available. That being said, this study suggests the potential for effects that are more sensitive than increased relative liver weight, including Leydig cell toxicity, and this possibility is considered in development of the RfD (see *Application of uncertainty factors* section, below).

Bodin et al. (2016) reported statistically significant effects on severity of insulitis and number of apoptotic cells in the pancreatic islets at 0.042 mg/kg/day, a lower dose than in the other studies. This study was not considered appropriate as the primary basis for RfD development for several reasons: it was conducted in a non-standard strain of mice that is susceptible to diabetes; the endpoints affected by PFUnDA have not been used as the basis for RfD development to our knowledge, and the data are presented graphically (numerical data are not presented). As is the case for Xin et al. (2022), this study suggests the potential for effects that are more sensitive than increased relative liver weight, and this possibility is considered in the development of the RfD.

Determination of Point of Departure (POD)

The first step in dose-response analysis is identification of a Point of Departure (POD), which is the dose within or close to the dose range used in the study from which extrapolation begins. As described below, if a Benchmark Dose can be developed, it is preferred for use as the POD. If BMD modeling does not give an acceptable fit to the data, the NOAEL (or LOAEL, if a NOAEL is not identified) is used as the POD. The BMD modeling presented below was performed using USEPA BMD Software Version 3.2.

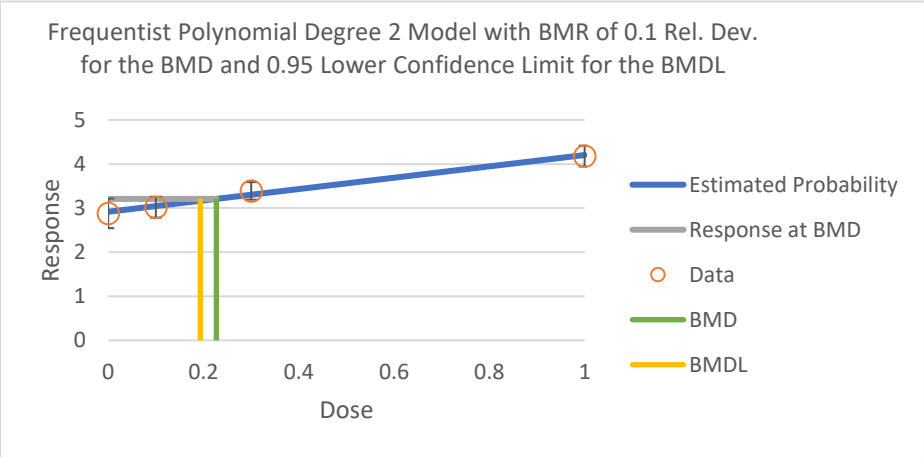
BMD modeling for a 10% change in relative liver weight, consistent with the Benchmark Response (BMR) for relative liver weight used in previous New Jersey PFAS risk assessments (DWQI, 2015; DWQI, 2017; NJDEP, 2021), was performed for the most sensitive dataset, adult male rats in Takahashi et al. (2014). The data are shown in Table 5.

Table 5. Relative liver weight (% of body weight) in male rats (Takahashi et al. 2014)

Dose	N	Mean	Std. Dev.
0	5	2.88	0.27
0.1	5	3.02	0.19
0.3	5	3.39	0.16
1	5	4.18	0.19

The BMD modeling results are provided in Appendix 2. The model with normal distribution and constant variance that provided the best fit was the polynomial degree 2 model, and this model was recommended by the BMD software. For this model, the AIC was the lowest (AIC = -3.35). This model provided a BMD of 0.23 mg/kg/day and a BMDL of 0.19 mg/kg/day. Because a restricted model with a normal distribution and constant variance fit the dataset, BMD modeling results from unrestricted models were not considered. Therefore, the BMDL of 0.19 mg/kg/day was selected as the POD for relative liver weight. The dose-response plot that is the basis for this BMDL is shown in Figure 2.

Figure 2. Graphical results for recommended BMDL model for increased relative liver weight in male rats (Takahashi et al., 2014).



Interspecies dosimetric adjustment

Because PFAS are excreted much more rapidly in rats than in humans, the same administered dose results in a much higher internal dose (i.e., body burden) in humans than in rats. Serum PFUnDA levels were not measured in any of the available toxicity studies, and the POD for increased relative liver weight identified above is based on administered doses to rats (mg/kg/day). To account for the much higher internal dose from a given administered dose in humans as compared to rats, the PODs from the rat studies were converted to human equivalent doses (HEDs) by adjusting for the ratio of estimated half-lives in humans and male rats. This approach, using the ratio of human:rodent half-lives to determine HEDs, has been used in the development of toxicity factors (RfDs and cancer slope factors) for other PFAS including for the chronic RfD for ClPFPECAs (NJDEP, 2021), short-term RfDs for PFOA and PFOS (USEPA, 2009), PFOA cancer slope factor (DWQI, 2017), and chronic and subchronic PFBS RfDs (MDH, 2020; USEPA, 2021e).

The mean human half-live values for PFUnDA reported in Zhang et al. (2013) are 12 years for men and older women and 4.5 years for women of childbearing age. Since the critical effect for PFUnDA is hepatic toxicity, which is not specific to women of childbearing age, the half-life for men and older women is relevant to HED development. The half-life of 12 years from Zhang et al. (2013) is based on urinary clearance, and consideration of fecal clearance would result in a

shorter half-life. However, the urinary clearance value for PFUnDA of 0.065 ml/day/kg from Zhang et al. (2013) is identical to the total clearance value of 0.065 ml/day/kg (0.060 ml/day/kg fecal clearance plus 0.005 ml/day/kg urinary clearance) estimated by Fujii et al. (2015).

Additionally, the human half-life estimates for all PFCAs in Zhang et al. (2013) are based on the volume of distribution for PFOA of 170 ml/kg reported by Thompson et al. (2010). However, Fujii et al. (2015) report that the volume of distribution of PFUnDA in mice is larger than for PFOA. Relevant to this topic, Zhang et al. (2013) discuss that the volumes of distribution for PFOA in different mammalian species are quite consistent as reported by Han et al. (2012), and this may also be true for other PFCAs including PFUnDA. This information suggests that the human volume of distribution of PFUnDA is likely to be larger than for PFOA, and a larger volume of distribution results in a longer half-life with a given clearance value, as shown below:

$$\text{Half-life (days)} = \frac{0.693 \times \text{Volume of Distribution (ml/kg)}}{\text{Clearance (ml/day/kg)}}$$

Where: 0.693 = natural log of 2

Taken together, the information on clearance values and volume of distribution discussed above indicates that, although uncertain, the human half-live of 12 years (4380 days) provided by Zhang et al. (2013) is appropriate for development of the interspecies dosimetric factor for PFUnDA.

The half-life of PFUnDA in rats has not been reported, and a half-life of 30 days in male rats was estimated from the relevant available information in the *Toxicokinetics* section above. While uncertain, this estimate appears reasonable based on half-lives in male rats reported for PFOA, PFNA, and PFDA of 6 days, 30 days, and 40 days, respectively (Ohmori et al., 2003). Therefore, it is considered appropriate for development of the interspecies dosimetric factor for PFUnDA.

The ratio of the estimated human and male rat half-lives (4380 days/30 days) is 146. This value is similar to the ratio of human and rat half-lives of 120 for PFOA that was used as the dosimetric adjustment factor in developing the cancer slope factor for PFOA by DWQI (2017). The human dose corresponding to the POD in male rats of 0.19 mg/kg/day (i.e., the HED) is (0.19 mg/kg/day)/146 = 0.0013 mg/kg/day or 1300 ng/kg/day.

The HED for the POD for increased relative liver weight for PFOA is 6100 ng/kg/day (DWQI, 2017). The 4.7-fold difference between the PFOA HED of 6100 ng/kg/day (based on data from male mice) and the PFUnDA HED of 1300 ng/kg/day for increased relative liver weight (based on data from male rats) is consistent with the relative potency factor (RPF) of 4 for increased relative liver weight for PFUnDA relative to PFOA, which is assigned a value of 1, from Bil et al. (2021). That being said, it should be noted that the RPFs developed by Bil et al. (2021) are all based on data from male rats.

Application of uncertainty factors

The RfD was developed by application of uncertainty factors (UFs) to the HED of 1300 ng/kg/day that corresponds with the BMDL for increased liver weight in male rats. The choice of uncertainty factors was consistent with current USEPA IRIS guidance (USEPA, 2002; USEPA, 2016b) and previous risk assessments developed by NJDEP. The UFs address specific factors for which there is uncertainty about the relationship of the HED derived from the rat POD to the protection of sensitive human subpopulations over a lifetime of exposure. UFs are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10, consistent with USEPA guidance (USEPA, 2002).

The five UFs shown below were considered. USEPA (2002) recommends that the total UF not exceed 3000 since a higher UF indicates that the level of uncertainty is too great to support RfD development. USEPA (2002) further notes that the maximum recommended total UF of 3000 applies only to the five UFs listed below and that it does not apply to other adjustment factors such as the interspecies toxicokinetic adjustment derived above.

- $UF_{intraspecies}$ – To account for the potential greater sensitivity of sensitive human subpopulations than the average human population. A full value of 10 is typically applied unless the endpoint is based on human data that include sensitive sub-populations.

- $UF_{duration}$ – Applied when a study of less than chronic duration is used to account for potential effects at lower doses with chronic exposure.

- $UF_{interspecies}$ – Applied when animal data are used to address the potentially greater sensitivity of humans than animals. Two factors of 3 each (i.e., one half on a log scale of the full default UF of 10) are normally applied to account for toxicokinetic and toxicodynamic differences. For PFUnDA, the interspecies toxicokinetic difference is accounted for with the ratio of half-lives in humans and rats. A UF of 3 (rather than a full value of 10) is therefore used to account for potential toxicodynamic differences between rodents and humans.

- UF_{LOAEL} – Applied when a LOAEL is used to estimate the corresponding NOAEL, when no NOAEL is identified in the study under consideration. A UF_{LOAEL} of 1 is used (i.e., no adjustment) when a BMDL is used since the BMDL is considered to be an estimate of the NOAEL.

- $UF_{database}$ – To account for potentially more sensitive effects, target organs, populations, or life stages that have not been fully evaluated. Examples of such database gaps include lack of data on reproductive, developmental, or immune system effects, as well as lack of sufficient data (e.g., from multiple species) for any specific effects that have been identified for the contaminant being evaluated or related contaminants.

The UFs used in the RfD for PFUnDA and the rationale for their selection are as follows:

- $UF_{intraspecies}$ = 10. The default value of 10 was used to account for potentially more sensitive human subpopulations.

- $UF_{interspecies}$ = 3. To account for interspecies toxicodynamic differences as discussed above.

- $UF_{duration}$ = 10. In the critical study (Takahashi et al., 2014), male rats were dosed for 42 days (less than subchronic duration).

  Although the New Jersey RfDs for PFOA and PFOS are based on studies of less than chronic duration, no adjustment for duration of exposure was made (i.e., a $UF_{duration}$ of 1). These RfDs were based on data on serum PFAS levels rather than administered dose, and available data indicated that the critical effect does not occur at lower serum PFAS levels or increase in magnitude with longer exposure at the same serum PFAS level. In these cases, the possibility that serum PFAS levels may continue to increase with longer exposure is taken into account by the use of serum PFAS levels as the dose metric.

  In contrast, the New Jersey RfDs for PFNA and ClPFPECAs, which are also based on studies of less than chronic duration, include a $UF_{duration}$ of 10 because the data indicated that the critical effect occurred at lower doses and/or increased in magnitude with longer exposure duration.

  For PFUnDA, the RfD is based on administered dose, because serum PFAS levels were not reported in the critical study. The critical study is of less than subchronic duration, no chronic data are available, and there is no information about whether or not the critical effect would occur at lower doses and/or increase in magnitude with longer exposure duration. As such, the default $UF_{duration}$ of 10 was applied.

- UF_{LOAEL} = 1. No adjustment was made because a BMDL is used.

- $UF_{database}$ = 3. The decreased absolute and relative spleen weight in male rats reported in Takahashi et al. (2014) suggests the potential for more sensitive types of immune system toxicity. Toxicity to Leydig cells in rats as reported by Xin et al. (2022) occurred a dose 3 times below the LOAEL for increased liver weight, and effects on the pancreas were reported in female mice from a strain susceptible to diabetes were reported at low doses (Bodin et al., 2016). Additionally, there are no data on other sensitive endpoints (e.g., effects on mammary gland development) which have been identified as a sensitive endpoints for other PFAS.

UF_{Total} = 1000

The RfD was derived from the HED of 1300 ng/kg/day (0.0013 mg/kg/day), which is based on a BMDL of 0.19 mg/kg/day, and the total UF of 1000 as follows:

Reference Dose = 1300 ng/kg/day / 1000 = 1.3 ng/kg/day

Fish consumption trigger levels

Fish consumption trigger levels for daily consumption that are based on an RfD for non-carcinogenic effects are developed with the following equation, as recommended by USEPA (2000):

$$\text{Daily trigger concentration (ng/g)} = \frac{\text{RfD (ng/kg/day) x 70 kg}}{\text{227 g}}$$

Where: Body weight = 70 kg

Meal size = 227 g

For consumption triggers that are less frequent than daily (weekly, monthly, once every three months, yearly), the daily (unlimited) consumption triggers were multiplied for the appropriate timeframe (i.e., 7-fold for weekly, 30-fold for monthly, 91-fold for once every 3 months (i.e., four times per year), 365-fold for yearly).

The equation shown above was used to develop triggers for PFUnDA, using the RfD of 1.3 ng/kg/day presented above. The consumption trigger for daily consumption is 0.40 ng/kg/day, and this value and the triggers for less frequent consumption are shown in Table 6. New Jersey fish advisory triggers were also previously developed for PFOA, PFOS, and PFNA using this same approach (NJDEP, 2018).

PFUnDA and other long-chain PFAS such as PFOS, PFNA, and PFOA cause developmental toxicity in laboratory animals. In humans, the developing fetus and infant/young child are considered susceptible subpopulations for the developmental effects of these PFAS (DWQI, 2015; DWQI, 2017; DWQI, 2018; USEPA, 2016a; USEPA, 2016c, USEPA, 2021c, USEPA, 2021d). Because PFUnDA and other long-chain PFAS have long human half-lives (several years), body burdens remain elevated for many years after exposure ends. Therefore, in women who have elevated body burdens when they become pregnant, these body burdens will remain elevated during pregnancy and lactation. As discussed in the *Biomonitoring* section above, PFUnDA, like other long-chain PFAS, is present in human breast milk. While numerical data are not available specifically for PFUnDA, serum levels of other long-chain PFAS in breast fed infants are higher than maternal serum levels and this is also be expected to be the case for PFUnDA.

For these reasons, it is not advisable for subgroups susceptible to developmental effects (i.e., pregnant and nursing women, young children, women of childbearing age) to receive large doses of PFUnDA or other long-chain PFAS, even if infrequent. Therefore, consistent with the approach used for advisory triggers for PFOA, PFOS, and PFNA (NJDEP, 2018), the advisory triggers for PFUnDA for consumption "Once Every 3 Months" or "Yearly" are not considered to be protective for individuals in these high-risk groups. For the general population (i.e., all others not in the high-risk group) these meal frequencies are allowed (Table 6). This approach has also been used for fish consumption advisories for other contaminants that cause developmental toxicity such as mercury and PCBs.

Table 6. Fish Tissue Concentrations Triggering Consumption Advisories for the General Population and the High-Risk Population (ng/g; µg/kg; ppb) for PFUnDA

Fish Advisory Consumption Frequency	General Population	High Risk Population
Unlimited (based on daily)	≤0.40*	≤0.40*
Weekly	0.40 - 2.8	0.40 - 2.8
Monthly	>2.8 - 12.0	>2.8 - 12.0
Once every 3 months**	>12.0 - 36.5	Not applicable
Yearly**	>36.5 - 146	Not applicable
Do not eat	>146	>12.0

These calculations are based on a meal size of 8 oz. (227 g), and a body weight of 70 kg.

* The reporting level for PFUnDA in the NJDEP study of PFAS in fish (Goodrow et al., 2020) was 0.5 - 1 ng/g.

** Advisories based on consumption frequency of "Once Every 3 Months" or "Yearly" are not applicable to individuals in high-risk groups (pregnant and nursing women, young children, women of childbearing age).

Discussion of uncertainties

The RfD presented in this document is based on currently available information and is intended to be used only for development of non-regulatory fish consumption advisory triggers. Development of the RfD for PFUnDA required the use of several assumptions, particularly for

the toxicokinetic parameters used in the interspecies toxicokinetic adjustment. If NJDEP considers development of regulatory standards for PFUnDA in the future, the Risk Subcommittee recommends that the basis for the RfD developed in this document be reevaluated to determine whether it is of sufficient certainty for regulatory use and that more recent scientific information that may have become available should be considered.

The uncertainty factors applied in the development of the RfD are intended to account for uncertainties associated with the following uncertainties: inter-individual and interspecies susceptibility to the toxicity of PFUnDA; lack of data on chronic exposure; and potentially more sensitive toxicological effects. Specific uncertainties associated with the RfD and fish consumption triggers for PFUnDA are discussed below.

- There are no data on potential carcinogenic effects of PFUnDA. Chronic exposure to other PFAS, including PFOA, PFOS, and GenX, causes tumors in rats, but other long-chain PFAS have not been tested for carcinogenicity. Additionally, PFOA, a long chain-PFCA structurally analogous to PFUnDA, is associated with several types of cancer in humans. The application of the database uncertainty factor is intended to account for the lack of data for non-carcinogenic effects that are potentially more sensitive than the critical effect for the RfD, but it does not account for lack of data on carcinogenicity

- Without additional toxicological data from species other than the rat, it is not possible to definitively determine whether the RfD for PFUnDA is sufficiently protective. Mice are more sensitive than rats to several PFAS including PFOA, PFNA, PFOS, and HFPO-DA (GenX), and this is potentially also the case for PFUnDA. The interspecies uncertainty factor is intended to account for this uncertainty.

- PFUnDA is detected in human breast milk, but no data on relationship between maternal exposure and breast milk concentration are available. It is known that exposure to the infant through breast milk is greater than maternal exposure for other long-chain PFAS. Without information on maternal transfer of PFUnDA to breast milk, it is not possible to definitively determine whether the RfD for PFUnDA is sufficiently protective for maternal exposures resulting in potentially higher exposures to breast fed infants.

- Uncertainties about the human relevance of effects observed in animals are inherent to all risk assessments based on animal data. As discussed above, the effects of PFUnDA observed in experimental animals are considered relevant to humans for the purposes of risk assessment.

- Available information indicates that the toxicological effects of PFUnDA (e.g., liver toxicity, decreased offspring growth) are also common to other PFAS that are detected in recreationally caught fish in NJ, including PFOS and PFNA. Therefore, toxicological interactions may occur when there is co-exposure to PFUnDA and these other PFAS through fish consumption. However, the potential for additive toxicity of PFUnDA and

other PFAS was not considered in the development of the fish consumption trigger levels for PFUnDA.

Acknowledgements: The TIBC Risk Subcommittee thanks Dr. Brian Pachkowski for his helpful input on an earlier draft of this document and Dr. Lori Lester for performing the Benchmark Dose modeling included in this document.

Citations

Bangma, J., Eaves, L. A., Oldenburg, K., Reiner, J. L., Manuck, T., Fry, R. C. (2020). Identifying Risk Factors for Levels of Per- and Polyfluoroalkyl Substances (PFAS) in the Placenta in a High-Risk Pregnancy Cohort in North Carolina. Environ. Sci. Technol. 54: 8158–8166.

Benninghoff, A.D., Bisson, W.H., Koch, D.C., Ehresman, D.J., Kolluri, S.K., Williams, D.E. (2011). Estrogenlike activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors *in vitro*. Toxicol. Sci. 120: 42-58.

Benninghoff A.D., Orner, G.A., Buchner, C.H., Hendricks, J.D., Duffy, A.M., Williams, D.E. (2012). Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicol Sci. 125: 69-78.

Bil, W., Zeilmaker, M., Fragki, S., Lijzen, J., Verbruggen, E., Bokkers, B. (2021). Risk Assessment of Per- and Polyfluoroalkyl Substance Mixtures: A Relative Potency Factor Approach. Environ. Toxicol. Chem. 40: 859–870.

Bodin, J., Groeng, E. C., Andreassen, M., Dirven, H., Nygaard, U. C. (2016). Exposure to perfluoroundecanoic acid (PFUnDA) accelerates insulitis development in a mouse model of type 1 diabetes. Toxicol. Rep. 3: 664–672.

CDC (2022a). Centers for Disease Control and Prevention. Biomonitoring Data Tables for Environmental Chemicals. March 24, 2022. https://www.cdc.gov/exposurereport/data_tables.html

CDC (2022b). Centers for Disease Control and Prevention. NHANES 2019-2020. Accessed May 28, 2022. https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2019

DWQI (2015). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorononanoic Acid (PFNA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee. June 22, 2015. http://www.nj.gov/dep/watersupply/pdf/pfna-health-effects.pdf

DWQI (2017). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee. February 15, 2017. https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf

DWQI (2018). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorooctane Sulfonate (PFOS). New Jersey Drinking Water Quality Institute Health Effects Subcommittee. June 5, 2018. https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf

Eick, S. M., Goin, D. E., Trowbridge, J., Cushing, L., Smith, S. C., Park, J. S., DeMicco, E., Padula, A. M., Woodruff, T. J., Morello-Frosch, R. (2021). Dietary predictors of prenatal per- and poly-fluoroalkyl substances exposure. J. Expo. Sci. Environ. Epidemiol. 10.1038/s41370-021-00386-6. Advance online publication. https://doi.org/10.1038/s41370-021-00386-6

Fujii, Y., Yan, J., Harada, K. H., Hitomi, T., Yang, H., Wang, P., Koizumi, A. (2012). Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere 86: 315-321.

Fujii, Y., Niisoe, T., Harada, K. H., Uemoto, S., Ogura, Y., Takenaka, K., Koizumi, A. (2015). Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. J. Occup. Health, 57: 1–12.

Goecke-Flora, C. M., Reo, N. V. (1996). Influence of carbon chain length on the hepatic effects of perfluorinated fatty acids. A 19F- and 31P-NMR investigation. Chem. Res. Toxicol. 9: 689–695.

Goodrow, S. M., Ruppel, B., Lippincott, R. L., Post, G. B., Procopio, N. A. (2020). Investigation of levels of perfluoroalkyl substances in surface water, sediment and fish tissue in New Jersey, USA. Sci. Total. Environ. 729: 138839.

Guruge, K. S., Noguchi, M., Yoshioka, K., Yamazaki, E., Taniyasu, S., Yoshioka, M., Yamanaka, N., Ikezawa, M., Tanimura, N., Sato, M., Yamashita, N., Kawaguchi, H. (2016). Microminipigs as a new experimental animal model for toxicological studies: comparative pharmacokinetics of perfluoroalkyl acids. J. Appl. Toxicol. 36: 68–75.

Han, X., Nabb, D. L., Russell, M. H., Kennedy, G. L., Rickard, R. W. (2012). Renal elimination of perfluorocarboxylates (PFCAs). Chem. Res. Toxicol. 25: 35−46 (cited in Zhang et al., 2013).

Haug, L. S., Thomsen, C., Brantsaeter, A. L., Kvalem, H. E., Haugen, M., Becher, G., Alexander, J., Meltzer, H. M., Knutsen, H. K. (2010). Diet and particularly seafood are major sources of perfluorinated compounds in humans. Environ. Int. 36: 772–778.

HIDOH (2021). Interim Soil and Water Environmental Action Levels (EALs) for Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs). Memorandum from R. Brewer to interested parties. April 18, 2021 (revised). https://health.hawaii.gov/heer/files/2021/11/PFASActionLevelsWAttachmentHIDOHApril-2021.pdf

ITRC (2022). Interstate Technology and Regulatory Council PFAS Water and Soil Values Table. Updated February 2022. https://pfas-1.itrcweb.org/wp-content/uploads/2022/03/ITRCPFASWaterandSoilValuesTables_FEB2022-FINAL.xlsx

Kärrman, A., Domingo, J. L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindström, G. (2010). Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. Environ. Sci. Pollut. Res. Int. 17: 750–758.

Klenow, S., Heinemeyer, G., Brambilla, G., Dellatte, E., Herzke, D., de Voogt, P. (2013). Dietary exposure to selected perfluoroalkyl acids (PFAAs) in four European regions. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 30: 2141–2151.

Kung, Y. P., Lin, C. C., Chen, M. H., Tsai, M. S., Hsieh, W. S., Chen, P. C. (2021). Intrauterine exposure to per- and polyfluoroalkyl substances may harm children's lung function development. Environ. Res. 192: 110178.

Lee, J. K., & Kim, S. H. (2018). Correlation between mast cell-mediated allergic inflammation and length of perfluorinated compounds. J. Toxicol. Env. Health. Part A, 81: 302–313.

Longsworth, S.G. (2022). Processes & Considerations for Setting State PFAS Standards. Updated March 2022. https://www.ecos.org/wp-content/uploads/2022/03/Standards-White-Paper_Updated_V3_2022_Final.pdf

Macheka, L. R., Abafe, O. A., Mugivhisa, L. L., Olowoyo, J. O. (2022). Occurrence and infant exposure assessment of per and polyfluoroalkyl substances in breast milk from South Africa. Chemosphere 288: 132601.

Mamsen, L. S., Jönsson, B., Lindh, C. H., Olesen, R. H., Larsen, A., Ernst, E., Kelsey, T. W., Andersen, C. Y. (2017). Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Sci. Total Environ. 596-597: 97–105.

Mamsen, L. S., Björvang, R. D., Mucs, D., Vinnars, M. T., Papadogiannakis, N., Lindh, C. H., Andersen, C. Y., Damdimopoulou, P. (2019). Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environ. Int. 124: 482–492.

McCord, J. P., Strynar, M. J., Washington, J. W., Bergman, E. L., Goodrow, S. M. (2020). Emerging chlorinated polyfluorinated polyether compounds impacting the waters of southwestern New Jersey identified by use of nontargeted analysis. Environ. Sci. Technol. Letters 7(12): 903–908.

Mertens, J.J., Sved, D.W., Marit, G.B., Myers, N.R., Stetson, P.L., Murphy, S.R., Schmit, B.,Shinohara, M., Farr, C.H. (2010). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. Int. J. Toxicol. 29, 358−371.

Motas Guzmàn, M., Clementini, C., Pérez-Cárceles, M. D., Jiménez Rejón, S., Cascone, A., Martellini, T., Guerranti, C., Cincinelli, A. (2016). Perfluorinated carboxylic acids in human breast milk from Spain and estimation of infant's daily intake. Sci. Total. Environ. 544: 595–600.

MDH (2022). Minnesota Department of Health. Toxicological Summary for: Perfluorobutane sulfonate. March 2022. https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfbssummary.pdf

NJDEP (2018). New Jersey Department of Environmental Protection. Recommended Fish Consumption Triggers for PFOS, PFNA, and PFOA. Memorandum from Gloria Post, Chair of Toxics in Biota Committee Risk Subcommittee, to Gary Buchanan, Chair of Toxics in Biota Committee. June 12, 2018.

NJDEP (2021). New Jersey Department of Environmental Protection. Technical Support Document: Interim Specific Ground Water Quality Criterion for Chloroperfluoropolyether Carboxylates (CAS Numbers 220182-27-4, 220207-15-8, 330809-92-2, and 329238-24-6). Public Version. October 20, 2021. https://www.nj.gov/dep/dsr/supportdocs/ClPFPECAs-tsd.pdf

Nyberg, E., Awad, R., Bignert, A., Ek, C., Sallsten, G., Benskin, J. P., (2018). Inter-individual, inter-city, and temporal trends of per- and polyfluoroalkyl substances in human milk from Swedish mothers between 1972 and 2016. Environ. Science Process. Impacts, 20: 1136–1147.

Ohmori, K., Kudo, N., Katayama, K., Kawashima, Y. (2003). Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184: 135–140.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, Zobel LR. 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect. 115:1298-305.

Post, G. B., Gleason, J. A., Cooper, K. R. (2017). Key scientific issues in developing drinking water guidelines for perfluoroalkyl acids: Contaminants of emerging concern. PLoS Biology 15: e2002855.

RIVM (2018). Netherlands National Institute for Public Health and the Environment. Mixture exposure to PFAS: A Relative Potency Factor approach. https://www.rivm.nl/bibliotheek/rapporten/2018-0070.pdf

Shi, Z., Zhang, H., Liu, Y., Xu, M., Dai, J. (2007). Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. Toxicol. Sci. 98: 206–215.

So, M.K., Yamashita, N., Taniyasu, S., Jiang, Q., Giesy, J.P., Chen, K., Lam, P.K. (2006). Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China. Environ. Sci. Technol. 40: 2924-2929.

Takahashi, M., Ishida, S., Hirata-Koizumi, M., Ono, A., Hirose, A. (2014). Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. J. Toxicol. Sci. 39: 97–108.

TCEQ (2016). Texas Council for Environmental Quality. Toxicity evaluations tables. January 14, 2016. https://www.tceq.texas.gov/assets/public/implementation/tox/evaluations/pfcs.pdf

Thépaut, E., Dirven, H., Haug, L. S., Lindeman, B., Poothong, S., Andreassen, M., Hjertholm, H., Husøy, T. (2021). Per- and polyfluoroalkyl substances in serum and associations with food consumption and use of personal care products in the Norwegian biomonitoring study from the EU project EuroMix. Environ. Res.195: 110795.

Thompson, J., Lorber, M., Toms, L. M. L., Kato, K.; Calafat, A. M., Mueller, J. F. (2010). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. Environ. Int. 36: 390–397.

Tilton, S.C., Orner, G.A., Benninghoff, A.D., Carpenter, H.M., Hendricks, J.D., Pereira, C.B., Williams, D.E. (2008). Genomic profiling reveals an alternate mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout. Environ. Health Perspect. 116: 1047-1055.

USEPA (2002). United States Environmental Protection Agency. A review of the Reference Dose and Reference Concentration processes. December 2002. https://www.epa.gov/risk/review-reference-dose-and-reference-concentration-processes-document

USEPA (2009). United States Environmental Protection Agency. Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). Junuary 8, 2009. https://www.epa.gov/sites/default/files/2015-09/documents/pfoa-pfos-provisional.pdf

USEPA (2016a). United States Environmental Protection Agency. Health Effects Support Document for Perfluorooctanoic Acid (PFOA). Office of Water. May 2016. https://www.epa.gov/sites/default/files/2016-05/documents/pfoa_hesd_final_508.pdf

USEPA (2016b). United States Environmental Protection Agency. Preamble to IRIS Toxicological Review of Ammonia, Noncancer Inhalation. EPA/635/R-16/163Fa. September 2016.

USEPA (2016c). United States Environmental Protection Agency. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). May 2016. https://www.epa.gov/sites/default/files/2016-05/documents/pfos_hesd_final_508.pdf

USEPA (2000). United States Environmental Protection Agency. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2: Risk Assessment and Fish Consumption Limits, Third Edition. Office of Water. EPA 823-B-00-008. November 2000.

USEPA (2021a). United States Environmental Protection Agency. Toxicological Review of Perfluorobutanoic Acid [CASRN 375-22-4] and Related Salts. External Review Draft. October 2021. https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=543579

USEPA (2021b). United States Environmental Protection Agency. Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3), Also Known as "GenX Chemicals". October 2021. https://www.epa.gov/system/files/documents/2021-10/genx-chemicals-toxicity-assessment_tech-edited_oct-21-508.pdf

USEPA (2021c). United States Environmental Protection Agency. Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water. External Peer Review Draft. November 2021. https://sab.epa.gov/ords/sab/apex_util.get_blob?s=10000792625908&a=100&c=1182706392071 4714&p=18&k1=2469&k2=&ck=gagJitcC33T9c2MP6gAF9fnVJHnrmF286KQ7TKCLT2zmsF CbFf9lxpviGQFwNNJ2MWRVW3hEXvfOhU5Zt3S_zg&rt=IR

USEPA (2021d). United States Environmental Protection Agency. Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water. External Peer Review Draft. November 2021. https://sab.epa.gov/ords/sab/apex_util.get_blob?s=10000792625908&a=100&c=1182706392071 4714&p=18&k1=2467&k2=&ck=Q9ZjTqo1ogC-jYj52n8EwxTnmhCeCbDCPBMV-tiXr1yzEFCAIeL7j2ltocvRFa-hfxk9E46Wda3CVEIX5JkusA&rt=IR

USEPA (2021e). United States Environmental Protection Agency. Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3). https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=542393

USEPA (2022). United States Environmental Protection Agency. Toxicological Review of Perfluorohexanoic Acid [CASRN 307244] and Related Salts. External Review Draft. February 2022. https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=544211

Washington, J. W., Rosal, C. G., McCord, J. P., Strynar, M. J., Lindstrom, A. B., Bergman, E. L., Goodrow, S. M., Tadesse, H. K., Pilant, A. N., Washington, B. J., Davis, M. J., Stuart, B. G., Jenkins, T. M. (2020). Nontargeted mass-spectral detection of chloroperfluoropolyether carboxylates in New Jersey soils. Science 368: 1103–1107.

WIDHS (2020). Wisconsin Department of Health Services. Summary and Scientific Support Documents for Cycle 11 Recommended Groundwater Standards. November 2020. https://www.dhs.wisconsin.gov/publications/p02807.pdf

Wolf, C.J., Schmid, J.E., Lau, C., Abbott, B.D. (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): Further investigation of C4-C12 compounds. Reprod Toxicol. 33: 546-551.

Yan, H., Li, C., Zou, C., Xin, X., Li, X., Li, H., Li, Y., Li, Z., Wang, Y., Chen, H., Ge, R. S. (2021). Perfluoroundecanoic acid inhibits Leydig cell development in pubertal male rats via inducing oxidative stress and autophagy. Toxicol. Appl. Pharmacol. 415: 115440.

Yu, C. H., Riker, C. D., Lu, S. E., & Fan, Z. T. (2020). Biomonitoring of emerging contaminants, perfluoroalkyl and polyfluoroalkyl substances (PFAS), in New Jersey adults in 2016-2018. Int. J. Hyg. Environ. Health 223: 34–44.

Yu, C. H., Weisel, C. P., Alimokhtari, S., Georgopoulos, P. G., Fan, Z. T. (2021). Biomonitoring: A tool to assess PFNA body burdens and evaluate the effectiveness of drinking water intervention for communities in New Jersey. Int. J. Hyg. Environ. Health 235: 113757.

Xin, X., Chen, H., Tian, F., Li, J., Yan, H., Yu, Y., Ma, F., Li, H., Wang, Y., Li, X., Zhu, Y., Ge, R. S. (2022). Effects of perfluoroundecanoic acid on the function of Leydig cells in adult male rats. Toxicol. Appl. Pharmacol. 439: 115903.

Zhang, Y., Beesoon, S., Zhu, L., Martin, J.W. (2013). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ. Sci. Technol. 47, 10619-27.

APPENDIX 1: Epidemiology studies reporting associations of health endpoints with PFUnDA

Bloom, M. S., Commodore, S., Ferguson, P. L., Neelon, B., Pearce, J. L., Baumer, A., Newman, R. B., Grobman, W., Tita, A., Roberts, J., Skupski, D., Palomares, K., Nageotte, M., Kannan, K., Zhang, C., Wapner, R., Vena, J. E., Hunt, K. J. (2022). Association between gestational PFAS exposure and Children's adiposity in a diverse population. Environ. Res. 203: 111820.

Callan, A. C., Rotander, A., Thompson, K., Heyworth, J., Mueller, J. F., Odland, J. Ø., Hinwood, A. L. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Sci. Total Environ. 569-570: 1107–1113.

Han, X., Meng, L., Zhang, G., Li, Y., Shi, Y., Zhang, Q., Jiang, G. (2021). Exposure to novel and legacy per- and polyfluoroalkyl substances (PFASs) and associations with type 2 diabetes: A case-control study in East China. Environ. Int. 156: 106637.

Impinen, A., Nygaard, U. C., Lødrup Carlsen, K. C., Mowinckel, P., Carlsen, K. H., Haug, L. S., Granum, B. (2018). Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ. Res. 160, 518–523.

Impinen, A., Longnecker, M. P., Nygaard, U. C., London, S. J., Ferguson, K. K., Haug, L. S., Granum, B. (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. Environ. Int. 124, 462–472.

Kang, H., Lee, H. K., Moon, H. B., Kim, S., Lee, J., Ha, M., Hong, S., Kim, S., Choi, K. (2018). Perfluoroalkyl acids in serum of Korean children: Occurrences, related sources, and associated health outcomes. Sci. Total Environ. 645, 958–965.

Kim, D. H., Kim, U. J., Kim, H. Y., Choi, S. D., Oh, J. E. (2016). Perfluoroalkyl substances in serum from South Korean infants with congenital hypothyroidism and healthy infants--Its relationship with thyroid hormones. Environ. Res. 147: 399–404.

Lind, P. M., Salihovic, S., van Bavel, B., Lind, L. (2017). Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ. Res. 15:, 157–164.

Luo, K., Liu, X., Nian, M., Wang, Y., Qiu, J., Yu, H., Chen, X., Zhang, J., for the Shanghai Birth Cohort (2021). Environmental exposure to per- and polyfluoroalkyl substances mixture and male reproductive hormones. Environ. Int. 152: 106496.

Ouidir, M., Mendola, P., Buck Louis, G. M., Kannan, K., Zhang, C., Tekola-Ayele, F. (2020). Concentrations of persistent organic pollutants in maternal plasma and epigenome-wide placental DNA methylation. Clin. Epigenetics, 12: 103.

Reardon AJF, Khodayari Moez E, Dinu I, Goruk S, Field CJ, Kinniburgh DW, MacDonald AM, Martin JW, The APrON Study (2019). Longitudinal analysis reveals early-pregnancy associations between perfluoroalkyl sulfonates and thyroid hormone status in a Canadian prospective birth cohort. Environ. Int. 29: 389-399.

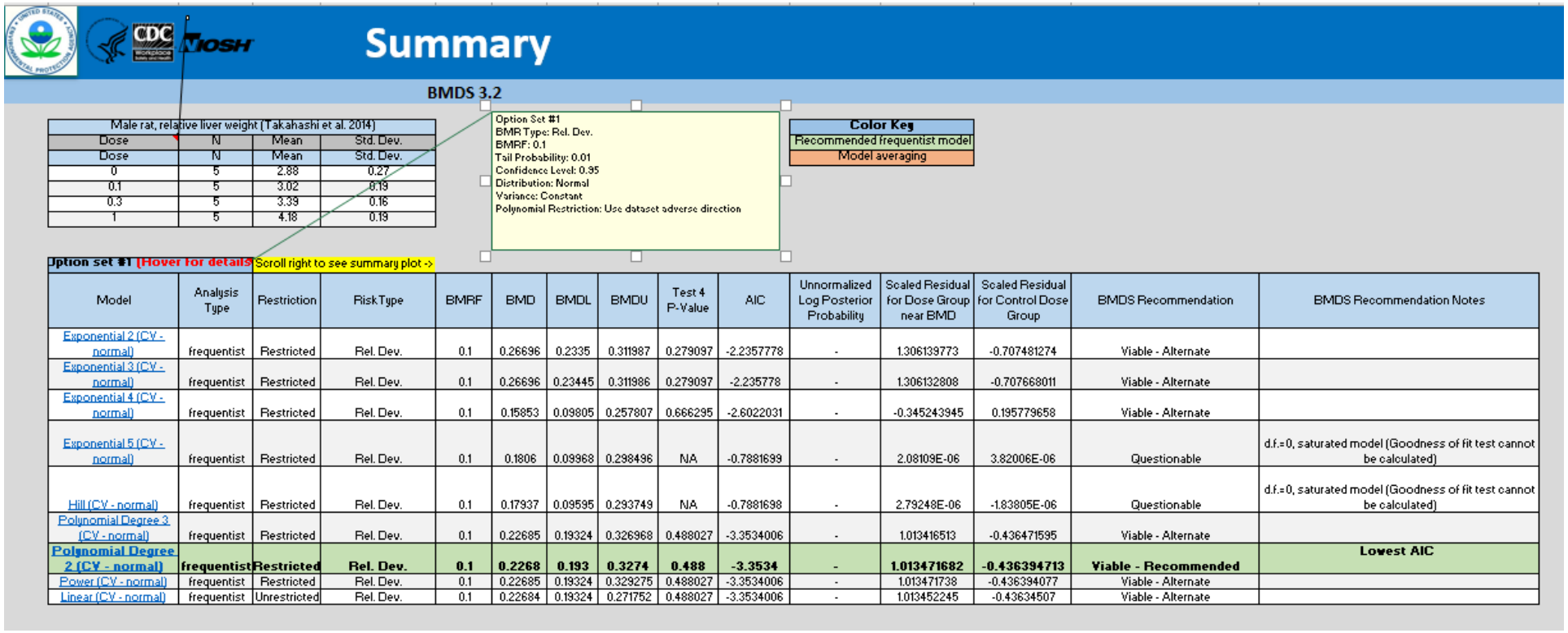
Schillemans T, Shi L, Donat-Vargas C, Hanhineva K, Tornevi A, Johansson I, Koponen J, Kiviranta H, Rolandsson O, Bergdahl IA, Landberg R, Åkesson A, Brunius C. (2021). Plasma metabolites associated with exposure to perfluoroalkyl substances and risk of type 2 diabetes - A nested case-control study. Environ. Int. 2021146:106180.

Tian, Y., Miao, M., Ji, H., Zhang, X., Chen, A., Wang, Z., Yuan, W., Liang, H. (2021). Prenatal exposure to perfluoroalkyl substances and cord plasma lipid concentrations. Environ. Pollution 268(Pt A): 115426.

Wang, Y., Rogan, W. J., Chen, P. C., Lien, G. W., Chen, H. Y., Tseng, Y. C., Longnecker, M. P., Wang, S. L. (2014). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ. Health Perspect. 122: 529–534.

Wang, Y., Adgent, M., Su, P. H., Chen, H. Y., Chen, P. C., Hsiung, C. A., Wang, S. L. (2016). Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan Maternal and Infant Cohort study. Environ. Health Perspect. 124: 1794–1800.

APPENDIX 2: Benchmark Dose (BMD) modeling results



Summary

BMDS 3.2

Male rat, relative liver weight (Takahashi et al. 2014)			
Dose	N	Mean	Std. Dev.
Dose	N	Mean	Std. Dev.
0	5	2.88	0.27
0.1	5	3.02	0.19
0.3	5	3.39	0.16
1	5	4.18	0.19

Option Set #1
BMR Type: Rel. Dev.
BMRF: 0.1
Tail Probability: 0.01
Confidence Level: 0.95
Distribution: Normal
Variance: Constant
Polynomial Restriction: Use dataset adverse direction

Color Key
Recommended frequentist model
Model averaging

Option set #1 (Hover for details) Scroll right to see summary plot ->

Model	Analysis Type	Restriction	Risk Type	BMRF	BMD	BMDL	BMDU	Test 4 P-Value	AIC	Unnormalized Log Posterior Probability	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Exponential 2 (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.26696	0.2335	0.311987	0.279097	-2.2357778	-	1.306139773	-0.707481274	Viable - Alternate	
Exponential 3 (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.26696	0.23445	0.311986	0.279097	-2.235778	-	1.306132808	-0.707668011	Viable - Alternate	
Exponential 4 (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.15853	0.09805	0.257807	0.666295	-2.6022031	-	-0.345243945	0.195779658	Viable - Alternate	
Exponential 5 (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.1806	0.09968	0.298496	NA	-0.7881699	-	2.08109E-06	3.82006E-06	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.17937	0.09595	0.293749	NA	-0.7881698	-	2.79248E-06	-1.83805E-06	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3 (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.22685	0.19324	0.326968	0.488027	-3.3534006	-	1.013416513	-0.436471595	Viable - Alternate	
Polynomial Degree 2 (CV - normal)	**frequentist**	**Restricted**	**Rel. Dev.**	**0.1**	**0.2268**	**0.193**	**0.3274**	**0.488**	**-3.3534**	**-**	**1.013471682**	**-0.436394713**	**Viable - Recommended**	**Lowest AIC**
Power (CV - normal)	frequentist	Restricted	Rel. Dev.	0.1	0.22685	0.19324	0.329275	0.488027	-3.3534006	-	1.013471738	-0.436394077	Viable - Alternate	
Linear (CV - normal)	frequentist	Unrestricted	Rel. Dev.	0.1	0.22684	0.19324	0.271752	0.488027	-3.3534006	-	1.013452245	-0.43634507	Viable - Alternate	