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Literature review and assessment of available toxicological data for PFAS

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Abstract: Literature review and assessment of available toxicological data for PFAS

Per- and polyfluoroalkyl substances (PFAS) are a large group of substances that have been widely used in articles since many years due to their unique properties. PFAS are not easily degradable and can remain in the environment for decades. They have been found in the environment and humans across Europe. Exposure to PFAS through drinking water is of significant concern due to potential adverse health effects. In 2020 the "Directive (EU) 2020/2184 of the European Parliament and of the Council on the quality of water intended for human consumption" was adopted. Among other things, the new parameter "sum of PFAS" was defined as the sum of 20 perfluorinated carboxylic and sulfonic acids with a chain length of 4 to 13 carbon atoms with a parameter value of 0.1 µg/l. In 2020, the European Food Safety Authority (EFSA) defined a tolerable weekly intake (TWI) of 4.4 ng/kg body weight for four individual PFAS. This value would lead to a drinking water concentration well below the above parameter value of the Drinking Water Directive and the values previously valid in Germany. The aim of this project was to evaluate the remaining 16 PFAS by carrying out literature searches on toxicological and epidemiological data that can be used as basis for the derivation of drinking water limit values. For the four PFAS assessed by EFSA a literature screening on potentially new toxicological and epidemiological data that might contradict with the EFSA assessment was carried out. Also, a possible grouping and the evaluation of PFAS according to the concept of relative potency factors was assessed as well as toxicological and epidemiological data for four alternative PFAS that are not subject to the parameter sum of PFAS of Directive (EU) 2020/2184.

Kurzbeschreibung: Literaturrecherche und Auswertung vorhandener toxikologischer Daten als Grundlage zur Ableitung von Trinkwasserleitwerten für PFAS

Per- und Polyfluoralkylsubstanzen (PFAS) sind eine große Gruppe von Substanzen, die aufgrund ihrer einzigartigen Eigenschaften seit vielen Jahren weit verbreitet in Produkten eingesetzt werden. PFAS sind nicht leicht abbaubar und können jahrzehntelang in der Umwelt verbleiben. Sie wurden in der Umwelt und bei Menschen in ganz Europa gefunden. Die Exposition von PFAS durch das Trinkwasser ist aufgrund möglicher gesundheitsschädlicher Auswirkungen von besonderer Bedeutung. Im Jahre 2020 wurde die „Richtlinie (EU) 2020/2184 des Europäischen Parlaments und des Rates über die Qualität von Wasser für den menschlichen Gebrauch“ verabschiedet. Unter anderem wurde der neue Parameter „Summe PFAS“ als Summe von 20 perfluorierten Carbon- und Sulfonsäuren mit einer Kettenlänge von 4 bis 13 Kohlenstoffatomen mit einem Parameterwert von 0,1 µg/l definiert. Bereits 2020 hat die Europäische Behörde für Lebensmittelsicherheit (EFSA) für vier einzelne PFAS-Stoffe eine tolerierbare wöchentliche Aufnahme (TWI) von 4,4 ng/kg Körpergewicht abgeleitet. Dieser Wert würde zu einer Trinkwasserkonzentration führen, die deutlich unter dem oben genannten Parameterwert der Trinkwasserrichtlinie und den bisher in Deutschland gültigen Werten liegt. Ziel dieses Projektes war es, die verbleibenden 16 PFAS durch Literaturrecherchen zu toxikologischen und epidemiologischen Daten zu bewerten, indem Grundlagen für die Ableitung von Trinkwasserleitwerten identifiziert wurden. Für die vier von der EFSA bewerteten PFAS wurde ein Literaturscreening auf potenziell neue toxikologische und epidemiologische Daten durchgeführt, die der EFSA-Bewertung widersprechen könnten. Außerdem wurde eine mögliche Gruppierung und Bewertung von PFAS nach dem Konzept der relativen Potenzfaktoren untersucht, sowie toxikologische und epidemiologische Daten zu vier alternativen PFAS, die nicht unter den Parameter „Summe PFAS“ entsprechend der Richtlinie (EU) 2020/2184 fallen.

Table of content

List of figures

List of tables

List of abbreviations

2-D DIGE	Two-dimensional differential gel electrophoresis
6:2 FTSA	6:2-Fluorotelomer sulfonic acid
Ach	Acetylcholine
ADME	Absorption, distribution, metabolism and excretion
ADONA	3H-Perfluoro-3-[(3-methoxy-propoxy) propanoic acid], ammonium salt
AF	Assessment factor
AFFF	Aqueous Film-Forming Firefighting Foam
AGD	Anogenital distance
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
APFB	Ammonium perfluoro butyrate
APO	Apolipoprotein
APrON	Alberta Pregnancy Outcomes and Nutrition
AST	Aspartate transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	Benchmark dose
BMI	Body mass index
BUN	Urea nitrogen
C604	Perfluoro([5-Methoxy-1,3-dioxolan-4-yl]oxy) acetic acid
CAS	Chemical abstracts service
CDC2	Cell division cycle protein 2
CONTAM	EFSA Panel on Contaminants in the Food Chain
CRE	Creatinine
DA	Dopamine
DevTox	Developmental toxicity
DNT	Development neurotoxicity
DPF	Days post fertilization
EFSA	European Food Safety Authority
FEPOS	Foetal Programming of Semen Quality
FOB	Functional observation battery
GCT	Paediatric germ cell tumours
GenX	Ammonium-2,3,3,3-tetrafluor-2-(heptafluorpropoxy)propanoate
GOW	Gesundheitlicher Orientierungswert (health related indicator value)
HBGV	Health-Based Guidance Value
HDL	High density lipoprotein
HED	Human equivalent dose
HFPO-DA	2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid
Hib	Haemophilus influenza type b
ITRC	Interstate Technology and Regulatory Council
iv	Intravenous

KPFB	Potassium perfluoro butyrate
LDL	Low density lipoprotein
LDL-C	Low density lipid cholesterol
LOAEL	Lowest observed adverse effect level
LUBW	Landes-Anstalt für Umwelt Baden-Württemberg (State Institute for the Environment of Baden-Württemberg)
MCLG	Maximum contaminant level goal
Na-PFHx	Sodium perfluorohexanoate
NHANES	US National Health and Nutrition Examination Survey
NIS	Sodium-Iodide Symporter
NMR	Nuclear magnetic resonance spectroscopy
NOAEL	No observed adverse effect level
NTP	National toxicology program
OECD	The Organisation for Economic Co-operation and Development
PBT	Persistent, bioaccumulative and toxic
PCE/EC	Polychromatic erythrocyte/ erythrocyte
PCR	Polymerase chain reaction
PFAS	Per- and polyfluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDoDS	Perfluorododecanesulfonic acid
PFDS	Perfluorodecanesulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptanesulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFHxSK	Perfluorohexane sulfonate potassium salt
PFNA	Perfluorononanoic acid
PFNS	Perfluorononanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPeA	Perfluoropentanoic acid
PFPeS	Perfluoropentanesulfonic acid
PFTrDA	Perfluorotridecanoic acid
PFTrDS	Perfluorotridecanesulfonic acid
PFUnDA	Perfluoroundecanoic acid
PFUnDS	Perfluoroundecanesulfonic acid
PLCO	Prostate, Lung, Colorectal and Ovarian
PND	Post-natal day
POD	Point of departure
PPAR	Proliferator activated receptor

RAC	Risk Assessment Committee
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Reference dose
ROB	Risk of bias
ROS	Reactive oxygen species
RPF	Relative potency factor
SD	Standard deviation
SDH	Sorbitol dehydrogenase
SDS	Standard deviation score
SELMA	Swedish Environmental, Longitudinal, Mother and Child, Asthma and Allergy
SGA	Small for gestational age
T3	Triiodothyronine
T4	Thyroxine
TBA	Total bile acids
T-Bil	Total bilirubin
TDAR	T-Cell-Dependent Antibody Response
TG	Triglyceride
TPO	Thyreoperoxidase
TPOAb	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
TWI	Tolerable weekly intake
TWLW	Trinkwasserleitwert (Drinking water guide value)
UBA	Umweltbundesamt (German Environment Agency)
UF	Uncertainty factor
US EPA	U.S. Environmental Protection Agency
vPvB	Very persistent and very bioaccumulative
WHO	World Health Organization
WST-1	Water-soluble tetrazolium salt
WTC	World Trade Center

Summary

The aim of the present project was the toxicological assessment of a total of 16 per- and polyfluorinated alkyl substances (PFAS) to propose starting points for deriving drinking water guideline values (TWLW). Extensive literature research was carried out for all individual substances in order to identify relevant toxicological and epidemiological studies. A previous assessment by the German Environment Agency (UBA) from 2016/2017 served as the basis for seven of the 16 substances.

Based on the available literature, a new starting point (POD) for the derivation of a TWLW could be identified for six of the 16 evaluated substances and assessment factors (AF) were proposed. AFs are applied to the POD as necessary. AFs are numerical values used to account for the differences and uncertainties when extrapolating experimental (animal or human) data to the relevant human exposure situation. The table below provides an overview of the results.

Table 1: Summary of new PODs for the derivation of TWLW

Proposal for a new point of departure (POD) for deriving a TWLW and proposed assessment factors (AF) (total value is obtained by multiplying the individual factors).

PFAS	Suggested POD for deriving a TWLW	Time AF	Interspecies AF	Intraspecies AF	Total AF
Substances already considered in 2016/2017					
PFBA	No new POD, Assessment of 2016 remains				
PFPeA	No new POD, Assessment of 2016 remains				
PFHxA	No new POD, Assessment of 2016 remains				
PFHpA	A LOAL = 0.5 mg/kg/d based on necrotic effects in the liver of mice could be identified. However, its use is not recommended, but rather referred to a BMD approach.	2 (subchronic 90-day exposure to chronic).	As a basis, an approximate half-life in humans of 62 days to 1.5 years (= 548 days) compared to about 0.19 days in mice (AF = half-life human/half-life days) could be used. and 2.5 (toxicodynamics)	10 (general population)	
PFDA	NOAEL = 0.125 mg/kg/d based on immunotoxicity in rats	6 (subacute to chronic)	84 (toxicokinetic PFDA) and 2.5 (toxicodynamics)	10 (general population)	12,600
PFBS	NOAEL = 50 mg/kg bw d	1	235 (toxicokinetic PFBS) and 2.5 (toxicodynamics)	10 (general population)	5,875
PFHpS	As in 2016, no POD could be identified.				
Substances with new evaluation					

PFAS	Suggested POD for deriving a TWLW	Time AF	Interspecies AF	Intraspecies AF	Total AF
PFUnDA	NOAEL = 0.1 mg/kg/d based on toxicity in the liver of rats.	6 (subacute to chronic)	84 (toxicokinetic PFDA) and 2.5 (toxicodynamics)	10 (general population)	12,600
PFDoDA	NOAEL = 0.1 mg/kg/d based on increase in relative liver weight in rats.	6 (subacute to chronic)	84 (toxicokinetic PFDA) and 2.5 (toxicodynamics)	10 (general population)	12,600
PFTrDA	LOEL = 1 mg/kg bw based on reduced testosterone levels in male puppies.	1	84 (toxicokinetic PFDA) and 2.5 (toxicodynamics)	10 (general population)	2,100
PFPeS	No POD could be identified.				
PFNS	No POD could be identified.				
PFDS	No POD could be identified.				
PFUnDS	No POD could be identified.				
PFDoDS	No POD could be identified.				
PFTrDS	No POD could be identified.				

Four other PFAS (PFOA, PFNA, PFHxS und PFOS) were already assessed by EFSA in 2020 and a TWI (tolerable weekly intake) for this group was derived. No new toxicological evaluations were performed for these substances; instead, current studies were checked for a possible change in the EFSA assessment. No toxicological studies were found for any of the individual substances that would influence the derived TWI. However, some epidemiological studies could be identified that could potentially influence the value. To check this, further modelling is necessary.

As regards the four PFAS alternatives the following assessment results could be obtained.

Table 2: Summary of new PODs for the derivation of TWLW

Proposal for a new point of departure (POD) for deriving a TWLW and proposed assessment factors (AF) (total value is obtained by multiplying the individual factors).

PFAS	Suggested POD for deriving a TWLW	Time AF	Interspecies AF	Intraspecies AF	Total AF
HPFO-DA (GenX)	NOAEL of 0.1 mg/kg bw/day	1	4 (allometric scaling factor) and 2.5 (toxicodynamics)	10 (general population)	100

PFAS	Suggested POD for deriving a TWLW	Time AF	Interspecies AF	Intraspecies AF	Total AF
ADONA	NOAEL of 10 mg/kg bw/day	1	4 (allometric scaling factor) and 2.5 (toxicodynamics)	10 (general population)	100

For 6:2 FTSA and C604 several options exist for the suggested POD as well as appropriate AFs. Please refer to chapter 22.5 and 23.5, respectively. In the following chapters, the results are presented in detail.

1 Background and objectives

Objectives in brief

The aim of this project was to provide the toxicological database for the derivation of drinking water guidance values for 16 PFAS. Some of these PFAS were already assessed in a previous work. Depending on previous assessments the objectives differed for the individual PFAS:

- Update of the assessment for 7 PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFBS, PFHpS) which were already evaluated by UBA in 2016/2017.
- New toxicological assessments for 9 PFAS (PFUnDA, PFDoDA, PFTrDA, PFPeS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS) which were not evaluated previously.

Additional objectives in this project were:

- Evaluation of recent toxicological findings for 4 PFAS (PFOA, PFNA, PFHxS, PFOS) that might change the assessment by EFSA in 2020.
- Evaluation of a possible assessment of PFAS using relative potency factors (RPFs)
- Evaluation of toxicological and epidemiological research data for 4 alternative PFAS (HPFO-DA (Gen-X), ADONA, 6:2 FTSA, C604)

Per- and polyfluoroalkyl substances (PFAS) are a large group of substances that have been widely used in articles for many years. As a result of the strong C-F bonds, PFAS have unique properties. These properties include a high resistance to external factors like extreme temperatures, pH, oxidation (non-flammable) and abrasion. They are found wherever extreme conditions prevail and particularly high demands are placed on materials. Their use spans over many different sectors ranging from fire-fighting foams to the manufacture of everyday articles like water-repellent outdoor jackets or stain-proofing agents. On the other side, PFAS are not easily degradable and can remain in the environment for decades. In addition, the use of PFAS has raised human and environmental concerns. In Europe, some PFAS are therefore classified as persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) under the REACH Regulation.

PFAS can be found in the environment and humans across Europe, whereby areas around industrial production, manufacturing and application sites have been found to be particularly contaminated. This has led to contaminated drinking water around PFAS manufacturing factories in Belgium, Italy and the Netherlands, and around airports and military bases with fire-fighting training sites in Germany, Sweden, Denmark, Norway and the United Kingdom. Exposure to these chemicals may lead to adverse health effects. People can be exposed to PFAS in different ways, including food, where these substances are most often found in drinking water, fish, fruit, eggs, and egg products.

In 2020 the "Directive (EU) 2020/2184 of the European Parliament and the Council on the quality of water intended for human consumption" was adopted. Among other things, the new parameter "sum of PFAS" was defined as the sum of 20 perfluorinated carboxylic and sulfonic acids with a chain length of 4 to 13 carbon atoms with a parameter value of 0.1 µg/l.

Four of the 20 individual PFAS were already assessed by the European Food Safety Authority (EFSA) in 2020. EFSA defined a tolerable weekly intake (TWI) of 4.4 ng/kg body weight for the group of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA),

perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS). This value would lead to a drinking water concentration well below the parameter value of the Drinking Water Directive and the values previously valid in Germany.

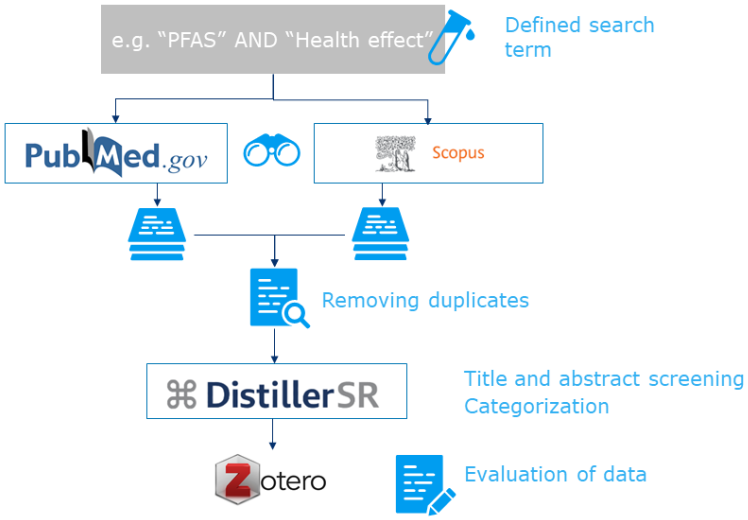
Considering the background, the German Environment Agency (UBA) initiated a project to check the toxicological database for the remaining 16 PFAS. The objective of this project was to review the current toxicological and epidemiological research data for the 16 PFAS and to process them so that they can be used as a basis for the derivation of drinking water guideline values. For 7 of the 16 substances the toxicological evaluation is based on the previous work of the UBA from 2016/2017, whereas for 9 substances a new compilation of the available data was carried out. Additionally, the project team looked for recent studies for the 4 PFAS (PFOA, PFNA, PFHxS, PFOS) assessed by EFSA that might influence the TWI and checked the possibility to apply relative potency factors (RPFs) as well as toxicological and epidemiological data for four alternative PFAS (HPFO-DA (GenX), ADONA, 6:2 FTSA, C604).

2 Project approach

2.1 Literature screening

Within this project data was collected by comprehensive literature screenings. The general methodology is shown in the following figure. Therefore, a specific term was defined depending on the objective of the research. All searches were carried out in parallel in two databases (Pubmed and Scopus) and the results were transferred to the Online Software Distiller SR for further processing. During the transfer duplicates were already removed automatically. The remaining duplicates were then removed manually. In Distiller SR articles were screened by title and abstract for relevance and categorized. The remaining relevant articles were transferred into a citation manager (Zotero) and the full texts were downloaded for a further evaluation of the data.

Figure 1: Methodology of literature screening.



Source: own illustration, Ramboll

2.1.1 Literature searches for individual PFAS

Combined literature searches were conducted for all PFAS and alternative PFAS to gather the database for the toxicological evaluation of each individual substance. Search terms were defined that consist of a substance identifier (substance name and acronyms) together with synonyms for common toxicological and health-related terms. The complete search terms are shown in Annex A.

The search period was limited accordingly for substances which were already assessed previously. For the 7 PFAS which were assessed by the UBA in 2016/2017, the search was limited to studies from 2017 onwards, whereas for the 4 PFAS substances evaluated by EFSA in 2020, the time frame of the search was limited to studies published 2020 onwards.

All search results were combined, and duplicates removed. Overall, 3560 individual scientific articles were identified. These articles were consequently screened for relevance based on their title and abstract. In this first screening 2419 articles could be excluded. Inclusion and exclusion criteria are summarized in Annex A.6. The most common exclusion reasons are shown in the following table.

Table 3: Most common exclusion criteria (>100 number of exclusions) in all combined literature searches on individual PFAS

Exclusion reason	Number of exclusions
No PFAS included	949
Environmental monitoring data	240
Human biomonitoring data (without epidemiological data)	193
Study on degradation and/or removal from the environment	198
Biomonitoring data (animals)	152
Ecotoxicity study	155
Bioaccumulation study	125

The remaining 1141 articles were categorized based on the study type (toxicological, toxicokinetic and/or epidemiological data) and the PFAS considered in the study. Then the full texts were downloaded, and the data was evaluated by an expert team of toxicologists and epidemiologists (see chapter 2.2).

2.1.2 Literature search on relative potency factors

A separate literature search was conducted on relative potency factors (RPFs) for a possible assessment of PFAS. The defined search term consists of a general term for PFAS and terms for the relative potency factor. The full search term is shown in Annex A.4. The search was conducted in PubMed, Scopus and google. The total number of records retrieved were 20 publications. After preliminary screening of the title/abstract 4 studies publishing relative potency factors addressing subsets rather than the broad class of PFAS were identified. An overview of these studies is presented in the respective subchapter.

2.2 Evaluation of toxicological and epidemiological studies for individual PFAS

Full texts of articles which were identified in the literature search for individual substances were evaluated by an expert team of toxicologists and epidemiologists for a potential point of departure (POD) that can be used for the deviation of a drinking water guideline value (TW_{LW}). The criteria which were considered for a potential POD are summarized in Annex A.6. If a new POD could be identified, a quality assessment of the respective study was conducted by a Risk of Bias (ROB) analyses (for more information see Annex A.7). The exclusion criteria for each study were tracked in a separate excel spreadsheet. All relevant findings were then summarized in specific subchapters of each individual PFAS. Even if studies were not relevant for a potential POD, toxicological or toxicokinetic relevant information could be extracted in some cases and was summarized. Secondary data (e.g., reviews, reports) was used to identify additional relevant articles. Also, non-peer reviewed literature was considered but highlighted as such.

2.2.1 Proposal of assessment factors

Assessment factors (AFs) are applied to the POD, when necessary, to obtain limit value. AFs are numerical values used to address the differences and uncertainties in the assessment of experimental (animal or human) data to the relevant human exposure situation. Under ideal

circumstances, these differences are addressed using substance-specific AFs derived from health hazard and/or toxicokinetic information on the substance. However, in many cases, the data needed to derive these substance-specific AFs are not available, so default AFs are most often used. The European Chemicals Agency has identified the following five areas of differences/uncertainties that should be addressed (ECHA, 2012).

Interspecies differences account for differences in responses observed in experimental animals vs. humans due to differences in toxicokinetics and toxicodynamics. If no substance-specific data are available, default values for differences in metabolic rate (i.e., allometric scaling based on body weight) and an additional factor of 2.5 to account for other interspecies differences is applied. The metabolic rate difference e.g., for rat to humans is 4, for mouse to human 7.

Intraspecies or intraindividual differences account for the different sensitivity to chemical exposures due to factors such as genetic differences in absorption and metabolism, age, gender, health status, and nutritional status. In order to cover sensitive subpopulations of the general population a default AF = 10 is applied.

Recommended AF for differences in duration of exposure are AF = 3 for subacute (28 days) to subchronic exposure, AF = 6 for subacute to chronic human exposure, and AF = 2 for subchronic (90 days) to chronic human exposure.

In addition, assessment factors can be also applied for deviations in the dose–response relationship (e.g. in general for NOAEL, AF = 1, deviations from the standard factor according to ECHA guidance) and for the quality of the whole data base.

3 Toxicological evaluation of perfluorobutanoic acid (PFBA)

3.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 4 and Table 5.

Table 4: Chemical identity of perfluorobutanoic acid (PFBA, CAS 375-22-4)

Name	Perfluorbutansäure
English Name	Perfluorobutanoic acid
Acronym	PFBA
Mol. Formula	C4HF7O2
Mol. Weight (g/mol)	214.0
CAS	375-22-4
EC	206-786-3

Table 5: Physicochemical properties of perfluorobutanoic acid (PFBA, CAS 375-22-4)

Properties	Value	Source
Density (g/cm^3)	1.651 (exp. at 20 °C)	ATSDR 2021 from (Lide, 2005)
Melting point (°C)	–17.5 (exp.)	ATSDR 2021 from (Lide, 2005)
Boiling point (°C)	121 (exp.)	ATSDR 2021 from (Lide, 2005)
Vapour Pressure (Pa)	1260 (exp. at 25 °C)	(Kwan, 2001)
Henry's Constant (Pa m^3/mol)	1.24 (exp. at 25 °C)	(Kwan, 2001)
	4.9 x 10^3 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	0.4 (exp. at 25 °C)	(Moroi et al., 2001)
Log Koc	1.88 (±0.11) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	2.14 x 10^5 (25 °C)	ATSDR 2021 from (Kwan, 2001)
	4.9 x 10^4 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated)

3.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews quantitative toxicological risk assessments of PFBA on human data determined by other institutions (Table 6) and drinking water limits for PFBA. Data was identified by general desk research.

Table 6: Summary of quantitative toxicological assessments of PFBA and corresponding health endpoints by other institutions

Agency	Quantitative assessment	Health endpoint	Value	Reference
ANSES	Chronic indicative toxicity values	liver effects	0.024 mg/kg bw per day	(ANSES, 2017)
US EPA	The oral reference dose (RfD)	liver and thyroid effects	1×10^{-3} mg/kg-day	(US EPA, 2021e)
Department of Health Minnesota	Short-term Non-Cancer Health Risk Limit ($nHRL_{Short\text{-}term}$) = 7 µg/l	Critical effect(s): Decreased cholesterol Co-critical effect(s): Increased relative thyroid weight, decreased serum total thyroxine (TT4), decreased dialysis free thyroxine (dFT4) Additivity endpoint(s): Hepatic (liver) system, Thyroid (E)	7 µg/l	(MDH, 2018)
Department of Health Minnesota	Subchronic Non-Cancer Health Risk Limit ($nHRL_{Subchronic}$) = $nHRL_{Short\text{-}term}$ = 7 µg/l	Critical effect(s): Liver weight changes, morphological changes in liver and thyroid gland, decreased TT4, decreased red blood cells, decreased haematocrit and haemoglobin Co-critical effect(s): Increased relative thyroid weight, decreased serum TT4 and dFT4, decreased cholesterol, delayed eye opening Additivity endpoint(s): Developmental, Haematological (blood) system, Hepatic (liver) system, Thyroid (E)	7 µg/l	(MDH, 2018)
Department of Health Minnesota	Chronic Non-Cancer Health Risk Limit ($nHRL_{Chronic}$) = $nHRL_{Short\text{-}term}$	Critical effect(s): Liver weight changes, morphological changes in liver and thyroid gland, decreased TT4, decreased red blood cells, decreased haematocrit and haemoglobin Co-critical effect(s): Increased relative thyroid weight, decreased serum TT4 and dFT4, decreased cholesterol, delayed eye opening Additivity endpoint(s): Developmental, Haematological (blood) system, Hepatic (liver) system, Thyroid (E)	7 µg/l	(MDH, 2018)

Table 7: Drinking water limits determined for PFBA or sum of PFAS

Country/ Institution	PFBA limit value in drinking water (µg/l)	Comment	Source
Germany/ UBA	10	Drinking water guidance value	(UBA, 2017)
Hawaii (USA)	7.6	Drinking water guideline level	(ECOS, 2022)
Wisconsin (USA)	10	Drinking water guideline level	(ECOS, 2022)
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFBA, drinking water guide value	(SFA, 2022)

The German UBA drinking water guide values (TW_{LW}, Trinkwasser-Leitwert) was calculated by using a POD of 6 mg/kg day and applying the following assessment factors: a factor of 10 for time extrapolation, and a factor of 8 for interspecies extrapolation for toxicokinetic differences, a factor of 2.5 for toxicodynamic differences and a factor of 10 for intraspecies differences. After reconsideration of a factor of 2.5 for toxicodynamic differences and higher quota of the drinking water portion of the tolerable dose, a tolerable dose between 3 µg/kg·d (with the assumption of toxicodynamic differences, EF_{total} 2,000) or a drinking water concentration of about 10 µg/l (drinking water quota 10%) and 7.5 µg/kg·d (without the assumption of toxicodynamic differences, EF_{total} 800) or about 52 µg/l (Drinking water quota 20%) are derived. With a 10% allocation of the lowest tolerable dose to drinking water consumption and 2 litres consumption per day and 70 kg body weight, a TW_{LW} of 10 µg/l is proposed as a precautionary idea of the Drinking Water Ordinance. It needs to be reconsidered that a widely accepted factor of 2 should be used for time extrapolation from subchronic to chronic exposure (e.g., UBA, ECHA). The current approach by the drinking water commission using a factor of 10 is quite conservative and perhaps needs to be discussed.

3.3 Toxicokinetics

3.3.1 Animal data

3.3.1.1 Data/studies reported in previous UBA evaluation

The absorption of PFBA was >95% in rats after an oral dose of 30 mg/kg b.w. The PFBA was excreted within 96 hours in ammonium perfluorobutyrate (APFB) treated mice mainly via urine (35% in males and 65-69% in females) and faeces (4-11% in males and 5-7% in females). The elimination after a gavage dose of 30 mg/kg b.w. APFB was faster in females (rats: 1.76 ± 0.26 h; mice: 3.08 ± 0.26 h) as compared to their male counterparts (rats: 9.22 ± 0.75 h; mice: 16.25 ± 7.19 h). Longer elimination time was observed with oral gavage as compared to intravenous administration. The plasma elimination in 10 mg/kg b.w. intravenous potassium perfluorobutyrate (KPFB) treated cynomolgus monkeys is approximately 40.32 (males) – 41.04 (females) hours (UBA, 2017).

3.3.1.2 New data

No relevant data was found.

3.3.2 Human data

3.3.2.1 Data/studies reported in previous UBA evaluation

The arithmetic mean elimination half-life (t½) for three male fluorine chemistry workers was 68 h and elimination half-lives for humans was found to be 72.16 h for seven males and 87 h for two females (Chang et al., 2008). This data was calculated by (ATSDR, 2009) indicating a mean t½ of 72 ± 38 h (from 56 and 118 h for two women) and a t½ of 75 ± 38 h on average for 12 subjects.

The PFBA serum concentrations were 73.2% of the 127 former and 68.0% of the 50 current production employees below the limit of quantification (0.5 ng/ml). Only 4% of the serum samples were above 2 ng/ml, a maximum of 6.2 ng/ml in the former and 2.2 ng/ml in the current employees (Chang et al., 2008).

3.3.2.2 New data

No human studies were available to inform the potential for PFBA exposure to affect sensitive subpopulations or life stage. In adult animals exposed subchronically, PFBA exposure was consistently observed to elicit stronger responses in male rats compared with female rats. The reason for this sex dependence is most likely due to differences in toxicokinetics between males and females. It does appear to be a clear sex dependence for some PFBA-induced health effects in adult rodents, the observed lack of sex-specific sensitivity for other effects in adult and immature rodents and the apparent lack of toxicokinetic differences between sexes in primates (and a single human occupational study) preclude the identification of males as a broadly sensitive subpopulation for PFBA-induced health effects in humans. Lastly, given the effects observed in pregnant mice (increased liver weights, full-litter resorptions) and the developing organism (foetal/postnatal death and delays in time to eye opening, vaginal opening, and preputial separation), that pregnancy and early life represent two sensitive life stages to PFBA exposure is possible (ATSDR, 2021).

Table 8: Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals (ATSDR, 2021)

Species, age, and sex	Route	Dose	Exposure duration	Elimination half-life	Reference
Human (n=3), adult, M	NA	NA	NA	81 hours (SD 41)	(Chang et al., 2008)
Human (n=9), adult, M (7), F (2)	NA	NA	NA	72 hours (SD 38)	(Chang et al., 2008)

3.4 Health effects in humans and/or animals

3.4.1 Relevant Animal data

3.4.1.1 Data/studies reported in previous UBA evaluation

3.4.1.1.1 Repeated dose toxicity

The critical study in the derivation of the drinking water limit for PFBA was a subchronic 90-day oral gavage study (J. L. Butenhoff, Bjork, et al., 2012). In this study Sprague Dawley rats

(10/sex/dose) were given 0, 1.2, 6, or 30 mg/kg bw/day of Ammonium perfluorobutyrate (APFB) as formulation MTDID-8391.

The formulation consisted of an aqueous solution of APFB with a nominal content of 33.2% which was converted to the PFBA content. In male rats, a dose-dependent increase in liver weight and hepatocellular hypertrophy (five animals with minimal and four animals with mild hypertrophy) were observed at 30 mg/kg bw/day in the 90-day study. An increase in absolute liver weight was also observed in a parallel 28-day study at 150 mg/kg bw/day. These effects reversed during the recovery phase of three weeks and were shown to be consistent with hepatic activation of peroxisome proliferator receptor. Dose-related thyroid follicular hyperplasia and hypertrophy (slightly in five out of ten animals) was also observed in males at 30 mg/kg bw/day. These effects did not fully reverse during the follow-up period of three weeks. These effects were less pronounced but also observed in a parallel 28-day study (only minimally in nine of ten animals) in the 30 mg/kg·d group, and (minimally/slightly in seven out of ten animals) in the 150 mg/kg·d group. A NOAEL of 6 mg/kg bw/day was identified in the 90-day study which was also shown as the NOAEL for the males in this parallel 28-day subacute study.

3.4.1.1.2 Carcinogenicity

No relevant data was found.

3.4.1.1.3 Mutagenicity

No relevant data was found.

3.4.1.1.4 Toxicity to reproduction

Treatment of APFB in CD1 mice at gavage doses of 35, 175 or 350 mg/kg·d during days 1 to 18 of gestation resulted in an increased number of dams with full foetal resorption (11.1% at 175 mg/kg·d and 29.6% at 350 mg/kg·d, compared to 6.8% in the controls) and an increased liver weight after day one which persisted only ten days after birth, slightly delayed eye opening (about 1.5 days, in all dose groups) and delayed onset of puberty in the high dose group (Das et al., 2008).

3.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

PFBA did not lead to the formation of 17β-oestradiol or testosterone in the steroidogenesis assay and also did not react in reporter gene tests with human oestrogen, androgen or Ah-Receptors (Rosenmai, 2014).

3.4.1.2 New data

3.4.1.2.1 Repeated dose toxicity

No relevant data was found.

3.4.1.2.2 Carcinogenicity

No relevant data was found.

3.4.1.2.3 Mutagenicity

Male mice (C57B1/6) were administered PFBA 5 mg/kg body weight for five weeks through drinking water (Crebelli et al., 2019). Markers of cell toxicity, oxidative stress and DNA strand breaks were measured in liver. Systemic genotoxicity was also assessed by the analysis of micronuclei in reticulocytes and spleen lymphocytes, and germ cell effects by the Comet assay on testis cells. Only mild liver hypertrophy, with no other signs of toxicity, genotoxicity and lower tendency to bioaccumulation were observed in PFBA treated mice. In this subacute study,

mice are exposed to only one dose group and there is no information on the histopathological changes in the liver of PFBA treated mice (for example number of animals affected or description of changes that were observed). In addition, no changes in liver weight, liver enzymes or hyperploid cells were reported in this study. Therefore, in the absence of any data for this endpoint, it is not used as a POD.

3.4.1.2.4 Toxicity to reproduction

No relevant data was found.

3.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

PFBA did not enhance oestrogen receptor alpha or beta activity, but enhanced dihydrotestosterone stimulated androgen receptor activity at concentrations above 10 µM in human cell lines (Behr et al., 2018). Blood serum concentrations of various PFAS (i.a., PFOA, PFOS, PFHxA, PFBA und PFBS) were reported to be in the general Western population in the range of 10 nM or below. The authors therefore concluded that endocrine effects in humans at exposure-relevant concentrations will not be observed.

PFBA at 0.0001 to 100 µM concentration did not induce acute cytotoxic effects or TSH-induced cAMP production in thyroid cells (Croce et al., 2019). PFBA was assessed for cytotoxicity, potential developmental, adipogenic and osteogenic toxicity in human mesenchymal stem cells (S. Liu et al., 2020). PFBA at 50 to 300 µmol/l did not yield cytotoxicity or increase in calcium concentration, mitochondrial membrane potential or expression of osteogenic differentiation, or increase in collagen secretion but resulted in significant increase in ROS and downregulated self-renewal markers and up regulated adipogenesis markers, genes and fatty acid synthase in human mesenchymal stem cells. HepG2 cells treated with PFBA at 10 µM to 100 µM had lowest cellular concentration but a significant positive relationship between cellular concentration and two-fold increase in PPAR-alpha activity was observed at the highest concentration (Rosenmai et al., 2018).

3.4.2 Relevant Human data

3.4.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

3.4.2.2 New data

Epidemiological studies of PFBA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFBA (ng/ml).

In a recent study (Grandjean et al., 2020) elevated plasma-PFBA concentrations were associated with an increased risk of a more severe course of COVID-19. However, PFBA exposure was analysed as >LOD/<LOD (presence/absence) which does not allow for the evaluation of exposure-response. The other PFAS considered in the study were analysed as continuous variables; without a quantified PFAS exposure (tertiles, quartiles, etc. with specified cut-points) associated with a relative risk estimate, exposure-response cannot be evaluated.

(Gao et al., 2019) conducted a cross-sectional study of maternal PFAS exposure, including PFBA, and birth outcomes. The study population included 132 pregnant women, with a mean age of 31.0 years, who delivered a child at a university hospital in Beijing during 2015 and 2016. Maternal blood samples for exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. Median concentration of PFBA was 0.10 ng/ml and 0.12 ng/ml in maternal serum and cord serum, respectively. Maternal PFBA > 0.132 ng/ml was associated with a 0.60 cm decrease in birth length (95% CI -1.03 to -0.17 cm)

when compared to maternal PFBA < 0.079 ng/ml (p for trend 0.004). There were no exposure-response associations between other maternal PFAS concentrations and birth length. There was no exposure-response association between PFBA in cord serum and birth length; There were no exposure-response associations between PFBA in maternal serum or PFBA in cord serum and other birth outcomes (birth weight, gestational age, and ponderal index). The POD from this epidemiological study, however, cannot be considered reliable until after the body of epidemiological studies on PFBA exposure and birth outcomes is synthesized and considered in the context of weight-of-evidence analysis. In addition, other PFAS were associated with birth outcomes. Cord serum PFDoDA > 0.316 ng/ml was associated with a 0.50 cm (95% CI 0.07–0.92) increase in birth length when compared to cord serum PFDoDA < 0.0184 ng/ml (p for trend=0.018). PFTrDA in cord serum 0.056-0.44 ng/ml was associated with an increase of 263 g (95% CI 52.9 – 474) in birth weight and PFTrDA in cord serum >0.44 ng/ml was associated with an increase birth weight of 161.1 g (95% CI -48.3–370.7) when compared to PFTrDA in cord serum ≤0.056 ng/ml (p for trend=0.044).

Table 9: Point of Departure (POD) for PFBA derived from epidemiological studies

Reference	Finding	Conc. in serum (ng/ml)	External dose (ng/kg-d)	Other PFAS measured
(Gao et al., 2019)	Decreases in birth length, *p*-for-trend=0.004, 132 maternal-child pairs, PFBA median maternal serum, 0.10 ng/ml	**> 0.132**	Not reported	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA 6:2 Cl-PFESA, PFBS, PFHxS, PFOS

3.5 Proposal for a (new) starting point for deriving a drinking water limit value

There is no recommendation to derive a new drinking water limit for PFBA on the basis that there is no relevant new information available for this substance that would change the NOAEL of 6 mg/kg bw/day previously recommended by (UBA, 2016) based on increased incidence of follicular hyperplasia/hypertrophy of the thyroid gland and hepatocellular hypertrophy in a 90-day study in male rats employed in the present assessment. This POD is supported by the evaluation of EPA-IRIS (US EPA, 2022c).

In (UBA, 2016) a drinking water quota of 10% and an overall assessment factor of 2000 was chosen:

- AF of 10 to account for the time extrapolation (subchronic to chronic exposure) Note: This approach is not in line with the ECHA guidance in which a factor of 2 is sufficient.
- AF of 8 for toxicokinetic differences (see detailed explanation in (UBA, 2017))
- AF of 2,5 for interspecies toxicodynamic differences
- AF of 10 for intraspecies differences (general population)

4 Toxicological evaluation of perfluoropentanoic acid (PFPeA)

4.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 10 and Table 11.

Table 10: Chemical identity of perfluoropentanoic acid (PFPeA, CAS 2706-90-3)

Name	Perfluorpentansäure
English Name	Perfluoropentanoic acid
Acronym	PFPeA
Mol. Formula	C5HF9O2
Mol. Weight (g/mol)	264.05
CAS	2706-90-3
EC	220-300-7

Table 11: Physicochemical properties of perfluoropentanoic acid (PFPeA, CAS 2706-90-3)

Properties	Value	Source
Density (g/cm^3)	1.713 (exp. at 20 °C)	(Kauck & Diesslin, 1951)
Melting point (°C)	–13.2 - –5.9 (exp.)	(M. Zhang et al., 2020)
Boiling point (°C)	139 (exp.)	(Kauck & Diesslin, 1951)
Vapour Pressure (Pa)	2720 (exp. at 25 °C)	(Kwan, 2001)
Henry's Constant (Pa m^3/mol)	1.5 (exp. at 25 °C)	(Kwan, 2001)
	1.8 x 10^4 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	0.4 (exp. at 25 °C)	(Moroi et al., 2001)
Log Koc	1.37 (±0.46) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	9.81 x 10^3 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated)

4.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFPeA (Table 12). Data was identified by general desk research. Except for the GOW of 3 µg/l determined by UBA only sum values for PFAS including PFPeA were found. No quantitative toxicological assessments of PFPeA on human data determined by other institutions were found.

Table 12: Drinking water limits determined for PFPeA or sum of PFAS

Country/institution	PFPeA limit value in drinking water (µg/l)	Comment	Source
Germany/UBA	3	Gesundheitlicher Orientierungswert (GOW)	(UBA, 2017)
Sweden/Swedish Food Agency	≤ 0,1	Sum of 20 PFAS including PFPeA, drinking water guide value	(SFA, 2022)
Hawaii (USA)	0.800	Drinking water guideline level	(ECOS, 2022)

4.3 Toxicokinetics

4.3.1 Animal data

4.3.1.1 Data/studies reported in previous UBA evaluation

No toxicity or toxicokinetic data was available for assessment in the previous UBA assessment. An extensive review carried out by EFSA (Bull et al., 2014) was cited in the report in which no relevant information on toxicokinetics, toxicology in laboratory animals, or toxicology in humans was found. Only two in-vitro studies were reported which are described below.

PFPeA activated the luciferase of both mouse and human PPARα plasmids in COS-1 cells in a concentration-dependent manner compared to controls. Mouse PPARα was only slightly more sensitive than human PPARα to PFPeA (Wolf et al., 2012). In a steroidogenesis test, PFPeA did not lead to the formation of 17β-oestradiol or testosterone with a human adrenal cortex carcinoma cell line (NCI-H295R) and also did not react in reporter gene tests with human oestrogen, androgen or Ah receptors (Rosenmai et al., 2016).

4.3.1.2 New data

In a pharmacokinetics study, groups of 3-5 male and female Sprague-Dawley rats were given a single administration of PFPeA at doses of 0.5, 3 or 10 mg/kg orally, 10 mg/kg b.w. intravenously via tail vein, or at 3 mg/kg orally for tissue analysis (no control groups were reported) to evaluate the pharmacokinetics of the compound (Choi et al., 2020). The presence of PFPeA in plasma, urine, faeces and nine tissue samples (brain, heart, liver, lung, spleen, kidney, gastrointestinal (GI) tract, adipose tissue and muscle) was investigated, however, metabolites of PFPeA were not assessed[1]. PFPeA exhibited gender specific differences in the elimination and the distribution of the substance, where male rats showed a higher internal exposure and slower elimination when administered the same dose as female rats. The t1/2 ranged from 12.26 to

[1] PFPeA, as other PFAS, is not expected to be metabolised.

17.31 hours in male rats and 3.95 to 5.79 hours in female rats after oral or iv administration of PFPeA. Gender specific differences were also observed in the tissue distribution of PFPeA, where males showed a lower concentration of PFPeA in all of the tissues sampled when compared to females, except for the GI tract where similar values were observed in both sexes. The AUCinf (area under the plasma concentration–time curve from zero to infinite) values reported for the administered doses ranged from 3.57 to 36.29 and 2.08 to 30.22 µg h/ml in males and females, respectively. Males also showed a higher maximum plasma concentration (Cmax) when compared to females: the values reported for the given doses ranged from 0.94 to 10.66 and 0.66 to 6.78 µg/ml, respectively. The higher AUCinf and Cmax values in males indicate that the concentration of PFPeA in blood is higher in males than females. The highest accumulation of PFPeA was found in the GI tract followed by the muscle and liver. Conversely, there was no significant difference in the protein binding ratio, the level of substance bound to proteins between male and female rats. Protein binding influences the bioavailability and distribution of the substance, which in turn can result in slower metabolism and excretion of the substance.

4.3.2 Human data

4.3.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

4.3.2.2 New data

No relevant data was found.

4.4 Health effects in humans and/or animals

4.4.1 Relevant Animal data

4.4.1.1 Data/studies reported in previous UBA evaluation

4.4.1.1.1 Repeated dose toxicity

No relevant data was reported.

4.4.1.1.2 Carcinogenicity

No relevant data was reported.

4.4.1.1.3 Mutagenicity

No relevant data was reported.

4.4.1.1.4 Toxicity to reproduction

No relevant data was reported.

4.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was reported.

4.4.1.2 New data

4.4.1.2.1 Repeated dose toxicity

No relevant data was found.

4.4.1.2.2 Carcinogenicity

No relevant data was found.

4.4.1.2.3 Mutagenicity

No relevant data was found.

4.4.1.2.4 Toxicity to reproduction

No relevant data was found.

4.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

4.4.2 Relevant Human data

4.4.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

4.4.2.2 New data

Epidemiological studies of PFPeA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFPeA (ng/ml). Logarithmic transformations are usually applied to data that are skewed, that is, they do not follow a normal distribution. Only one epidemiological study was found that evaluated associations between exposure and outcome using untransformed PFPeA exposure data.

In a cross-sectional study of maternal PFAS exposure and birth outcomes, (Gao et al., 2019) did not find any dose-response relationships between PFPeA measured in maternal serum or cord serum and birth length, birth weight, gestational age or ponderal index of newborns. The study population included 132 pregnant women, with a mean age of 31.0 years, who delivered a child at a university hospital in Beijing during 2015 and 2016. Maternal blood samples for the exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. Median levels of PFPeA were 0.05 and 0.04 ng/ml in maternal serum and cord serum, respectively. A total of 14 PFAS were measured in blood and cord serum, including PFPeA.

There are no dose-response data from epidemiological studies suitable for identifying a point of departure to derive a drinking water limit for PFPeA.

4.5 Proposal for a (new) starting point for deriving a drinking water limit value

There were no suitable human toxicological data to justify a TWLW for PFPeA. A GOW of 3.0 µg/l was proposed by the previous UBA assessment, which was based on values recommended by the (UBA, 2011) and (Wilhelm et al., 2010).

There is no recommendation to derive a new drinking water limit for PFPeA on the basis that there are no relevant new animal studies or human studies available for this substance that would change the GOW of 3.0 µg/l determined by UBA.

5 Toxicological evaluation of perfluorohexanoic acid (PFHxA)

5.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 13 and Table 14.

Table 13: Chemical identity of perfluorohexanoic acid (PFHxA, CAS 307-24-4)

Name	Perfluorhexansäure
English Name	Perfluorohexanoic acid
Acronym	PFHxA
Mol. Formula	C6HF11O2
Mol. Weight (g/mol)	314.06
CAS	307-24-4
EC	206-196-6

Table 14: Physicochemical properties of perfluorohexanoic acid (PFHxA, CAS 307-24-4)

Properties	Value	Source
Density (g/cm^3)	1.789 (exp. at 20 °C)	ATSDR 2021 from (Lide, 2005)
Melting point (°C)	7.8-14.8 (exp.)	(M. Zhang et al., 2020)
Boiling point (°C)	136 (exp.)	(Schindler et al., 2013)
Vapour Pressure (Pa)	44 (exp. at 20 °C)	(Schindler et al., 2013)
Henry's Constant (Pa m^3/mol)	0.93 (exp. at 25 °C)	(Kwan, 2001)
	6.7 x 10^4 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	<1.6 (exp.)	(Vierke et al., 2013)
Log Koc	1.31 (±0.29) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	15.7 x 10^3 (25 °C)	ATSDR 2021 from (Kwan, 2001)
	1.89 x 10^3 (ext. 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated)

5.2 Quantitative toxicological assessments on human data and drinking water limits determined by other institutions

This chapter reviews quantitative toxicological risk assessments of PFHxA on human data determined by other institutions (Table 15) and drinking water limits for PFHxA (Table 16). Data was identified by general desk research.

Table 15: Summary of quantitative toxicological assessments of PFHxA and corresponding health endpoints by other institutions

Agency	Quantitative assessment	Health endpoint	Value	Reference
ANSES (2017)	Chronic toxicity reference values (TRV)	kidney effects in rodents	0.32 mg/kg bw per day	(EFSA, 2020)
US EPA	Chronic Oral Reference Dose (RfD) for non-cancer effect	decreased offspring body weight	4×10^{-4} mg/kg-day	(US EPA, 2021e)
Department of Health Minnesota	Short-term Non-Cancer Health-Based Value ($nHBV_{Short\text{-}term}$)	Critical effect: Decreased total T4 Co-critical effect: Decreased pup body weight Additivity endpoint: Developmental, Thyroid [E]	0.2 µg/l	(MDH, 2021)
Department of Health Minnesota	Subchronic Non-Cancer Health-Based Value ($nHBV_{Subchronic}$) = nHBVShort-term = 0.2 µg/l	Critical effect: Nasal epithelium degeneration Co-critical effect: Decreased bilirubin Additivity endpoint: Hepatic (liver) system, Respiratory system	0.2 µg/l	(MDH, 2021)

Table 16: Drinking water limits determined for PFHxA or sum of PFAS

Country/institution	PFHxA limit value in drinking water (µg/l)	Comment	Source
Germany/UBA	6	Drinking water guide value	(UBA, 2017)
Hawaii (USA)	4.0	Drinking water guideline level	(ECOS, 2022)
Michigan (USA) /DTMB	400	Drinking water guideline level	(ECOS, 2022)
Minnesota (USA)	0.2	Drinking water guideline level	(ECOS, 2022)
Wisconsin (USA)	150	Drinking water guideline level	(ECOS, 2022)
Sweden/Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFHxA,	(SFA, 2022)

Country/institution	PFHxA limit value in drinking water (µg/l)	Comment	Source
		drinking water guide value	

5.3 Toxicokinetics

5.3.1 Animal data

5.3.1.1 Data/studies reported in previous UBA evaluation

The half-life (t½) of PFHxA varies depending on the species, e.g., t½ in rodents is approximately 1 to 2 hours, in cynomolgus monkeys 1 to 2 days and in humans it has been estimated to be between 14 to 49 days (Chengelis, Kirkpatrick, Myers, et al., 2009; Nilsson, Kärrman, Westberg, et al., 2010; Nilsson et al., 2013; Nilsson, Kärrman, Rotander, et al., 2010; Russell et al., 2013).

There was no oxidative DNA damage induction or potential for formation of reactive oxygen species (ROS) in human hepatoma HepG2 cells at concentrations of up to 2000 µM PFHxA (Eriksen et al., 2010). Mouse and human PPARα were activated in the presence of PFHxA, with the mouse PPARα being only slightly more sensitive than the human receptor (Buhrke et al., 2013; Wolf et al., 2008). Binary mixtures of PFHxA and PFOA resulted in an additive effect at low concentrations with respect to PPARα activation (Wolf et al., 2014). PFHxA showed a slight induction of peroxisome β-oxidation mediated effects such as β-oxidation and liver enlargement (Kudo et al., 2006). There was no hormone production (17β-oestradiol or testosterone) in a steroidogenesis test using human adrenal cortex carcinoma cell line (NCI-H295R) nor was there reaction in reporter gene tests with human oestrogen, androgen or aryl hydrocarbon (Ah) receptors indicating that this substance is unlikely to perturbate the endocrine system (Rosenmai et al., 2016).

5.3.1.2 New data

Female CD-1 mice (n=4-6) were administered with a single instance of radioactive labelled PFHxA either in the tail vein at a concentration of 4.44 MBq (120 µCi) in 100 µL or 3.7 MBq (100µCi) in 100 µL by oral gavage on gestation day 18 to study the uptake and distribution of PFHxA (Bartels et al., 2019). Rapid uptake of PFHxA was observed in placentae followed by transport into foetuses after tail vein administration. A slower uptake was observed following oral gavage exposure, however transport across the placenta was still reported. PFAS uptake was found in all tissues examined in this study, with the highest uptake being exhibited in the blood for PFHxA via tail vein and the lungs via oral gavage.

Male and female Hsd:Sprague Dawley rats (n=1/dose) were given a bolus iv dose of 40 mg/kg bw and oral gavage dose of 40, 80 and 160 mg/kg bw as a single administration (Dzierlenga et al. 2020). Females administered PFHxA had a shorter half-life (2 h vs. 9 h) than males and faster clearance with a smaller plasma AUC.

Male Wistar rats were given a single administration by oral gavage of PFHxA at a dose of 100 µg/kg bw and a sub-chronic administration in drinking water at a concentration of 1, 5 or 25 µg/l for 1 or 3 months duration to evaluate the toxicokinetic profile of the substance (Iwabuchi et al., 2017). The substance was very rapidly absorbed, distributed and eliminated from the tissues with nearly the same tissue t½ of 2-3 hours. Considering serum Vd (the volume of distribution), and the tissue delivery, PFHxA was mainly in the serum with the lowest delivery to

the brain; and no tissue accumulation was observed in the chronic studies as estimated from the single dose study.

Maximum serum levels of PFHxA were reached within 15 - 30 min after oral administration of 2 or 100 mg/kg bw of 14C-labelled PFHxA to mice and rats. 14C-labelled PFHxA could be detected after 30 to 120 minutes primarily in plasma, kidneys, liver and bladder, and to a lesser extent in erythrocytes, bone and marrow, brain, fat, heart and other internal organs including reproductive organs (Gannon et al., 2011).

Following a single oral dose of microencapsulated PFHxA (3 mg/kg bw) to micromini-pigs, the maximum blood concentration was reached in less than 12 h (Guruge et al., 2016).

In a separate study, PFHxA was fed (47.8µg/kg dry weight) to fattening pigs (average weight 83 kg) mixed with six other perfluorocarboxylic and sulfonic acids (PFBS, PFHxS, PFOS, PFHpS, PFHpA, PFOA) for 25 days. Approximately 97% of the dose administered in food could be balanced in blood and edible parts and therefore absorption considered to be high and practically complete. Blood plasma contained the largest proportion of perfluoro-compounds, followed by muscle and fat tissue, which contained the same concentrations. Lower proportions (7 and 2 %) were found in the liver and kidneys, respectively. PFHxA exhibited a saturation curve, which increased only slightly from the 10th day onwards (Numata et al., 2014).

PFHxA was not metabolised in mammals, including humans (ATSDR, 2021). There was no metabolite formation in *in vitro* rat hepatocyte culture studies, nor have the analysis of plasma, stool or urine samples from rats that had previously received PFHxA once orally dosed at 2 or 100 mg/kg (Gannon et al., 2011).

Based on studies in rats, mice, monkeys and pigs PFHxA is rapidly excreted and primarily in urine. Elimination from serum can be described by a biphasic kinetics in which ≥ 99% of the administered dose was eliminated in a first (α) phase and a small proportion in a slower second (ß) phase. In male and female rats > 99% of 14C-PFHxA was excreted in urine within 12 h after single or repeated administration; in mice > 99% was excreted within 24 h (females) to 48 h (males) (Gannon et al., 2011).

Rats were administered single oral doses of 40, 80 or 160 mg PFHxA/kg bw intravenously to calculate biphasic elimination kinetics (NTP, n.d.). For the first and second phase of elimination from plasma after oral administration, the half-lives calculated were α-Phase: 2.35 ± 1.27, α-Phase: 1.78 ± 5.74 and α-Phase: 1.46 ± 0.26 at 40, 80 and 160 mg/kg bw in males, respectively and ß-Phase: 9.33 ± 20.8, ß-Phase: 5.74 ± 4.59 and ß-Phase: 13.7 ± 14.2 at 40, 80 and 160 mg/kg bw in males, respectively. In females, α-Phase: 1.37 ± 2.23, α-Phase: 1.12 ± 0.13 and α-Phase: 2.35 ± 1.27 at 40, 80 and 160 mg/kg bw, respectively and ß-Phase: 2.27 ± 2.13, ß-Phase: 5.46 ± 2.64 and ß-Phase: 12.2 ± 23.6 at 40, 80 and 160 mg/kg bw, respectively. Following intravenous administration, the first and second half-lives were 0.66 and 8 h (M) and 0.34 and 7.3 h (F) for males and females, respectively.

The elimination pharmacokinetics of PFHxA in mouse, rat, microminipig, pig, monkey and human has been complied in an industry report (low reliability) summarising the alpha and beta phase elimination rates together with the proportion of $PFHx^-$ (anion of PFHxA) that is eliminated during each phase (Anonym, 2016). The elimination kinetics of PFHxA is consistent across the range of mammalian species included in this review. Minor differences between typical laboratory species (mice, rats), larger mammals (microminipigs, pigs and monkeys) and humans have been reported. There is an extremely rapid initial rate of elimination (i.e., alpha phase), which accounts for elimination of more than 99.7% of this substance from blood in less than 24 hours in mice, rats and monkeys. While the α-phase of elimination is not available for

the microminipig, pig or human, the report authors state given that consistent alpha and beta kinetic behaviour seen across mammalian species, similar elimination response for these species is scientifically reasonable. The PFHxA elimination in the beta phase is slower in mammals, with half-lives in the range of 50-122 hours (2-5 days) in mice, rats, microminipigs, pigs, monkeys and humans. Almost all of the PFHxA is eliminated in the alpha phase, with less than 0.3% of the PFHxA administered dose accounting for the slower beta phase.

5.3.2 Human data

5.3.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

5.3.2.2 New data

No relevant data was found.

5.4 Health effects in humans and/or animals

5.4.1 Relevant Animal data

5.4.1.1 Data/studies reported in previous UBA evaluation

5.4.1.1.1 Repeated dose toxicity

Groups of ten Crl:CD(SD) rats were administered PFHxA by oral gavage doses of 0, 10, 50 or 200 mg/kg bw/day (Chengelis, Kirkpatrick, Radovsky, et al., 2009). At a dose of 200 mg/kg bw/day changes were seen with lower red blood cell parameters, higher reticulocyte counts and reduced globulin content. In the males of the two upper dose groups, increased liver enzyme values were found, and in the highest dose group reduced total protein, an increased albumin/globulin ratio and reduced cholesterol and calcium concentrations in the serum were noted. There was also minimal centrilobular hypertrophy of the liver cells and, correlated with higher liver weight and slightly increased peroxisomes at the top dose. A NOAEL of 50 mg/kg bw/day in females and 200 mg/kg bw/day in males was identified by the study authors based on liver histopathology and liver weight changes.

The critical study in the derivation of the drinking water limit for PFHxA was a chronic oral gavage study in which Sprague Dawley rats (60/sex/dose and 70/sex/top dose) were given 0, 2.5, 15 or 100 mg/kg bw/day for males and females were given 0, 5, 30 or 200 mg/kg bw/day PFHxA daily for a duration of 104 weeks (Klaunig et al., 2015). A NOAEL of 15 mg/kg bw/day in males and 30 mg/kw bw/day in females was identified (NICNAS, n.d., 2015). The drinking water limit was calculated from a NOAEL of 15 mg/kg bw/day in males based on low urine pH values identified in a 2-year study in rats. The following factors were applied: an interspecies extrapolation (for the toxicokinetics corresponding to the human/rat elimination half-lives: 768 h (32 d) / 2.35 h ≈ factor 327, and for toxicodynamic a factor of 2.5) and the intraspecies extrapolation (100.5 or 3.16 for toxicokinetic and toxicodynamic) to determine a human equivalent dose (HED) of 1.84 µg/kg bw/day for a 70 kg body weight adult drinking 2 litres of drinking water per day and 10% allocation of exposure per day attributed to drinking water. In comparison, the WHO is considering 60 kg body weight and a standard allocation factor of 20% (changed from 10 to 20% in 2008) (WHO, 2008, 2017). These assumptions correspond to WHO guidance for deriving generic health-based guidance values for substances in water (WHO, 2017). A drinking water limit of 6.42 µg/l was calculated. A factor of 327 for toxicokinetic considerations was considered justified given that the mean t½ for the rat of 2.35 h (Chengelis,

Kirkpatrick, Myers, et al., 2009) and a mean t½ for humans of 32 days (Russell et al., 2013) gives an approximate difference of 327.

5.4.1.1.2 Carcinogenicity

No relevant data was reported.

5.4.1.1.3 Mutagenicity

Sodium perfluorohexanoate (Na-PFHx) was negative for mutagenesis in the Ames test employing Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA at concentrations of 333, 667, 1000, 3333 and 5000 µg/plate in the presence and absence of a metabolic activation system (Loveless et al. 2009). Na-PFHx did not cause chromosome aberrations in peripheral human lymphocytes at concentrations of 5 to 3860 µg/ml) (Loveless et al. 2009).

5.4.1.1.4 Toxicity to reproduction

Sprague Dawley rats (n=55/sex for control and top dose, n=45/sex for the low and mid dose groups) were given daily gavage doses of 0, 20, 100 or 500 mg/kg Na-PFHx in a 90-day reproduction study (Loveless et al., 2009a). Subgroups of five animals per dose and sex (including the control group) were then observed for a recovery period of one and up to three months. Nasal lesions were observed at doses of 100 mg/kg bw/day and above and a NOAEL of 20 mg/kg bw/day was identified on this basis. No effects were observed with regard to the neurobehavioral parameters. Hepatic peroxisomal β-oxidation was induced and the study authors identified a NOEL of 20 mg/kg bw/day for male rats and 100 mg/kg bw/day for female rats.

Rats were dosed with Na-PFHx from 70 days before cohabitation to throughout pregnancy and lactation for a total of 4 months (Loveless et al., 2009a). No effects on reproductive toxicity parameters were observed. A maternal NOAEL of 20 mg/kg bw/day was identified based on reduced body weight at higher doses (500 mg/kg bw/day) in the parental generation. The NOAEL for reproductive toxicity was 100 mg/kg bw/day based on reduced body weight in the first generation.

In a developmental toxicity study, pregnant rats were dosed during gestation days 6 – 20 (Loveless et al., 2009a). The maternal and foetal NOAEL was 100 mg/kg bw/day based on body weight effects at 500 mg/kg bw/day.

Twenty females were administered by oral gavage doses of 0, 100, 350 or 500 mg/kg bw/day and a separate group of 20 females were administered by oral gavage doses of 0, 7, 35 or 175 mg/kg bw/day, on the day 6th to 18th day of pregnancy (Iwai & Hoberman, 2014a). At doses of 350 and 500 mg/kg bw/day mortality was observed and in the remaining animals increased salivation and, compared to the controls changes in body weight gain were reported. The body weights of the offspring were reduced at all doses, permanently only at the two highest doses of 350 and 500 mg/kg bw/day. At these two doses stillbirths, reduced viability indices (viability) and developmental delay were also observed. At a dose of 175 mg/kg bw/day stillbirths as well as mortality and reduced body weights in the offspring was seen. The NOAEL for maternal and reproductive toxicity was 100 mg/kg bw/day ammonium perfluorohexanoate (equivalent to approximately 95 mg/kg bw/day PFHxA) as identified by the study authors.

5.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was reported.

5.4.1.2 New data

5.4.1.2.1 Repeated dose toxicity

No relevant data was found.

5.4.1.2.2 Carcinogenicity

No relevant data was found.

5.4.1.2.3 Mutagenicity

No relevant data was found.

5.4.1.2.4 Toxicity to reproduction

The study (Iwai & Hoberman, 2014b) was re-evaluated in (Iwai et al., 2019). Female Crl:CD-1(ICR) mice (20/group) were given the ammonium salt of PFHxA (i.e., APFHx) at doses of 0, 100, 350 or 500 mg APFHx /kg bw/day during Phase 1 and 0, 7, 35 or 175 mg APFHx /kg bw/day during Phase 2 during gestation days 6 to 18 by oral gavage to assess developmental toxicity potential (Iwai et al., 2019). In Phase 1, 13 of 80 (16%) of F0-generation female mice were found dead, including 3 in the control group and 6 in the low-dose group, which the study authors noted were within the historical range for the testing facility. No mortality occurred in Phase 2 animals. While the mortality seen in Phase 1 was largely unrelated to ammonium perfluorohexanoate exposure. The outcomes from Phase 2 were considered by the study authors to be more indicative of developmental end points of interest in F1-generation pups given that there was an absence of maternal health effects. The incidence of stillborn pups was considered by the study authors to be reflective of developmental toxicity. There were no stillbirths in the 2 lower dose groups and the control group of Phase 2. The incidence of stillborn pups in Phase 2 of the study was 1.2% (3 of 241 delivered pups) at the high-dose group (175 mg/kg bw/day). However, in the Phase 1 control group, the incidence of stillborn pups was 1.8% (4 of 221 delivered pups). This occurrence of stillbirths in the control group, at a higher incidence rate than the Phase 1 treatment group reported for the same study, indicated that the observation of stillbirths in Phase 2 high-dose group is unlikely to be attributable to in utero ammonium perfluorohexanoate exposure and therefore not indicative of a developmental effect. When control data for both Phase 1 and Phase 2 studies were pooled results indicated that the difference in stillbirths (i.e., 0.9% for controls and 1.2% for the high dose group 175 mg/kg bw/day) was not statistically significant ($p = 0.169$). The study authors identified an unbounded NOAEL of 175 mg/kg bw/day for maternal ammonium perfluorohexanoate exposure based on an array of developmental toxicity end points. However, they stated that this is an unbounded NOAEL for developmental effects, meaning that the study did not establish a dose at which developmental effects may occur.

5.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

5.4.2 Relevant Human data

5.4.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

5.4.2.2 New data

Epidemiological studies of PFHxA in blood serum typically evaluated health outcomes or biomarkers of effect in relation to log-transformed continuous measures of PFHxA (ng/ml). Only

three epidemiological studies were found that evaluated associations between exposure and outcome using untransformed PFHxA exposure data. Log transformations are used for continuous data that have skewed distributions, such as concentrations of substances in blood. Log transformation of skewed data typically makes the distribution more like a normal distribution which fits the assumptions for linear regression. However, the log transformed data are not directly interpretable; the response is described per log-unit increase. Back transformation is required to arrive at the original scale (e.g., PFAS concentrations measured in blood; publications generally do not provide enough information to back transform the data and additional analysis is required.

In a cross-sectional study of maternal PFAS exposure and birth outcomes, (Gao et al., 2019) reported that PFHxA measured in maternal serum or cord serum was not related to birth length, birth weight, gestational age or ponderal index when PFHxA exposure was categorized into tertiles of exposure. The study population included 132 pregnant women, with a mean age of 31.0 years, who delivered a child at a university hospital in Beijing during 2015 and 2016. Maternal blood samples for the exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. Median concentrations of PFHxA were 0.17 and 0.18 ng/ml in maternal serum and cord serum, respectively. A total of 14 PFAS were measured in blood and cord serum, including PFHxA.

(T.-W. Lin et al., 2020) conducted a cross-sectional study of 397 adults aged 55-75 years from communities in Taiwan during 2016-2017. The communities were located near a river where wastewater from semiconductor and electronics industries had discharged PFAS. Study participants must have lived in their communities for 10 or more years to be eligible for participation in the study. Median serum PFHxA concentrations were 0.38 ng/ml. No statistically significant exposure-response associations were detected between quartiles of PFHxA and metabolic syndrome status or triglyceride levels, uric acid, or total cholesterol. Results were adjusted for age, sex, smoking status, and drinking status.

(Ou et al., 2021) conducted a case-control study of 158 cases of congenital heart defections (CDH) and 158 controls nested within a cohort of 11,578 newborns. Pregnant women were recruited at a hospital in Guangzhou, China during 2014-2018. Screening for congenital anomalies occurred between 18 and 26 weeks of gestation. Maternal blood plasma was collected before delivery and cord blood plasma was collected during delivery. The median PFHxA in maternal plasma was 0.015 ng/ml for cases and 0.020 ng/ml for controls. Odds ratios for CHDs, adjusted for maternal age, parity, and infant sex, were not increased when cases and controls with maternal PFHxA serum ≥ 0.033 ng/ml (the 75th percentile for controls) were compared to cases and controls with maternal PFHxA serum <0.033 ng/ml. Ou et al. (2021) described this study as a pilot study intended to generate hypotheses regarding gestational PFAS exposure and congenital heart defects. There were a total of 11 PFAS measured in this study.

Based on these study results, there is a lack of adequate dose-response data from epidemiological studies suitable for identifying a point of departure to derive a drinking water limit for PFHxA.

5.5 Proposal for a (new) starting point for deriving a drinking water limit value

There is no recommendation to derive a new drinking water limit for PFHxA on the basis that there is no relevant new information available for this substance that would change the NOAEL of 15 mg/kg bw/day in males based on low urine pH values identified in a 2-year study in rats employed in the present assessment. The observed lowered urine pH value at 100 mg/kg

bw/day was significant, but can be seen as negligible (after 52 weeks: control pH 6.8 ± 0.35, highest dose 6.5 ± 0.39) and the urine pH value is still in the normal range (Tannehill-Gregg et al., 2009). Lowering of urine pH is not typically considered to be an adverse effect, but rather an adaptive response. The project team recommends reviewing this when considering an update.

Alternatively, a NOAEL of 30 mg/kg bw/day from a chronical toxicity study on nephro- and hepatotoxicity in female rats can be used (Klaunig et al., 2015). At the next higher dose of 200 mg/kg bw/day papillary necrosis were observed in female rats (tubular necrosis). This can be ranked as adverse effect. The study is discussed in detail in an unpublished report (UBA & Voss, 2020).

6 Toxicological evaluation of perfluoroheptanoic acid (PFHpA)

6.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 17 and Table 18.

Table 17: Chemical identity of perfluoroheptanoic acid (PFHpA, CAS 375-85-9)

Name	Perfluorheptansäure
English Name	Perfluoroheptanoic acid
Acronym	PFHpA
Mol. Formula	C7HF13O2
Mol. Weight (g/mol)	364.06
CAS	375-85-9
EC	206-798-9

Table 18: Physicochemical properties of perfluoroheptanoic acid (PFHpA, CAS 375-85-9)

Properties	Value	Source
Density (g/cm^3)	1.792 (exp. at 20 °C)	(Kauck & Diesslin, 1951)
Melting point (°C)	19.1-32.8 (exp.)	(M. Zhang et al., 2020)
Boiling point (°C)	175 (at 742 mm Hg)	ATSDR 2021 from (Kauck and Diesslin 1951)
Vapour Pressure (Pa)	1170 (exp. at 25 °C)	(Kwan, 2001)
Henry's Constant (Pa m^3/mol)	0.57 (exp. at 25 °C)	(Kwan, 2001)
	2.5 x 10^5 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	<1.6 (exp.)	(Vierke et al., 2013)
Log Koc	1.63 (±0.15) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	4.37 x 10^5 (25 °C)	ATSDR 2021 from (Kwan, 2001)
	356 (ext. at 25 °C)	(M. Kim et al., 2015)
	1.2 x 10^5 (exp. at 21.6 °C)	(Kaiser et al., 2006)

(exp. = experimental, ext. = extrapolated)

6.2 Quantitative toxicological assessments on human data and drinking water limits determined by other institutions

This chapter reviews drinking water limits for PFHpA (Table 19). Data was identified by general desk research. Except for the GOW of 0,3 µg/l determined by UBA only sum values for PFAS including PFHpA were found. No quantitative toxicological assessments of PFHpA on human data determined by other institutions were found.

Table 19: Drinking water limits determined for PFHpA or sum of PFAS

Country/institution	PFHpA limit value in drinking water (µg/l)	Comment	Source
Germany/UBA	0.3	Gesundheitlicher Orientierungswert (GOW)	(UBA, 2017)
Colorado (USA)/ DPHE	0,07	Lifetime health advisory, drinking water	(Cape Fear PUA 2018)
Hawaii (USA)	0.040	Drinking water guideline level	(ECOS, 2022)
Massachusetts / Maine (USA)	0.020	Sum of 6 PFAS including PFHpA, drinking water guideline level	(ECOS, 2022)
Vermont (USA)	0.020	Sum of 5 PFAS including PFHpA, drinking water guideline level	(ECOS, 2022)
Sweden/Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFHpA, drinking water guide value	(SFA, 2022)

The Landesanstalt für Umwelt Baden-Württemberg (LUBW, 2014) provides the following analogy to the question of a so called Geringfügigkeitsschwelle (GFS): Similar to the case of PFBA, a key difference for various perfluoroalkane carboxylic acids is the rate of excretion from the human body, which depends on the number of perfluorinated carbon atoms. The LUBW also leans on the consideration of (Lud et al., 2010), who consider the following gradation of excretion rates to be possible for carboxylic acids with three to seven perfluorinated carbon atoms: PFBA ≤ PFPeA ≤ PFHxA << PFHpA = PFOA. Furthermore, analogously to the procedure for PFBA, a comparable potency of PFOA (and PFOS) is also assumed for PFHpA and due to the same, extremely slow excretion rate, the drinking water value for PFOA is adopted for PFHpA.

6.3 Toxicokinetics

6.3.1 Animal data

6.3.1.1 Data/studies reported in previous UBA evaluation

The half-life of PFHpA in rats was reported to be 0.10 ± 0.05 days for the males (according to (ATSDR, 2009, 2021), 2.4 ± 1.2 h) and 0.05 ± 0.01 days measured for the females (according to (ATSDR, 2009, 2021), 1.2 ± 0.2 h).

6.3.1.2 New data

Similar to rats, mice also showed a rapid elimination of PFHpA in urine. In NJcI mice, PFHpA was rapidly and almost completely eliminated in the urine after 24 hours (99% in males and 66% in females) and a small amount was excreted via faeces (3% in males and 13% in females) for iv injection. Similar values were observed for gavage administration. A volume distribution of 0.07 L/kg for male and 0.08 L/kg for female was reported as well as a clearance rate of 248.8 ml/day/kg in males and 166.7 ml/day/kg in females. When calculating the half-live with the formula $t_{1/2}$ = ln2 x Volume distribution/Clearance, this results in a half-live of 0.29 days (7 hours) for female and 0.19 days (4.7 hours) for male mice (Fujii et al., 2015).

6.3.2 Human data

6.3.2.1 Data/studies reported in previous UBA evaluation

In humans, a half-life of 1.2 years for a collective of 31 men and older women (>50 years) from the population of China was reported. Younger women had a similar elimination half-life (mean 1.5 years, standard deviation 0.8 years; n=12) (ATSDR, 2009, 2021).

6.3.2.2 New data

Different data on half-lives of PFHpA in humans were found in the literature. (Y. Zhang et al., 2013) reported a half-life of 1.2 (male) to 1.5 (female) years in human for PFHpA when comparing half-lives of different PFAS in males and females. Other information on half-lives in humans is reported by (Y. Xu et al., 2020). Here, a half-live of PFHpA of 62 days with a 95% confidence interval of 51 to 80 days was indicated.

6.4 Health effects in humans and/or animals

6.4.1 Relevant Animal data

6.4.1.1 Data/studies reported in previous UBA evaluation

6.4.1.1.1 Repeated dose toxicity

No relevant data was found.

6.4.1.1.2 Carcinogenicity

No relevant data was found.

6.4.1.1.3 Mutagenicity

No relevant data was found.

6.4.1.1.4 Toxicity to reproduction

No relevant data was found.

6.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant toxicological data to derive a TWLW is available for PFHpA. The UBA proposed in their report a GOW of 0.3 µg/l (UBA, 2011).

After interpolating its guide values for assessable PFAS according to the chain length for PFHpA, the (UBA, 2011) recommends a GOW of 0.3 µg/l (Wilhelm et al., 2010).

The sensitivity of mice and human PPARα was tested in COS-1 transfected cells with mouse or human PPARα plasmids by (Wolf et al., 2012). In this study, mouse PPARα was only slightly more sensitive to PFHpA than human PPARα.

Furthermore, PFHpA showed the third strongest effects of peroxisome β-oxidation, which is closely related to PPARα activity, compared to different PFAS *in vivo* (Kudo et al., 2006).

6.4.1.2 New data

6.4.1.2.1 Repeated dose toxicity

A search of the available data on the website of the European Chemicals Agency (ECHA) led to a reference to a combined 90-day repeated-dose toxicity study and reproductive/developmental toxicity screening study (OECD TG 408 & 422) in CD1 mice from 2017, but the author is not named2. The study prompted the ECHA risk assessment committee (RAC) to classify the substance as toxic to reproduction.

The test substance (PFHpA, purity > 99.3 %) was administered by gavage (vehicle: deionised water) at doses of 0, 0.5, 10 and 50 mg/kg bw/day. The F0 generation consisted of 20 mice/sex/dose with 5 additional female mice in the control and high dose groups (for the purpose of gender comparison). Adult animals (~6 weeks old at baseline) were exposed for 90 days prior to mating. Males were further exposed during mating, resulting in exposure durations between 109 and 113 days, while females were exposed until the 20th day of lactation (i.e., 130 - 142 days). The 5 females in the control group and the group with the highest dose introduced for the sex comparison were exposed for 109 days. No clinical signs were observed in the F0 generation and there were no effects on survival, body weight/body weight gain, feed intake, reproductive parameters mating index, fertility index, implantation sites, gestation, parturition or oestrous cycle (except a slight prolongation of the reproductive parameter pre-coital interval (observed at all dose groups, not statistically significant)), functional observation battery (FOB) behaviour or motor activity. There were also no effects on organ weights, except for a statistically significant increase in liver weight in the middle and highest dose groups. Liver-related biochemical markers in the blood were significantly impaired in the highest dose males (marked increases in aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and triglycerides), less so in the highest dose unmated females (increases in ALP and triglycerides) and on day 21 of lactation there was no increase in ALP or triglycerides. No effect was observed on day 21 of lactation in females at the highest dose. Liver-related biochemical markers in the blood were also affected after 75 days in male and female animals. Significant microscopic liver changes were observed in males and females at all doses tested, with a dose-dependent increase in frequency and severity. At the low dose, necrosis was observed in one male (minimal) and four females (3 minimal, 1 mild) of the F0 generation.

F1 pups were randomly selected for F1 generation. While there were no effects on the number of litters, average litter size or anogenital distance as well as no evidence of nipple retention in F1 males after PND 13, postnatal survival and pup body weight were reduced. Liver weights were also significantly increased in the F1 generation in males in the middle and highest dose groups and in females in the highest dose group. Other organ weight changes were a statistically significant decrease in absolute and relative weight of the adrenal gland in females in the highest dose group and in absolute weight in the low dose group. In addition, absolute brain weight was statistically significantly reduced at the highest dose. No histopathological correlates were described for these organs.

The macroscopic liver findings were confirmed by microscopic examination. Centrilobular hypertrophy was observed in all animals, while necrosis was observed in males and females at the intermediate and highest doses. A dose-dependent increase was evident in these observations. It is also noted that serum T4 levels were reduced at the mid and highest dose of

[2] [04.01-ML-014.03] (europa.eu)

F0 males (females not analysed). A slight dose-dependent decrease in serum T4 levels was also observed in F1 males, while a slight increase was seen in females. No corresponding findings in the thyroid gland were reported.

The applicant considered the liver-related effects observed at 10 mg/kg bw/day to be sufficiently severe to support classification as STOT RE. After correction for the duration of exposure (109 days), an effective dose of 12.1 mg/kg bw/day was calculated. This value is above the upper guideline value for classification in category 1. The observed effects on the liver are adverse and, where necrosis occurred, irreversible and were observed in male and female animals in two generations. As only one study on PFHpA is available, the effects have only been demonstrated in one species. However, RAC points out that similar liver toxicity has also been demonstrated for the closely related substances PFOA and PFNA, although no detailed assessment has been provided by the applicant. Based on the observed dose-dependent increase in hepatocellular necrosis from 0.5 mg/kg body weight/day, RAC supports the applicant's proposal to classify PFHpA as STOT RE 1; H372 (liver) (ECHA, 2020b).

6.4.1.2.2 Carcinogenicity

No relevant data was found.

6.4.1.2.3 Mutagenicity

No relevant data was found.

6.4.1.2.4 Toxicity to reproduction

Reproductive effects of PFHpA were investigated by (Z. Li et al., 2021). In this study 35-day-old Sprague Dawley male rats (8 per group) were exposed to PFHpA by gavage with 0 (corn oil), 10, 50 and 100 mg/kg bw/day for 21 days. PFHpA did reduce the testis weight, relative testis weight and epididymis weight at the highest tested dose. Serum testosterone, luteinizing hormone and follicle-stimulating hormone levels were significantly increased at the highest tested dose. Also, the sperm production at 100 mg/kg bw was suppressed and Leydig cell hyperplasia was observed. The expression of CYP11A1, Hsd3b1 and CYP17A1 was downregulated and the follicle-stimulating hormone (FSH) receptor (Fshr) in the Sertoli cells was upregulated at the highest tested dose. The number of HSD11B1 positive Leydig cells and SOX9 positive Sertoli cells was not affected by PFHpA. An increase of BCL2 and phosphorylation of AKT1, AKT2, ERK1/2 and JNK was observed after PFHpA treatment, whereas BAX levels were decreased. No effect on SIRT1 and PFC-1-α levels were observed. The authors concluded that PFHpA induces Leydig cell hyperplasia due to an increase in the secretion of LH through negative feedback after the downregulation of the expression of steroidogenic enzymes and inhibiting testosterone production in Leydig cells. They assume that this proliferation is mediated by the increase of BCL2 and phosphorylation of AKT, ERK1/2 and JNK and decreasing BAX level (Z. Li et al., 2021). It should be noted that the effects in Leydig cells observed in the rat might be not relevant for humans, even a hyperplasia may become a tumour. It is known that there are differences in the biology of the Leydig cells between rats and humans, especially type and number of receptors, which might have an effect in the differences of observed rates of Leydig cell tumours in rats and humans. Steinbach et al. (2015) compared both human and rodent exposure data, indicating that humans have a noticeably lower incidence of Leydig cell tumours compared to rats. Furthermore, they indicated that the mechanism of a Leydig cell tumour induction is different between rats and humans. These findings support that rodent Leydig cell tumours are not relevant to human health (Steinbach et al., 2015). Therefore, this study is not used for the derivation of TWLW, since Leydig cell hyperplasia was considered as the adverse effect in rats after PFHpA exposure.

The combined 90-day repeated-dose toxicity study and reproductive/developmental toxicity screening study (OECD TG 408 & 422) in CD1 mice from Anonymous (2017) mentioned above investigated also the reproductive and developmental toxicity of PFHpA. No effects on survival, body weight/body weight gain, food consumption, clinical signs, mating index, fertility index, implantation sites, gestation, parturition or oestrous cycle were observed. Behaviour in the functional observation box (FOB) and motor activity was similar in all groups. A slight increase in pre-coital interval (observed at all dose groups, not statistically significant) was investigated (pre-coital interval in control, low, mid and top dose was 2.2d, 2.9d, 2.7d and 2.9d, respectively). Effects on development was observed in F1 animals. A reduced pup survival was observed from birth to PND 4 (99.6%, 95%, 99.6% and 89.3% in the control, low, mid and top dose groups, respectively). On PND 21 the indices were 99.3%, 99.4%, 98.7% and 87.8%, respectively, and indicated that a further decrease was seen in the mid and top doses. Only at the top dose between PND4 and 21, these effects were outside of the historical control data. At the high dose, mean pup body weight was statistically significantly decreased from PND 1 in males (except PND 22) and from PND 4 to 21 in females. Female pups from the mid dose also had significantly lower body weight compared to the control animals on PND 43. Cleft palate (palatine plates not joined for the entire length) was only found in dead animals (no evidence of milk in stomach, necropsy on PND 0 or 1) but with no dose response relationship. In the mid and top dose groups an increase in the number of pups with missing digits (left and/or right limbs) was observed as well as pups with malrotated forelimbs) and a small stature (ECHA, 2020b).

6.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

Data for a steroidgenesis test with a human adrenal cortex carcinoma cell line is available, which shows that PFHpA did not lead to a formation of 17β-oestradiol or testosterone and also did not react in reporter gene tests with human oestrogen, androgen or Ah receptors (Rosenmai et al., 2016).The effects of PFHpA on mast cell-mediated allergic inflammation in the presence of high-affinity immunoglobulin (Ig) E receptor cross-linking were examined by (J.-K. Lee & Kim, 2018). PFHpA did not induce cytotoxicity in IgE-stimulated mast cells at doses up to 500 µM. It also did not change the histamine release, degranulation and the intracellular calcium concentration of mast cells. Furthermore, no change in expression of pro-inflammatory cytokines at both mRNA and protein levels and no change in the secretion of TNF-α was observed after PFHpA treatment. PFHpA did also not induce NF-κB activation in IGE-stimulated RBL-2H3 cells. No significant effects on the ovalbumin-induced active systemic anaphylaxis was observed for PFHpA by measuring the rectal temperature in mice (J.-K. Lee & Kim, 2018).

PFHpA did significantly induce PPARα activity in HepG2 cells at a concentration of 30 µM (2-fold) and 100 µM (3-fold) (Rosenmai et al., 2018).

6.4.2 Relevant Human data

6.4.2.1 Data/studies reported in previous UBA evaluation

No relevant data was reported.

6.4.2.2 New data

Epidemiological studies of PFHpA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFHpA (ng/ml). Epidemiological studies that evaluated exposure-response relationship using untransformed PFHpA measures are described below.

In a cross-sectional study of maternal PFAS exposure and birth outcomes, (Gao et al., 2019) did not find any dose-response relationship between PFHpA measured in maternal serum or cord

serum and birth length, birth weight, gestational age or ponderal index. The study population included 132 pregnant women, with a mean age of 31.0 years, who delivered a child at a university hospital in Beijing during 2015 and 2016. Maternal blood samples for the exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. Median levels of PFHpA were 0.04 ng/ ml and 0.04 ng/ml in maternal serum and cord serum, respectively. A total of 14 PFAS were measured in blood and cord serum, including PFHpA.

(Kvalem et al., 2020) conducted a cross-sectional and longitudinal analysis of asthma and allergies in a birth cohort. Subjects in the cohort were born between January 1992 and March 1993 at two hospitals in Oslo, Norway for the Environment and Child Asthma Study. PFAS in serum were collected in 378 children at 10 years of age and diagnoses of asthma and allergies were measured at 10- and 16-years. Median serum PFHpA concentration at 10 years was 0.11 ng/ml. In the cross-sectional analysis, there were no exposure-response associations between PFHpA measured at 10 years and atopic dermatitis, asthma, rhinitis, or a positive skin prick test measured at 10 years (relative risk estimates were presented per IQR increase of 0.13 ng/ml PFHpA). Similarly, there were no statistically significant exposure-response associations between PFHpA exposure measured at 10 years and diagnoses of atopic dermatitis, rhinitis, or a positive skin prick test for 10-16 years or for asthma measured at 16 years. The results were adjusted for sex, maternal smoking in pregnancy, passive and active smoking status, parental atopy, maternal education, number of siblings, physical activity, BMI, and stage of puberty. This study measured 9 PFAS in blood serum, including PFHpA.

(H.-W. Lin et al., 2020) conducted a case-control study of paediatric germ cell tumours and maternal PFAS exposure. Cases were 42 patients less than 16 years old (mean age 29 months) diagnosed with germ cell tumours at a paediatrics hospital in Shanghai between 2014 and 2017. A total of 42 controls were recruited from patients diagnosed with mycoplasma/bacterial pneumonia and asthma at the same hospital and matched to cases on age and sex. Serum samples were collected for PFAS analysis one week after diagnosis. Among the cases and controls, median PFHpA concentrations were 0.268 ng/ml and 0.232 ng/ml, respectively. The odds ratio was 4.78 (95% CI 0.38–60.77) per increase of 1 ng/ml of PFHpA; however, this result was imprecisely estimated and not statistically significant as indicated by the wide 95% confidence interval that did not exclude 1.0. Other PFAS measured in this study included PFBS, PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFOSA.

(Reardon et al., 2019) studied plasma PFAS and thyroid hormones in 494 women from the Alberta Pregnancy Outcomes and Nutrition (APrON) longitudinal Canadian pregnancy cohort, 2009-2012. PFAS measurements were collected during the second trimester of pregnancy. Median PFHpA concentrations in blood was 0.08 ng/ml. Thyroid hormones were measured in the women at <13 weeks, 14-26 weeks, 27-40 weeks gestation as well as 3 months post-partum. PFAS concentrations were measured in blood during the second trimester. No exposure-response relationship was detected between PFHpA and thyroid hormones in the main analysis. The results were adjusted for maternal age, ethnicity, history of smoking, and diagnosed thyroid condition. A total of 25 PFAS were measured in this study.

(B. Wang et al., 2017) conducted a case-control study of endometriosis-related infertility in Chinese reproductive age women during 2014-2015. Cases were 157 women with surgically confirmed endometriosis and controls were 178 women who were seeking infertility treatment due to male reproductive dysfunction. Median concentrations of PFHpA in cases and controls were 0.09 ng/ml and 0.10 ng/ml, respectively. In an exposure-response analysis, endometriosis-related infertility was not associated with plasma PFHpA concentrations categorized by tertiles (2nd, >0.08–0.11 ng/ml, and 3rd tertiles >0.11–0.66 ng/ml compared to 1st tertiles, 0.04–0.08

ng/ml) after the results were adjusted for age, BMI, household income, and education. Other PFAS measured in the study included PFBS, PFHxS, PFOS, PFHpA, PFOA, PFNA, PFUnDA, and PFDoDA.

As can be seen from the results above, epidemiological studies that evaluated exposure-response relationships in relation to untransformed measures of PFHpA do not have data that clearly demonstrate an exposure-response relationship exists with an adverse health outcome. From these studies, there are no suitable data for identifying a POD for use in deriving a drinking water limit value for PFHpA.

6.5 Proposal for a (new) starting point for deriving a drinking water limit value

Based on the new data from the repeated dose study in mice (GLP guideline study), necrotic effects in the liver at the lowest dose in adult mice are considered to be adverse which results in a LOAEL of 0.5 mg/kg/day. However, since the dose spacing between 0.5 and 10 mg/kg/day is very large, the use of the LOAEL as a starting point would not be recommended. According to the ECHA guidance (ECHA, 2012) a LOAEL can only be used as a starting point in case of a very steep dose-response curve, which is not provided at this dose spacing. Therefore, the use of the BMD approach would be recommended, which is also preferred over the NOAEL/LOAEL approach according to ECHA. The $BMDL_{05}$ (lower confidence interval of the benchmark dose for a 5% effective level) is used by the Indoor Exposure Committee as a value “rather than” a NOAEL.

Basis for the calculation of the BMDL: Data of hepatocellular necrosis in male rats (ECHA, 2020a, p. 35) (Histopathological changes seen in F0 males), Benchmark analysis was conducted using PROAST online[3].

$BMDL_{05}$: 6.63 mg/kg bw/day (recommended)

$BMDL_{10}$: 8.32 mg/kg bw/dayAccording to the ECHA guidance, an assessment factor can be calculated as follows:

- AF of 1 for dose-response relationship when using the BMD_{10} concept
- AF of 2 to account for the time extrapolation (adjusting for subchronic 90-day exposure to chronic exposure).
- AF for interspecies differences based on an approximate half-live in humans, considering 62 days to 1.5 years (=548 days) compared to approximately 0.19 days in mice (AF = half-live humans/half-live days) for PFHpA (please refer to section 5.3.1)- AF of 2.5 for intraspecies toxicodynamic differences.
- AF of 10 to account for intraspecies differences (general population).

[3] https://proastweb.rivm.nl/Analysis/New

7 Toxicological evaluation of perfluorodecanoic acid (PFDA)

7.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 20 and Table 21.

Table 20: Chemical identity of perfluorodecanoic acid (PFDA, CAS 335-76-2)

Name	Perfluordecansäure
English Name	Perfluorodecanoic acid
Acronym	PFDA
Mol. Formula	C10HF19O2
Mol. Weight (g/mol)	514.084
CAS	335-76-2
EC	206-400-3

Table 21: Physicochemical properties of perfluorodecanoic acid (PFDA, CAS 335-76-2)

Properties	Value	Source
Density (g/cm^3)	1.76-1.82 (mod.)	(USEPA, 2020)
Melting point (°C)	79.7-82.9 (exp.)	(M. Zhang et al., 2020)
Boiling point (°C)	218 (exp.)	(Kauck & Diesslin, 1951)
Vapour Pressure (Pa)	0.23 (ext. at 25 °C)	(Kaiser et al., 2005)
Henry's Constant (Pa m^3/mol)	1.2 x 10^7 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	<1.6 (exp.)	(Vierke et al., 2013)
	2.6 (exp. at 25 °C)	(Moroi et al., 2001)
Log Koc	2.96 (±0.15) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	260 (exp. at 22.4 °C)	(Kaiser et al., 2006)
	2.1 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated, mod = modelled)

7.2 Quantitative toxicological assessments on human data and drinking water limits determined by other institutions

This chapter reviews drinking water limits for PFDA (Table 22). No quantitative toxicological assessments of PFDA on human data determined by other institutions were found.

Table 22: Drinking water limits determined for PFDA or sum of PFAS

Country/institution	PFDA limit value in drinking water (µg/l)	Comment	Source
Germany/UBA	0.1	Drinking water guide value	(UBA, 2017)
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFDA, drinking water guide value	(SFA, 2022)
Hawaii (USA)	0.004	Drinking water guideline level	(ECOS, 2022)
Wisconsin (USA)	0.3	Drinking water guideline level	(ECOS, 2022)
Massachusetts/ Maine (USA)	0.020	Sum of 6 PFAS including PFDA, drinking water guideline level	(ECOS, 2022)

7.3 Toxicokinetics

7.3.1 Animal data

7.3.1.1 Data/studies reported in previous UBA evaluation

The half-life after a single injection of 48.64 mmol/kg body weight was measured in Wistar rats as 39.92 ± 8.62 d for males and 58.57 ± 5.84 d for females; the difference between the sexes was statistically significant (Ohmori et al., 2003).

7.3.1.2 New data

The major route of excretion of PFDA is renal elimination and to a smaller extent biliary and faecal excretion (ECHA 2018). Serum elimination half-lives for PFDA have been reported in rats and mice. In humans the half-life in males and females were 12 years and 4.5 years, respectively. In rats, serum half-lives were 40 and 59 days in males and females, respectively.

Male and female Sprague Dawley rats were administered a single dose of PFDA of 2, 10, or 20 mg/kg via oral gavage, or 2 mg/kg bw intravenous (n = 3/sex/various time points) (Dzierlenga et al., 2020). Plasma concentration profiles were best described using a two-compartment model and are presented in Table 23 below. Following single injections in male rats the Cmax/dose was 3.65 mM/mmol/kg, α and β half-lives were 27 and 854 hours, AUC/dose was 1875 mM/h/mmol/kg, CL was 0.534 ml/h/kg and V1 and V2 were 274 and 355 ml/kg. In female rats following injection of PFDA, the AUC/dose was higher at 3065 mM/h/mmol/kg and V2 was lower at 186 ml/kg, but other parameters were comparable to male rats. Following oral gavage administered 2 mg/kg by oral gavage, male rats reached a Cmax/dose of 3.76 mM/mmol/kg by 8.27 hours. The initial (α) and terminal (β) half-lives were 175 and 1620 hours, AUC was 3220mM_h/mmol/kg, CL was 0.310 ml/h/kg, and V1 and V2 were 259 and 327 ml/kg. In female

rats administered 2 mg/kg the Cmax/dose was higher (5.20 mM/mmol/kg), as was the AUC (5200mM_h/mmol/kg), with slower CL(0.192 ml/h/kg) and V1 and V2 (189 and 88 ml/kg). Both males and females exhibited a bioavailability of 170% (estimated by dividing the dose-normalised gavage AUC by the dose-normalised iv AUC), which the authors suggest may be due to increased reabsorption by intestinal transporters. Concentrations of PFDA were highest in the liver of male and female rats administered 10 mg/kg via oral gavage, and the lowest concentrations were noted in the brain. In the male rats there was a steady increase in the liver: plasma ratio (>4 after 36 hours), while in females the maximum liver: plasma ratio was 3. Kidney: plasma ratios in both male and females ranged from 0.5 to 1.0. Also, in both males and females, PFDA was poorly distributed to the brain, with brain: plasma ratios of <0.1.

(Eryasa et al., 2019) evaluated the partitioning of PFDA detected in maternal serum, umbilical cord sera and cord whole blood in 151 matched mother-child pairs from two Faroese birth cohorts. Cord: maternal serum (transplacental transfer) and serum: whole cord blood (blood partitioning) rations were estimated for PFDA. The median transplacental transfer ratio for PFDA was 0.36. Overall, gestational diabetes was a strong predictor of transplacental transfer ratio, with significantly higher transplacental transfer and blood partitioning in mothers with gestational diabetes.

(S.-J. Kim et al., 2019) developed and validated pharmacokinetic (PBPK) models for detecting PFDA toxicokinetics in male and female rats and applied the model to humans to explore potential pharmacokinetic sex differences. In order to develop the PBPK model, male and female rats (5/sex/group) were given a dose of 1 mg/kg PFDA via oral or intravenous administration. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 (h), and at 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 days. A PBPK model for PFDA was developed by adapting the methodology of a model previously developed by the authors for PFHxS. General pharmacokinetic and pharmacodynamic properties of PFAS were considered to develop a PBPK model for PFDA, which consisted of compartments for blood, filtrate, storage, tissues interconnected via blood flow, and a storage compartment for the path from liver to faces (the major excretion route of PFDA). The major tissue compartments considered for the model were the GI tract (absorption site), liver (accumulative site), kidney (a renal reabsorption by filtration compartment). Menstrual blood loss was also considered as one of the excretion routes for developing the PBPK model. Results indicated gender differences in the elimination half-life and volume of distribution in rats. The tissue–plasma partition coefficients were the highest in the liver in both male and female rats. The PBPK model for PFDA in male and female rats was extrapolated to a human PBPK model based on human physiological parameters. The external doses were calculated at 3.35 ng/kg/day (male) and 17.0 ng/kg/day (female) for PFNA and 0.530 ng/kg/day (male) and 0.661 ng/kg/day (female) for PFDA. The authors conclude that the results of this study provide valuable insight into human health risk assessment regarding PFDA exposure.

Table 23: Pharmacokinetic Properties of PFDA as reported by (Dzierlenga et al., 2020)

Parameter	Male				Female			
	iv	gavage			iv	gavage		
	2 mg/kg	2mg/kg	10 mg/kg	20 mg/kg	2 mg/kg	2mg/kg	10 mg/kg	20 mg/kg
C_{max}[a] (mM)	0.0142 ± 0.00144	0.0146 ± 0.0007	0.0825 ± 0.0053	0.158 ± 0.007	0.0163 ± 0.0024	0.0202 ± 0.0012	0.107 ± 0.006	0.241 ± 0.019

Parameter	Male				Female			
	iv	gavage			iv	gavage		
	2 mg/kg	2mg/kg	10 mg/kg	20 mg/kg	2 mg/kg	2mg/kg	10 mg/kg	20 mg/kg
C_{max}/Dose (mM/mmol/kg)	3.65 ± 0.37	3.76 ± 0.18	4.24 ± 0.27	4.07 ± 0.18	4.20 ± 0.62	5.20 ± 0.30	5.52 ± 0.32	6.20 ± 0.50
T_{max}[a] (h)	NA	8.27 ± 0.63	9.06 ± 0.85	10.0 ± 0.06	NA	9.01 ± 0.80	9.29 ± 0.88	10.8 ± 1.2
$\alpha T_{1/2}$ (h)	27 ± 17	175 ± 31	123 ± 40	111 ± 24	5.92 ± 4.64	295 ± 110	298 ± 116	226 ± 81
$\beta T_{1/2}$ (h)	854 ± 61	1620 ± 220	995 ± 80	1070 ± 60	904 ± 83	1240 ± 290	1260 ± 330	1240 ± 270
K_{10} $T_{1/2}$(h)	356 ± 44	579 ± 40	478 ± 38	485 ± 27	506 ± 81	685 ± 50	681 ± 55	569 ± 55
AUC_{∞}[a] (mM/h)	7.29 ± 0.43	12.53 ± 0.56	58.7 ± 2.3	115 ± 3	11.9 ± 0.9	20.2 ± 1.0	107 ± 6	202 ± 12
AUC_{∞}/Dose (mM/h/mmol/kg)	1875 ± 110	3220 ± 145	3020 ± 120	2960 ± 80	3065 ± 225	5200 ± 250	5500 ± 290	5200 ± 300
CL (ml/h/kg)	0.534 ± 0.031	0.310 ± 0.014	0.331 ± 0.013	0.338 ± 0.009	0.327 ± 0.024	0.192 ± 0.009	0.182 ± 0.010	0.192 ± 0.010
V_1[b] (ml/kg)	274 ± 28	259 ± 13	228 ± 16	236 ± 12	238 ± 35	189 ± 11	178 ± 11	158 ± 13
V_2[c] (ml/kg)	355 ± 69	327 ± 44	183 ± 30	220 ± 23	186 ± 57	87.9 ± 23.9	85.8 ± 25.3	112 ± 27
F[d] (%)	NA	172	161	158	NA	170	179	170

NA, not applicable; C_{max}, peak concentration; C_{max}/dose, dose-corrected peak concentrations; AUC, Area under the curve; CL, clearance; $\alpha T_{1/2}$ initial half- life; $\beta T_{1/2}$, terminal half-life; $K_{10} T_{1/2}$ elimination half-life; V_1, apparent volume of central distribution; V_2, apparent volume of peripheral distribution; F, bioavailability (calculated by dividing the dose adjusted gavage AUC by the iv AUC).

[a] Predicted using two compartment model

[b] Volume of distribution for the central compartment

[c] Volume of distribution for the peripheral compartment

[d] Estimated by dividing dose-normalized gavage AUC by dose-normalized iv AUC

7.3.2 Human data

7.3.2.1 Data/studies reported in previous UBA evaluation

No relevant data was found.

7.3.2.2 New data

No relevant data was found.

7.4 Health effects in humans and/or animals

7.4.1 Relevant Animal data

7.4.1.1 Data/studies reported in previous UBA evaluation

7.4.1.1.1 Repeated dose toxicity

No relevant data was found.

7.4.1.1.2 Carcinogenicity

No relevant data was found.

7.4.1.1.3 Mutagenicity

No relevant data was found.

7.4.1.1.4 Toxicity to reproduction

Only one in vivo toxicity study is reported in the existing UBA assessment. In this study, (Harris and Birnbaum, 1989) administered PFDA to pregnant mice on gestation days 6 through 15 at doses of 0, 0.03, 0.3, 1, 3, 6.4, or 12.8 mg/kg/day. Treatment with PFDA resulted in significant decreases in maternal body weight on gestation day 18 at the highest dose tested and significantly increased liver weights at 1 mg/kg/day. Resorption rates per litter were increased 19.1% at 6.4 mg/kg/day and 41.7% at 12.8 mg/kg/day. Foetal body weights were significantly decreased in offspring of dams treated with 1 mg/kg/day and higher, and the number of live foetuses was significantly decreased at the highest dose tested.

7.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

The existing UBA assessment identified several *in vitro* studies for PFDA investigating genotoxicity (Buhrke et al., 2013), oxidative stress and DNA damage (Wielsøe et al., 2015); activation of peroxisome proliferator-activated receptor α (PPARα) (Corsini et al., 2012; Kudo et al., 2000; Wolf et al., 2008, 2012). (Buhrke et al., 2013) reports no significant increases in the number of revertants following *in vitro* genotoxicity tests conducted in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA 1538 at a concentration of 5 µmol/plate both with and without metabolic activation. (Wielsøe et al., 2015) reported that PFDA increased reactive oxygen species (ROS) formation in human liver cells but produced only weak DNA damage in the comet assay.

Results of *in vitro* studies in human peripheral blood lymphocytes and human leukaemia cells (THP-1) indicated PFDA inhibited tumour necrosis factor -α, interleukins-6 and -10 and interferon-γ (Corsini et al., 2012). In THP-1 cells PFDA inhibited NF-κB activation (a specific transcription factor) and prevented I-κB degradation (I-κB as an enzyme complex is part of the NF-κB transduction cascade), but did not activate the peroxisome proliferator-activated receptor α (PPARα)In addition, results of in vitro studies indicated PFDA induced the formation of 17β-oestradiol in a human adrenal cortical carcinoma cell line (NCI-H295R); however, progesterone and testosterone levels were not affected and PFDA did not react in reporter gene tests with human androgen or Ah receptors (Rosenmai et al., 2014). (Kjeldsen & Bonefeld-Jørgensen, 2013) reported that PFDA did not show any oestrogenic effects in oestrogen-sensitive gene-transformed breast carcinoma cells but PFDA acted as a concentration-dependent antagonist in the androgen receptor (AR) transactivation assay.

According to the existing UBA assessment there were no studies relevant to the assessment with subchronic or prolonged oral exposure. Therefore, it was not possible to derive a TWLW based on human toxicology. In the existing UBA assessment a GOW of 0.1-0.3 µg/l was recommended

for PFDA according to the available data (*in vitro* genotoxicity negative, *in vitro* immunotoxicity positive), estimated according to the GOW concept (Grummt et al., 2013; UBA, 2003). The GOW of 0.1 µg/l was recommended on the basis of the proposed classification as suspected carcinogen, supported by the proposed classification as toxic to reproduction in category Repr. 1B.

7.4.1.2 New data

7.4.1.2.1 Repeated dose toxicity

No relevant data was found

7.4.1.2.2 Carcinogenicity

No relevant data was found

7.4.1.2.3 Mutagenicity

No relevant data was found

7.4.1.2.4 Toxicity to reproduction

No relevant data was found

7.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

To assess the immunotoxicity of PFDA, female Harlan Sprague-Dawley rats were administered PDFA via oral gavage daily at doses of 0, 0.125, 0.25, 0.5, 1, 2 mg/kg/day (Frawley et al., 2018). In addition, female B6C3F1/N mice were administered PFDA via oral gavage once per week for 4 weeks at doses of 0, 0.3125, 0.625, 1.25, 2.5 or 5 mg/kg/week. Both rats and mice were evaluated for effects on immune cell population in spleen and bone marrow, as well as innate, humoral-, and cell-mediated immunity. In addition, mice were evaluated for resistance to the Influenza virus. In rats, treatment-related hepatic necrosis and enlargement of the liver were reported at doses of 0.5 mg/kg/day and higher after exposure for 28 days. In mice, significant liver enlargement was reported at weekly doses of 0.625 mg/kg/day and higher. Also in rats, phagocytosis by fixed tissue macrophages was significantly decreased in the liver at 0.25 mg/kg/day and higher. Immune cell populations were significantly decreased in mice at doses of 1.25 mg/kg/day and higher in mice. In both rats and mice, PFDA-induced effects on humoral- and cell-mediated immunity, host resistance, and bone marrow progenitor cells were limited. The authors concluded that exposure to PFDA may induce adverse effects in the rat liver in a manner consistent with other PFAS chemicals and may alter the balance of T-cell and T-helper cell populations in lymphoid tissues in mice. Based on the results of this study, a NOAEL of 0.125 mg/kg/day in rats following subacute exposure to PFDA could be assumed based on treatment related effects in the liver, as well as immunotoxicity reported in rats at higher doses.

7.4.2 Relevant Human data

7.4.2.1 Data/studies reported in previous UBA evaluation

The previous UBA states that overviews of the human toxicological data on PFDA can be found in (ATSDR, 2009), (Bull et al., 2014) and (SCA, 2015).

7.4.2.2 New data

Epidemiological studies of PFDA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFDA (ng/ml). Studies that provided results in relation to continuous or categorized measures of untransformed PFDA concentrations in blood are discussed below.

(Averina et al., 2021) reported an increased odds ratio of dyslipidaemia in relation to PFDA in a cross-sectional study of 940 adolescents in Norway during 2010-2011. In this study dyslipidaemia was defined as total cholesterol ≥5.17 mmol/l and/or low density lipoprotein (LDL) cholesterol ≥3.36 mmol/l and/or apolipoprotein (apo) B ≥ 1.10 g/l. The geometric mean PFDA in blood serum was 0.27 ng/ml and 0.19 ng/ml in girls and boys, respectively, and the difference in means was statistically significant. After adjusting for age, sex, and diet, Averina et al. (2021) reported that higher serum PFDA was statistically significantly associated with dyslipidaemia for PFDA concentrations greater than or equal to 0.158 ng/ml (Odds ratio [OR] 2.34, 95% CI 1.08 – 5.05 for those exposed to 0.158–0.207 ng/ml; OR 2.09, 95% CI 1.01 – 4.74 for those exposed to 0.208–0.288 ng/ml; and OR 2.36, 95% CI 1.08-5.16 for those exposed to 0.289 – 1.89 ng/ml) when compared to those exposed to 0.016–0.157 ng/ml. A statistical test for trend was not reported. The cross-sectional design measured PFAS and biomarkers of dyslipidaemia concurrently. Such a study design cannot evaluate causal relationships. It is not known whether the reported associations between PFAS exposure and lipid metabolism are causal or if an underlying biological process explains the reported associations (for example, enterohepatic cycling of bile acids that affects both cholesterol levels and PFAS levels, as reported by (EFSA, 2020).

(Bach et al., 2018) studied a subset of 1,251 women from the longitudinal Danish National Birth Cohort, recruited during 1996-2002. Median serum PFDA in this cohort was 0.2 ng/ml; after adjusting for age, pre-pregnancy BMI, socioeconomic status, and interpregnancy interval (among parous women only), the authors reported no exposure-response associations between quartiles of plasma PFDA and fecundability ratio among either nulliparous or parous women (highest quartile of PFDA was 0.23-0.87 ng/ml compared to the lowest quartile of PFDA, <0.14 ng/ml).

(Blake et al., 2018) examined the association of several indicators of chronic disease among 210 members of the Fernald Community Cohort in Ohio, who were potentially exposed to PFAS-contaminated drinking water. Study participants were recruited from a medical surveillance program for residents who may have been exposed to uranium dust from living within five miles of a former US Department of Energy uranium processing site. The study participants were selected based on low exposure to uranium (not exposed to uranium above background levels). The study participants were followed longitudinally from 1990-2008. The median serum PFDA in this cohort at first measurement was 0.10 ng/ml. Adjusting for sex, year of measurement, age, income, education, marital status, and BMI, Blake et al. (2018) found no association between PFDA in serum and changes in thyroid hormones (TSH 11%, 95% CI -4.45% to 28.8% and Total T4 per 0.13 ng/ml PFDA (the interquartile range). A total of 8 PFAS were measured in serum: PFOA, PFNA, PFDA, PFOS, PFHxS, MePFOSA, PFOSA, and EtPFOSA.

(W. Cao et al., 2018) performed a longitudinal analysis of PFAS in umbilical cord blood and possible associations with gestational and postnatal growth among 337 mother-child pairs recruited in China from 2013-2015. Median cord serum level of PFDA was 0.1 ng/ml (25th percentile, 0.06; 75th percentile, 0.15). Preferred participants with ≥3 available serum samples collected in different calendar years were used. After adjusting for infant sex, maternal age, income, parity, and some paternal factors (depending on analysis), Cao et al. (2018) reported no exposure-response associations between PFDA and birth weight, birth length or ponderal index, or postnatal growth (weight, length and head circumference measured at follow up appointment during the first year after birth). This study measured 11 PFAS in cord serum: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFHxS, PFOS, and PFDS.

(J. V. R. Christensen et al., 2021) examined PFAS exposure and anogenital distance among 463 mother-child pairs in the longitudinal Faroese Birth Cohort 5, originally recruited from 2007-

2009. The mean PFDA concentration in maternal serum, measured two weeks after expected term date, was 0.3 ng/ml. Adjusting for parity, maternal education, and child's weight and age at examination, the authors found no association between maternal PFDA and anogenital distance in girls. Among boys, PFDA was associated with a 1.3 mm increase in anogenital distance (95% CI 0.3 – 2.4) in the fourth quartile of exposure compared to the lowest quartile.

(Gao et al., 2019) conducted a cross-sectional study of maternal PFAS exposure and birth outcomes. The study population included 132 pregnant women with a mean age of 31 years who visited the Affiliated Hospital of Capital Medical University in Beijing for delivery between 2015 and 2016. Maternal blood samples for exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. The median concentration of PFDA was 0.47 ng/ml and 0.21 ng/ml in maternal serum and cord serum, respectively. Models were adjusted for maternal age, height, weight, BMI, and gestational age, depending on the analysis. Neither maternal serum PFDA (highest tertile >0.55 ng/ml) nor cord serum PFDA (highest tertile >0.259 ng/ml) were statistically significantly associated with birth weight, birth length, gestational age, or ponderal index.

(M. H. Harris et al., 2021) assessed PFAS exposure in a cross-sectional study, including PFDA, in mother-child pairs who were recruited for a birth cohort study, Project Viva, in Massachusetts. Blood samples were collected from pregnant mothers during 5 to 21 weeks gestation, with a median of 10 weeks. A total of 1,116 children aged 6 to 10 years old participated in follow up visits between 2007 and 2010 to assess behaviour and executive function abilities. Of these included children, blood samples were collected from 702 during these visits. Because more than 30% of maternal PFDA samples were below the limit of detection (LOD, 0.1 ng/ml), only PFDA in child samples was analysed. Childhood PFDA concentrations showed a median of 0.3 ng/ml. Higher levels of PFDA in children were associated with higher parent-rated scores for behavioural problems. Compared to the first quartile (PFDA <0.2 ng/ml), the study investigators reported that the fourth quartile (PFDA ≥0.5 ng/ml) of PFDA plasma concentration showed a borderline statistically significant increase in behavioural problems (β=1.1, 95% CI 0.1 – 2.1). However, there was no exposure-response association reported for teacher-rated scores of behavioural problems in relation to PFDA. Results were adjusted for year of blood draw, maternal race/ethnicity, age, education, parity, KBIT-2 score (a measure of maternal IQ), paternal education, annual household income, HOME-SF score (a questionnaire-based measure of cognitive stimulation and emotional support in the child's home), child's sex and age at mid-childhood behavioural assessment, and breastfeeding duration.

(Inoue et al., 2019) conducted a cross-sectional study of PFAS exposure, including PFDA, and thyroid hormones in pregnant women. Subjects were recruited from the Danish National Birth Cohort, which enrolled pregnant women at their first antenatal care visit in Denmark between 1996 to 2002. Plasma samples were collected at entry for 1,366 pregnant women between 5- and 19-weeks gestation (median, 8 weeks gestation). The median PFDA concentration was 0.17 ng/ml. After adjusting for age, SES, BMI before pregnancy, parity, smoking status, and birth year, the study investigators found no association between PFDA and thyroid stimulating hormone (TSH) or free thyroxine (fT4) when PFDA was analysed as a continuous variable (per IQR increase of 0.09 ng/ml) or when analysed as a categorical variable (PFDA concentrations 0.13 - <0.17, 0.17 - <0.22, or ≥0.22 ng/ml compared to maternal PFDA concentrations < 0.13 ng/ml).

(Jensen et al., 2020) conducted a cohort study of prenatal PFAS exposure and markers of adiposity and plasma lipids in infants. Subjects were recruited from the Danish-based Odense Child Cohort which enrolled pregnant women in Southern Denmark at less than 16 weeks gestation between 2010 and 2012. Maternal serum samples were collected during pregnancy to measure PFAS exposure. The median maternal PFDA serum concentration was 0.26 ng/ml.

Infant BMI, ponderal index, and waist circumference were measured or calculated at birth. Lipid assays were performed using non-fasting blood samples collected at 3 and 18 months of age in the children. In total, 613 mother-child pairs had available data for adiposity markers at birth, using calculated standard deviation scores (SDSs) for body mass index (BMI), waist circumference, and ponderal index. Of the 613 with adiposity markers, 84 had data for lipid biomarkers (using calculated standard deviation scores for total cholesterol, LDL, HDL, and triglycerides) at both 3 and 18 months. When adiposity measurements at birth, 3 months, and 18 months were pooled, PFDA was associated with increased BMI SDS (β=0.42 per ng/ml increase, 95% CI 0.01 – 0.84) and increased ponderal index SDS (β=0.60 per ng/ml increase, 95% CI 0.18 – 1.02), but was not associated with waist circumference. When the three time points were analysed separately, PFDA was only significantly associated with increased ponderal index SDS at 3 months (β=0.61 per ng/ml, 95% CI 0.14 – 1.07) and 18 months (β=0.59 per ng/ml, 95% CI 0.07 – 1.10). When stratified by sex, only ponderal index SDS in females remained significant (β=1.02 per ng/ml, 95% CI 0.40 – 1.64). PFDA was significantly associated with increased body fat percentage SDS at 3 months (β=0.40 per ng/ml, 95% CI 0.04 – 0.75) but not at 18 months and not when stratified by sex. PFDA was significantly associated with increased total cholesterol SDS at 18 months (β=1.06 per ng/ml, 95% CI 0.08 – 2.03) but not at 3 months and not with LDL SDS, HDL SDS, or triglycerides SDS. When stratified by sex, PFDA was significantly associated with increased total cholesterol SDS in females (β=1.29 per ng/ml, 95% CI 0.17 – 2.42), increased LDL SDS in girls (β=1.51 per ng/ml, 95% CI 0.34 – 2.68), and increased triglycerides SDS in males (β=4.44 per ng/ml, 95% CI 2.37 – 6.53). These results were adjusted for maternal age at delivery, maternal parity, BMI before pregnancy, maternal smoking status, breastfeeding duration, and maternal education.

(Kvalem et al., 2020) studied 378 children who participated in the Environment and Child Asthma cohort based in Oslo, which initially recruited healthy newborns born during 1992 and 1993. The present study included a cross-sectional analysis at the 10-year follow up and a longitudinal analysis at 16-year follow up. The authors examined associations of 19 PFAS with asthma, allergies, lung function, and airway infections. Median serum PFDA was 0.18 ng/ml. The authors adjusted for BMI, puberty status, physical activity, maternal education, and age, depending on analysis. The authors found no consistent associations between PFDA at 10 years and diagnoses of atopic dermatitis or rhinitis, positive skin-prick test, or lung function, at 10-year or at 16-year follow-up.

PFDA serum at 10 years was associated with a reduced risk of atopic dermatitis in female children at 16 years (RR=0.45 per IQR, 95% CI 0.29 – 0.69, IQR=0.13 ng/ml). No other associations were seen between PFDA at 10 years and asthma, rhinitis, or a positive skin-prick test.

(H.-W. Lin et al., 2020) conducted a case-control study of PFAS exposure and paediatric germ cell tumours (GCT) among 42 cases and 42 sex- and age-matched controls recruited from 2014-2017 in Shanghai, China. Among the cases, who had a mean age of 29 months, the median serum PFDA concentration was 0.794 ng/ml; among the controls, who had a mean age of 22 months, median serum PFDoDA was 0.641 ng/ml. Adjusting for maternal factors during pregnancy such as cosmetics use and living near farmland, the authors reported no exposure-response association between PFDA and increased odds of paediatric germ cell tumours. This study assessed 10 PFAS including PFDA.

(Petersen et al., 2022) conducted a cross-sectional analysis to examine plasma PFAS and male reproduction biomarkers, including semen quality, testicular volume, and reproductive hormones in 1,041 Danish men. The men were recruited between 2017 and 2019 as part of the Foetal Programming of Semen Quality (FEPOS) cohort. Median PFDA concentration was 0.18

ng/ml. No clear associations were detected between PFDA and measures of semen quality or testicular volume. For reproductive hormones, the highest tertile (0.22-1.73 ng/ml) of PFDA was associated with a 12% increase in FSH levels (95% CI 2% - 23%) compared to the lowest tertile (0.06-0.15 ng/ml) in a model adjusted for personal smoking status, maternal smoking status, body mass index, family's occupational status during pregnancy, time of day for blood sample collection, and batch for PFAS analyses.

(Shearer et al., 2021) conducted a nested case-control study to examine serum PFAS and risk of renal cell carcinoma. A total of 324 cases and 324 controls matched on age at enrolment, sex, race and ethnicity, study centre, and year of blood draw were identified from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, a study of 55–74-year-old adults from 10 US cities recruited between 1993 and 2001. In logistic regression models, PFDA was categorized as ≤LOD (≤0.1 ng/ml), >0.1-0.2, >0.2-0.3, and >0.3-2.1 ng/ml. The distributions of other PFAS were based on quartiles of serum concentration in controls; however, the authors reported that cut points for PFDA were not based on quartiles because more than a quarter of PFDA measurements were below the limit of detection. There was no exposure-response relationship observed between renal cell carcinoma and PFDA categories of increasing exposure when compared to the referent group (PFDA ng/ml < 0.1 ng/ml (limit of detection[LOD]) (p for trend=0.20). The results were adjusted for body mass index, smoking, hypertension history, estimated glomerular filtration rate, previous freeze-thaw cycle, and year of blood draw. A total of 10 PFAS were measured in this study.

(Timmermann et al., 2022) conducted a cross-sectional study among Greenlandic children ages 7-12 to investigate whether exposure to endocrine-disrupting chemicals, including seven PFAS, was associated with changes in diphtheria and tetanus vaccine antibodies after receiving immunization. The authors measured seven PFAS, including PFDA, among children at a median age of 9.9 years; median serum PFDA was 0.49 ng/ml. After adjusting for time since vaccination, breastfeeding duration, and area of residence, the authors observed no significant association between tetanus or diphtheria antibody concentrations and child serum PFDA. Among a subset of 175 children with known vaccination records, after adjusting for area of residence and duration of breastfeeding, the study investigators found that the association between PFDA and the odds of not being protected against diphtheria (as measured by antibody concentrations < 0.1 IU/ml) were statistically significant (OR 5.08, 95% CI 1.32-19.51 per ng/ml increase). Other PFAS measured in this study included PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA, and PFUnDA.

(B. Wang et al., 2017) conducted a case-control study of endometriosis-related infertility in Chinese reproductive age women during 2014-2015. Cases were 157 women with surgically confirmed endometriosis and controls were 178 women who were seeking infertility treatment due to male reproductive dysfunction. Median concentrations of PFDA in cases and controls were 1.29 ng/ml and 1.34 ng/ml, respectively. In an exposure-response analysis, endometriosis-related infertility was not associated with plasma PFDA concentrations categorized by tertiles (2nd, >0.95–1.79 ng/ml, and 3rd tertiles >1.79–11.2 ng/ml compared to 1st tertile, 0.09–0.95 ng/ml) after the results were adjusted for age, BMI, household income, and education. Other PFAS measured in the study included PFBS, PFHxS, PFOS, PFHpA, PFOA,PFNA, PFUnDA, and PFDoDA.

(Wikström et al., 2020) studied a cohort of 1533 mother-child pairs in the Swedish Environmental, Longitudinal, Mother and child, Asthma and allergy (SELMA) study to assess associations between PFAS exposure in maternal serum measured during first trimester of pregnancy (median, 10 weeks) and birth outcomes such as birth weight (BW), birth weight for gestational age, and birth weight small for gestational age (SGA). The median PFDA in blood serum was 0.26 ng/ml. Maternal PFDA concentrations >0.34 ng/ml (4th quartile) compared to

maternal PFDA concentrations <0.19 ng/ml (1st quartile) were associated with a decrease in birthweight of 58 grams (95% CI: -89, -4). These results were adjusted for sex, gestational age, maternal weight, parity, and cotinine level. In sex-stratified analyses, a statistically significant decrease in birthweight was seen in girls (-116 g, 95% CI -204 to -27) but not boys (-27 g, 95% CI -118 to 64). The authors concluded that there was a significant effect by the PFAS analysed on birth weight related outcomes in girls but not in boys. A total of 8 PFAS were measured in the study.

Table 24: Point of Departure (POD) for PFDA derived from epidemiological studies

Reference	Finding	POD (ng/ml)	External dose (ng/kg-d)	Other PFAS measured
Averina et al. (2021)	Dyslipidemia, 940 Adolescents, Norway PFDA geometric mean boys, 0.19 ng/ml girls, 0.27 ng/ml p<0.0001 for difference in means between sexes	>0.158	Not reported	PFBS, PFPS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS, PFOSA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA
Wikstrom et al. (2019)	Decreases in birth weight 1533 mother-child pairs, Sweden PFDA median, maternal serum, 1st trimester, 0.26 ng/ml	>0.34	Not reported	PFOS, PFOA, PFHxS, PFNA, PFDA, PFUnDA, PFHpA, PFDoDA

7.5 Proposal for a (new) starting point for deriving a drinking water limit value

7.5.1 Selected study

In the previous UBA assessment it was not possible to derive a TWLW based on human toxicology and a GOW of 0.1-0.3 µg/l was recommended for PFDA according to the available data (*in vitro* genotoxicity negative, in vitro immunotoxicity positive), estimated according to the GOW concept (Grummt et al., 2013; UBA, 2003). The GOW of 0.1 µg/l was recommended on the basis of the proposed classification as suspected carcinogen, supported by the proposed classification as toxic to reproduction in category Repr. 1B.

Based on new data from a subacute study conducted in rats and mice, the starting point for a TWLW is the lowest NOAEL of 0.125 mg/kg/day in rats based on the results of the study conducted by Frawley et al. (2018).

7.5.2 Proposal of assessment factors/modification factors with justification

An assessment factor (ECHA 2010) of overall 12,600 is proposed with the following justifications for its derivation:

- AF of 6 to account for the time extrapolation (adjusting for subacute 28-day exposure to chronic exposure).

- AF of 84 based on an approximate half-life in humans of 4,380 days (ATSDR 2021) compared to approximately 52 days in rats (Dzierlenga et al. 2020) (4380 days/52 days) derived for PFDA for intraspecies toxicokinetic differences.
- AF of 2.5 for interspecies toxicodynamics differences.
- AF of 10 to account for intraspecies differences (general population).

This would result in a total factor of 12,600 and a human-related tolerable dose of 10 ng/kg bw x d.

8 Toxicological evaluation of perfluoroundecanoic acid (PFUnDA)

8.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 25 and Table 26.

Table 25: Chemical identity of perfluoroundecanoic acid (PFUnDA, CAS 2058-94-8)

Name	Perfluorundecansäure
English Name	Perfluoroundecanoic acid
Acronym	PFUnDA
Mol. Formula	C11HF21O2
Mol. Weight (g/mol)	564.085
CAS	2058-94-8
EC	218-165-4

Table 26: Physicochemical properties of perfluoroundecanoic acid (PFUnDA, CAS 2058-94-8)

Properties	Value	Source
Density (g/cm^3)	1.76-1.85 (mod.)	(USEPA, 2020)
Melting point (°C)	67.7-101.2 (exp.)	(M. Zhang et al., 2020)
Boiling point (°C)	160 (exp.)	(Sigma-aldrich, n.d.)
Vapour Pressure (Pa)	0.10 (ext. at 25 °C)	(Kaiser et al., 2005)
Henry's Constant (Pa m^3/mol)	3.8 x 10^7 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	<1.6 (exp.)	(Vierke et al., 2013)
	3.12 (exp. at 30 °C)	(Moroi et al., 2001)
Log Koc	3.56 (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	92.3 (exp. at 21,9 °C)	(Kaiser et al., 2006)
	0.60 (exp. at 25 °C)	(Inoue et al., 2012)
	0.5 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated, mod = modelled).

8.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFUnDA (Table 27). Data was identified by general desk research. Only sum values for PFAS including PFUnDA were found. No quantitative toxicological assessments of PFUnDA on human data determined by other institutions were found.

Table 27: Drinking water limits determined for PFUnDA or sum of PFAS

Country/institution	PFUnDA limit value in drinking water (µg/l)	Comment	Source
Sweden/Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFUnDA, drinking water guide value	(SFA, 2022)
Hawaii (USA)	0.010	Drinking water guideline level	(ECOS, 2022)
Wisconsin (USA)	3	Drinking water guideline level	(ECOS, 2022)

8.3 Toxicokinetics

8.3.1 Animal data

8.3.1.1 New data

An overview of the data on PFAS in general can be found in (Bull et al., 2014); no information for PFUnDA on toxicokinetics, toxicology in laboratory animals was found.

Information about ADME of PFUnDA was found in the Agency for Toxic Substances and Disease Registry (ATSDR) report (ATSDR, 2021).

It was reported that quantitative estimate of the fractional absorption of orally administered perfluoroalkyls in animals was >95% for PFUnDA. In male and female mice, comparison of the 24-hour area under the curve (AUC) for oral and intravenous administration showed that 90–100% of the oral dose was absorbed for PFUnDA as cited in (ATSDR, 2021).

PFUnDA may accumulate preferentially in the liver and faecal excretion is predominant route of elimination for PFUnDA (EFSA, 2020).

Guruge *et al* (2016) investigated pharmacokinetics of PFUnDA given as a part of mixture of 10 PFAAs to Micromini pig (MMPig). Three female MMPigs received orally a single gelatine capsule filled with a nominal mixture of 30 mg kg bw of each of the 10 PFAAs (including PFUnDA), while control MMPigs were given a capsule without PFAAs. The mean elimination half-live ($t^{1/2}$) for PFUnDA was 38.5 days in female micromini pig blood. The liver was the greatest site of accumulation of PFUnDA with mean concentration of 18 420 ± 4870 ng/g at 21 days after a single oral exposure. PFUnDA was also found to accumulate in kidney, spleen and muscle tissues (Guruge et al., 2016).

8.3.2 Human data

8.3.2.1 New data

The renal clearances for PFUnDA in humans (three males and five females) was found to be 0.005±0.00 ml/day/kg; mean±SD as cited in (ATSDR, 2021).

In (EFSA, 2020) was also reported that urinary clearance decreased as a function of chain length, from 0.67 ml/day/kg day for PFHpA to 0.005 ml/day/kg for PFUnDA.

European Food Safety Authority (EFSA) scientific opinion regarding risk to human health related to the presence of perfluoroalkyl substances in food reported some information about ADME of PFUnDA (EFSA, 2020). Half-life of PFUnDA in human (young females and in a group of males and older females) were 4.0 years/7.4 years respectively (EFSA, 2020).

PFUnDA can bind to human serum albumin, among PFCA, the binding strength follows the order PFHpA < PFOA = PFNA < PFDA > PFUnDA (EFSA, 2020).

Median serum (or plasma) to whole blood ratio in human of PFHxS, PFOS, PFOAPFNA and PFUnDA was approximately 2 (EFSA, 2020).

Available total clearance value in humans (sum of urinary and biliary clearance) for PFUnDA was estimated at approximately 0.07 ml/kg per day (EFSA, 2020).

Information about half-life of PFUnDA in human was found in RAC Opinion (ECHA, 2018). It was reported that serum elimination half-lives of PFUnDA (C11 PFCA) in humans were found to be 12 years in males and 4.5 years in females.

8.4 Health effects in humans and/or animals

8.4.1 Relevant Animal data

8.4.1.1 New data

8.4.1.1.1 Repeated dose toxicity

Repeated dose toxicity of PFUnDA was tested according to the OECD test guideline 422 (Takahashi et al., 2014). PFUnDA was suspended in corn oil and administered by gavage to Crl:CD(SD) rats at 0 (vehicle: corn oil), 0.1, 0.3 or 1.0 mg/kg bw/day. Twelve males per dose group were dosed for 42 days, beginning 14 days prior to mating. After the administration period, 5 of 12 males per group were reared for the recovery period of 14 days. Females were exposed for 41-46 days, beginning 14 days before mating throughout the mating and gestation period until day 4 of lactation. Females in the satellite group received PFUnDA for 42 days, followed by the recovery period of 14 days. No deaths were observed in any of the groups. Body weight gain was inhibited in both sexes at 1.0 mg/kg/day, and there was a statistically significant decrease in fibrinogen in both sexes (200±230 mg/dL for males and 228±42 mg/dL in females) and shortening of the activated partial thromboplastin time in males. In males, liver weight was increased at 0.3 mg/kg/day and above and in females at 1.0 mg/kg/day. This change was still observed after a recovery period of 14 days. In both sexes, centrilobular hypertrophy of hepatocytes was observed at 0.3 mg/kg/day and above and focal necrosis was observed at 1.0 mg/kg/day. Additional results on reproduction and developmental toxicity are discussed in the respective subchapter.

8.4.1.1.2 Carcinogenicity

No data available.

8.4.1.1.3 Mutagenicity

(Wielsøe et al., 2015) demonstrated that PFUnDA statistically significantly increased generation of intracellular reactive oxygen species (ROS) with lowest observed effect concentration (LOEC) of 2 x 10^{-6} M. However in the publication was reported that exposure to PFUnDA did not induce DNA damage in the *in vitro* Comet assay conducted with HepG2 cell line (Wielsøe et al., 2015).

8.4.1.1.4 Toxicity to reproduction

Reproduction and developmental toxicity of PFUnDA was tested according to OECD 422 (Takahashi et al., 2014). PFUnDA was suspended in corn oil and administered by gavage to Crl:CD(SD) rats at 0 (vehicle: corn oil), 0.1, 0.3 or 1.0 mg/kg bw/day. Twelve males per dose group were dosed for 42 days, beginning 14 days prior to mating. After the administration period, 5 of 12 males per group were reared for the recovery period of 14 days. Females were exposed for 41-46 days, beginning 14 days before mating throughout the mating and gestation period until day 4 of lactation. Females in the satellite group received PFUnDA for 42 days, followed by the recovery period of 14 days. No deaths were observed in any of the groups. As results for reproductive/developmental toxicity, body weight of pups at birth was lowered and body weight gain at 4 days after birth was inhibited at 1.0 mg/kg/day, while no dose-related changes were found in the other parameters. The NOAEL for reproductive/developmental toxicity was considered to be 0.3 mg/kg bw/day (Takahashi et al., 2014).

(Yan et al., 2021) investigated effects of PFUnDA on Leydig cell development in pubertal male rats. Sprague-Dawley rats were orally (gavage) dosed with PFUnDA at 0, 1, 5, and 10 mg/kg bw/day from postnatal day (PND) 35 to PND 56. No mortality and morbidity were observed in the study. Body weight and weights of the testes and epididymis were statistically significantly reduced at dosed of 5 and 10 mg/kg bw/day. Serum testosterone and luteinizing hormone (LH) levels were statistically significantly reduced by PFUnDA at ≥1 mg/kg bw/day while serum follicle-stimulating hormone (FSH) levels were lowered at 5 and 10 mg/kg bw/day. The Leydig cell (LC) numbers were reduced at 5 and 10 mg/kg bw/day. It was shown that PFUnDA delays LC development by multiple mechanisms, such as preventing of transcription of pituitary gene expression leading to low LH and FSH levels, downregulation of steroidogenesis- related genes, inducing of oxidative stress and increasing autophagy. No NOAEL could be derived in the study. LOAEL could be estimated as 1 mg/kg bw/day (Yan et al., 2021).

The effects of PFUnDA on Leydig cell function were investigated in the *in vivo* study conducted on rats (Xin et al., 2022). 10 male Sprague-Dawley rats/group at age of 56 days were orally (gavage) exposed to 0 (corn oil), 0.1, 0.5, 1, or 5 mg/kg bw/day PFUnDA for 28 days. PFUnDA statistically significantly reduced body weight about 15% and 55% at 1 and 5 mg/kg respectively when compared to the control. Moreover, exposure to PFUnDA statistically significantly reduced relative epididymis weight at 1 and 5 mg/kg and the relative testis weight at 5 mg/kg. Treatment with PFUnDA statistically significantly reduced serum T levels at doses of 0.5 and 1 mg/kg bw/day. According to the authors the number of Leydig cell decreases starting from dose 0.1 mg/kg bw/day. However, this conclusion cannot be reconstructed based on the available study material. Therefore, the project team sees no reason for a LOAEL of 0.1 mg/kg bw/day.

Additionally, the expression of LC genes and pituitary genes upon the treatment with PFUnDA were measured in the study. It was concluded that PFUnDA inhibits Leydig cell function possibly via AKT/ERK1/2/mTOR signalling pathways (Xin et al., 2022).

8.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

Effects of PFUnDA on mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) were investigated in *in vitro* study conducted with transiently transfected COS-1 cells (Wolf et al., 2012).

A significant positive relationship between the PPARα activity and the cellular PFAS concentration (including PFUnDA) was reported in the *in vitro* study conducted in human hepatocellular carcinoma (HepG2) cells (Rosenmai et al., 2018).

Thyroid hormone (TH) disruption potential of PFUnDA was evaluated in the *in vitro* assay conducted on the rat pituitary cell line (GH3). Cells were exposed to 3, 10, or 30 µM of PFUnDA. It was found that PFUnDA upregulated thyroid-stimulating hormone beta (Tshβ) gene and therefore could increase thyroid hormone synthesis through stimulation by Tsh (J. Kim et al., 2021).

In the *in vitro* assay conducted with primary cultures of rat cerebellar granule neurons (CGNs) PFUnDA caused reductions in cell viability (LC_{50} of 15 µM for MTT and LC_{50} of 40 µM for trypan blue assays) with 30–60 min after exposure (Berntsen et al., 2017).

NOD mice were exposed to PFUnDA in drinking water (3, 30 and 300 µg/l) at mating, during gestation and lactation and until 30 weeks of age. The exposure doses correspond to about 0.417, 4.17 and 41.7 µg/kg bw/day (calculated based on mean mouse weight of 23 g and mean measured volume of drinking water consumption of 3.2 ml/day at 10 weeks of age). NOD mice upon PFUnDA exposure had altered cytokine secretion in splenocytes at 3 µg/l PFUnDA. (e.g. INF-γ, TNF-α, IL-1b, IL-2, IL-4, IL-6, IL-10, IL-13, and IL-17) (Bodin et al., 2016) and as cited in (Neagu et al., 2021).

Lee et al. reported that in *in vitro* assay PFUnDA at concentration 100 µM increased release of histamine and β-hexosaminidase by up-regulation of intracellular calcium levels in IgE-stimulated mast (RBL-2H3) cells. In addition, PFUnDA enhanced gene expression of pro-inflammatory cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 by activation of nuclear factor-κB in IgE-stimulated mast cells. In ovalbumin (OVA)-induced model of systemic anaphylaxis in the presence of hypothermia, PFUnDA exacerbated allergic symptoms accompanied by elevation in serum histamine, TNF-α, IgE, and IgG_1 (J.-K. Lee & Kim, 2018).

PFUnDA was found to be an immunotoxic compound *in vitro*, inducing largest reduction in phagocytosis of rat isolated peritoneal macrophages (PCM). However PFUnDA had no effect on phagocytic function in neither rat nor human monocyte derived macrophages (MDM) measured *in vitro* (Berntsen et al., 2018).

Several *in vitro* mechanistic studies for PFUnDA were evaluated and summarized in EFSA scientific opinion (EFSA, 2020). Using *in vitro* assays conducted in rat H4IIE hepatoma cells the impact of PFUnDA on transcript levels of seven genes, five of them involved in fatty acid and cholesterol synthesis, were investigated. The PPARα-regulated peroxisomal 3-ketoacyl-CoA thiolase was downregulated by PFUnDA (EFSA, 2020). Utilizing a radioligand-binding assay the competition between the binding of T4 and PFAS to the human variant of TTR was assessed a. The binding potency (IC_{50}) for PFUnDA was 22 µM (EFSA, 2020). The binding affinities of 15 PFAS (including PFUnDA) to the ligand binding domain of TRα were investigated. PFUnDA was identified as binder (IC_{50}=18–20 µM). Therefore it could be concluded that PFUnDA is able to interfere with thyroid hormone function on the level of thyroid hormone receptors (EFSA, 2020). Additionally, the effect of PFAS (including PFUnDA) on proliferation control of rat pituitary GH3

cells was investigated in the T-screen assay. PFUnDA significantly decreased GH3 cell proliferation, and this effect was dose-dependent (EFSA, 2020).

8.4.2 Relevant Human data

8.4.2.1 New data

Epidemiological studies of PFUnDA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFUnDA (ng/ml). Epidemiological studies that evaluated exposure-response relationship using untransformed PFUnDA measures are described below.

(Averina et al., 2021) performed a cross-sectional study of 940 adolescents in Norway in 2010-2011. In this population, the geometric mean of PFUnDA among girls was 0.17 ng/ml; among boys the geometric mean of PFUnDA was 0.14 ng/ml. After adjusting for age, sex, and dietary factors, the authors reported that higher serum PFUnDA was also associated with dyslipidaemia, but the association was not consistent.

(W. Cao et al., 2018) performed a longitudinal analysis of PFAS in umbilical cord blood and possible associations with gestational and postnatal growth among 337 mother-child pairs recruited in China from 2013-2015. Median cord serum levels of PFUnDA 0.08 ng/ml. Adjusting for infant sex, maternal age, income, parity, and some paternal factors (depending on analysis), the authors reported PFUnDA was associated with increased birth length in the highest exposure category (>0.11 ng/ml) compared to the lowest (>0.06 ng/ml; β=0.41, 95% CI 0.06–0.77) and was inconsistently associated with increased birth weight. Postnatal growth, measured at a mean of 19.7 months, was not associated with PFUnDA.

(K. Y. Christensen et al., 2019) studied cross-sectional data from the NHANES survey, 2007-2014, to examine the association between PFAS and metabolic syndrome in 2,975 US adults aged 20 years or older. The median serum concentration of PFUnDA was 0.1 ng/ml in all years of NHANES data (2007-2008, 2009-2010, and 2011-2012) except for 2013-2014, when the median was 0.07 ng/ml. Adjusting for age, sex, income, race/ethnicity, alcohol, smoking, and survey cycle, the authors reported the fourth quartile of PFUnDA exposure (between 0.1 and 0.3 ng/ml and above, depending on NHANES survey cycle) was associated with decreased odds of metabolic syndrome compared to the first quartile (between 0.07 and 0.1 ng/ml, depending on NHANES survey cycle) (OR 0.63, 95% CI 0.42–0.95).

(Donat-Vargas, Bergdahl, Tornevi, Wennberg, Sommar, Kiviranta, et al., 2019) performed a prospective nested case-control study examining the association between serum levels of PFAS and risk of type 2 diabetes (T2D) among 124 case-control pairs from a sub-cohort of the Northern Sweden Health and Disease Study, which was initiated in 1985. The authors analysed serum concentrations of PFUnDA (median, 0.16 ng/ml in cases, 0.18 ng/ml in controls). In analyses adjusted for sex, age, year of sample, red and processed meat intake, fish intake, and BMI, the study reported no statistically significant association between baseline PFUnDA serum concentration and risk of T2D.

(Donat-Vargas, Bergdahl, Tornevi, Wennberg, Sommar, Koponen, et al., 2019) examined associations between plasma PFAS and cardiometabolic risk factors among 187 participants in the Northern Sweden Health and Disease Study; participants were adults free of diabetes and were assessed at baseline between 1990-2003 and followed up after ten years. Median PFUnDA at baseline was 0.19 ng/ml and at follow-up was 0.22 ng/ml. Adjusting for age, gender, education, BMI, sample year, smoking, alcohol, diet, and physical activity, the authors observed

no association between PFUnDA and hypertension, per one standard-deviation increase (0.19 ng/ml for PFUnDA).

(Gao et al., 2019) conducted a cross-sectional study of maternal PFAS exposure and birth outcomes. The study population included 132 pregnant women with a mean age of 31 years who visited the Affiliated Hospital of Capital Medical University in Beijing for delivery between 2015 and 2016. Maternal blood samples for exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. The median level of PFUnDA in maternal serum was 0.45 ng/ml; in cord serum, median PFUnDA was 0.26 ng/ml. Models were adjusted for maternal age, height, weight, BMI, and gestational age, depending on the analysis. Neither maternal serum PFUnDA (highest tertile >0.61 ng/ml) nor cord serum PFUnDA (highest tertile >0.31 ng/ml) were statistically significantly associated with birth weight, birth length, gestational age, or ponderal index.

(Goudarzi et al., 2016) conducted a longitudinal cohort study examining prenatal exposure to 11 PFAS and allergic diseases in early childhood. The study population included 1,558 mother-child pairs enrolled in the Hokkaido Study on Environment and Children's Health, recruited in Japan from 2003-2009. Maternal blood samples were collected at 23 to 32 gestational weeks; the median plasma concentration of PFUnDA was 1.43 ng/ml. Adjusting for maternal age and education, parental allergies, breastfeeding, older siblings, passive smoking, and day care attendance, the authors reported no association of PFUnDA with allergic diseases at age 4 years.

(M. Huang et al., 2018) conducted a cross-sectional study of 12 perfluoroalkyl chemicals and cardiovascular disease in the 10,859 participants from the US population using cross-sectional NHANES data from 1999-2014. The median serum concentration of PFUnDA in the total study population was 0.20 ng/ml. Adjusting for BMI, diabetes, hypertension, family history, total energy intake, serum cotinine, cholesterol, serum protein, and estimated glomerular filtration rate, the authors found that PFUnDA was associated with increased odds of the presence of total cardiovascular disease, which included congestive heart failure, coronary heart disease, angina pectoris, heart attack, or stroke (OR 1.55, 95% CI 1.11–2.16 for the fourth quartile of exposure, >=0.20 ng/ml, compared to the first quartile, <0.13 ng/ml. When cardiovascular outcomes were analysed individually, PFUnDA was consistently associated only with coronary heart disease (OR 2.02, 95% CI 1.36–3.00 for the fourth quartile compared to the first).

(Kvalem et al., 2020) studied 378 children who participated in the Environment and Child Asthma Study based in Oslo, Norway, which initially recruited healthy newborns from 1992-1993. The present study included a cross-sectional analysis at the 10-year follow-up and a longitudinal analysis at the 16-year follow up; the authors examined associations of 19 PFAS with asthma, allergies, lung function, and airway infections. Median serum PFUnDA at 10 years was 0.16 ng/ml. The authors adjusted for BMI, puberty status, physical activity, maternal education, and age, depending on analysis. The authors found no consistent associations between PFUnDA at 10 years and diagnoses of atopic dermatitis or rhinitis, positive skin-prick test, or lung function, at 10-year or at 16-year follow-up.

(C.-Y. Lin et al., 2013) conducted a cross-sectional study of four PFAS, including PFUnDA, and thyroid hormones among 551 adolescents and young adults aged 12-30 years in Taiwan, originally recruited from 1992-2000. Participants were re-contacted and had plasma PFAS, and thyroid hormones measured between 2006-2008. The median serum PFUnDA level in this population was 6.46 ng/ml. After adjusting for age, sex, smoking, and alcohol use, the authors reported no significant differences in either serum free thyroxine (T4) or thyroid stimulating hormone (TSH) across quartiles of serum PFUnDA exposure (highest quartile >13.91 ng/ml).

(T.-W. Lin et al., 2020) conducted a cross-sectional study of 397 adults between 55-75 years of age from PFAS-exposed communities in Taiwan from 2016-2017 to examine the association between nine serum PFAS and metabolic syndrome and related biomarkers (uric acid, fasting blood sugar, total cholesterol, and triglycerides). The median serum PFUnDA among participants was 1.41 ng/ml. In models stratified by sex and adjusted for age, smoking, and drinking, no statistically significant associations were detected between PFUnDA and metabolic syndrome status, uric acid, fasting blood sugar, or measures of cholesterol. PFUnDA was associated with decreased triglycerides in both men (β = -55.16 mg/dL, 95% CI -99.24 to -11.08) and women (β= -29.64 mg/dL, 95% CI -58.97 to -0.3) for the highest exposure quartile (>2 ng/ml) compared to the lowest exposure quartile (<1 ng/ml).

(Okada et al., 2014) conducted a longitudinal study of prenatal PFAS exposure and childhood allergic diseases among 2,062 mother-child pairs originally enrolled in the Hokkaido Study on Environment and Children's Health from 2003–2009 in Japan. Median maternal plasma PFUnDA in this cohort was 1.40 ng/ml. Analyses were adjusted for maternal age and education, breastfeeding, siblings, passive smoking, day care attendance, and parental allergies. Among girls, the odds of total allergic diseases in the first 24 months were statistically significantly decreased for the top quartile of prenatal PFUnDA exposure (>1.87 ng/ml) compared to the bottom quartile (<1.02 ng/ml) (OR 0.58, 95% CI 0.39–0.86); this association was not statistically significant among boys. A similar pattern was observed for PFUnDA at 24 months and eczema, which was included in analyses of total allergic diseases; there were decreased odds among girls in the top quartile compared to the bottom quartile (OR 0.50, 95% CI 0.30–0.81), but no association among boys. No statistically significant associations were detected for PFUnDA and eczema at 12 months of age or wheezing at 12 or 24 months of age. This study evaluated 11 PFAS in total.

(Shearer et al., 2021) conducted a nested case-control study to examine serum PFAS and risk of renal cell carcinoma. A total of 324 cases and 324 controls matched on age at enrolment, sex, race and ethnicity, study centre and year of blood draw were identified from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, a study of 55–74-year-old adults from 10 US cities recruited between 1993 and 2001. In logistic regression models, PFUnDA was categorized as ≤LOD (≤0.1 ng/ml), >0.1-0.2, >0.2 ng/ml. The distributions of other PFAS were based on quartiles of serum concentration in controls; however, the authors reported that cut points for PFUnDA were not based on quartiles because more than a quarter of PFUnDA measurements were below the limit of detection. Adjusting for BMI, smoking, hypertension, estimated glomerular filtration rate, calendar year of blood draw, and previous freeze-thaw cycle, the authors observed no exposure-response association between PFUnDA and renal cell carcinoma (highest exposure category >0.2-1.7 ng/ml) (p for trend=0.9). A total of 10 PFAS were measured in this study.

(Timmermann et al., 2022) conducted a cross-sectional study among Greenlandic children ages 7-12 to investigate whether exposure to endocrine-disrupting chemicals, including seven PFAS, was associated with changes in diphtheria and tetanus vaccine antibodies after receiving immunization. The authors measured seven PFAS, including PFUnDA, among children at a median age of 9.9 years; median serum PFUnDA was 0.42 ng/ml. After adjusting for time since vaccination, breastfeeding duration, and area of residence, the authors observed no significant association between tetanus or diphtheria antibody concentrations and child serum PFUnDA. Among a subset of 175 children with known vaccination records, after adjusting for area of residence and duration of breastfeeding, the study investigators found that the association between PFUnDA and the odds of not being protected against diphtheria were not statistically significant (OR = 2.61, 95% CI 0.99, 6.90 per ng/ml increase).

(Wang et al., 2014) conducted cross-sectional and longitudinal analyses of the association between maternal serum concentrations of nine PFAS and thyroid hormones among 285 pregnant women and 116 newborns. Pregnant women were recruited from 2000-2001 in Taiwan. Maternal serum PFAS were measured in the third trimester; median PFUnDA was 3.26 ng/ml. Analyses of maternal thyroid hormones were adjusted for maternal age, previous live births, and education; analyses of cord blood thyroid hormones were additionally adjusted for infant sex and type of delivery. In cross-sectional analyses, maternal PFUnDA was associated with decreased free thyroxine (T4) (β=-0.004 ng/dL, 95% CI -0.007 to -0.002) and decreased total thyroxine (T4) (β=-0.062 ng/dL, 95% CI -0.097 to -0.026) per one ng/ml increase in PFUnDA. PFUnDA was not associated with statistically significant changes in maternal total triiodothyronine (T3) or in thyroid-stimulating hormone (TSH). In longitudinal analyses, maternal PFUnDA was associated with decreased total T4 (β=-0.052 µg/dL, 95% CI -0.095 to -0.010) and total T3 (β=-0.001 ng/dL, 95% CI -0.001, -0.0002) in cord blood, per one ng/ml change in PFUnDA. PFUnDA was not associated with statistically significant changes in free T4 or TSH in cord blood.

(Wikström et al., 2020) studied 1,533 mother-infant pairs from the Swedish Environmental, Longitudinal, Mother and child, Asthma and allergy (SELMA) study to assess associations between prenatal PFAS exposure and birth outcomes. The median maternal serum concentration of PFUnDA was 0.23 ng/ml. After adjusting for maternal weight, cotinine, and parity, the authors found no association of PFUnDA with decreased birthweight or increased odds of small for gestational age (highest exposure quartile >0.33 ng/ml).

8.5 Proposal for a starting point for deriving a drinking water limit value

8.5.1 Selected study

The guideline conform repeated dose and reproduction/developmental toxicity screening test (OECD 422, GLP) conducted on rats (Takahashi et al., 2014) was selected as most suitable study to derive a drinking water limit value. Based on the result of this study and information reported in (ATSDR, 2021) liver was suggested as a sensitive target organ for PFUnDA. The liver effects upon exposure to PFUnDA could be initiated by activation of the nuclear hormone receptor PPARα observed in the *in vitro* studies (Wolf et al., 2012), (Rosenmai et al., 2018) and as cited in (ATSDR, 2021). Humans are less sensitive to PPARα agonists than rats and mice (ATSDR, 2021) and the derivation of assessment criteria from rodent data could therefore lead to more conservative values. The lowest NOAEL of 0.1 mg/kg /day based on the liver effects (centrilobular hypertrophy of hepatocyte and increased liver weights) is proposed as the starting point for a TWLW.

8.5.2 Proposal of assessment factors/modification factors with justification

An assessment factor (ECHA 2010) of overall 12,600 is proposed with the following justifications for its derivation:

- AF of 6 to account for the time extrapolation (adjusting for subacute 42-day exposure to chronic exposure).
- AF of 84 based on an approximate half-life in humans of 4,380 days (ATSDR 2021) compared to approximately 52 days in rats (Dzierlenga et al., 2020) (4,380 days/52 days) derived for PFDA (please refer to Section 7.5).
- AF of 2.5 for interspecies toxicodynamics differences.

- AF of 10 to account for intraspecies differences (general population).

This would result in a total factor of 12,600 and a human-related tolerable dose of 8 ng /kg bw/day.

9 Toxicological evaluation of perfluorododecanoic acid (PFDoDA)

9.1 Chemical and physical information

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 28 and Table 29.

Table 28: Chemical identity of Perfluorododecanoic acid (PFDoDA, CAS 307-55-1)

Name	Perfluordodecansäure
English Name	Perfluorododecanoic acid
Acronym	PFDoDA
Mol. Formula	C12HF23O2
Mol. Weight (g/mol)	614.10
CAS	307-55-1
EC	206-203-2

Table 29: Physicochemical properties of perfluorododecanoic acid (PFDoDA, CAS 307-55-1)

Properties	Value	Source
Density (g/cm^3)	1.77-1.87 (mod.)	(USEPA, 2020)
Melting point (°C)	108-110 (exp.)	(USEPA, 2020)
Boiling point (°C)	245-249 (exp.)	(USEPA, 2020)
Vapour Pressure (Pa)	0.1 (exp. at 25 °C)	(Bhhatarai & Gramatica, 2011)
Henry's Constant (Pa m^3/mol)	1.7 x 10^8 (exp. at 25 °C)	(M. Kim et al., 2015)
pKa	No data	
Log Koc	3.6 to 3.8 (± 0.6) (exp.)	(Munoz et al., 2015)
Water Solubility (mg/l)	0.52 (exp. at 25 °C)	(Inoue et al., 2012)
	0.07 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated, mod = modelled).

9.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFDoDA (Table 30). Data was identified by general desk research. Only sum values for PFAS including PFDoDA were found. No quantitative toxicological assessments of PFDoDA on human data determined by other institutions were found.

Table 30: Drinking water limits determined for PFDoDA or sum of PFAS

Country/institution	PFDoDA limit value in drinking water (µg/l)	Comment	Source
Sweden/Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFDoDA, drinking water guide value	(SFA, 2022)
Hawaii (USA)	0.013	Drinking water guideline level	(ECOS, 2022)
Wisconsin (USA)	0.5	Drinking water guideline level	(ECOS, 2022)

9.3 Toxicokinetics

9.3.1 Animal data

9.3.1.1 New data

(ATSDR, 2021) reports the oral absorption of PFDoDA is >95% and in male and female mice, the 24-hour area under the curve (AUC) for oral administration was 90 to 100% of the oral dose.

9.3.2 Human data

9.3.2.1 New data

ATSDR (2021) reported renal clearance in humans was 0.005 ml/day/kg with biliary clearances much higher than total body clearance in humans.

Cord:maternal (transplacental transfer) and serum:whole cord blood (blood partitioning) ratios were estimated for several PFAS including PFDoDA in a group of 151 mother- newborn pairs from two successive Faroese birth cohorts (Eryasa et al., 2019). The relationship of these ratios with physicochemical properties of PFAS was examined using multivariable regression analyses. PFDoDA exhibited low serum:whole cord blood (blood partitioning) ratios with a geometric mean in maternal serum from cohort 3 of 0.03 ng/ml. Geometric means from cohort 3 and 5 in cord serum were 0.02 and 0.05 ng/ml, respectively; and in whole cord blood they were 0.02 and 0.03 ng/ml, respectively. Results showed decreasing transplacental transfer with increasing carbon chain length from PFHpA (C7) to PFDoDA (C12).

9.4 Health effects in humans and/or animals

9.4.1 Relevant Animal data

9.4.1.1 New data

9.4.1.1.1 Repeated dose toxicity

(Kato et al., 2015) conducted a combined repeated dose toxicity test and reproductive/developmental test in accordance with OECD guideline 422. Male and female Crl:CD(SD) rats (12/sex/group) were administered PFDoDA at doses of 0, 0.1, 0.5, or 2.5 mg/kg/day via oral gavage for 42 to 47 days. Twelve male rats in each dose group were administered PFDoDA for 42 days beginning 14 days prior to mating. On day 42, seven males in the control and high dose group and all males receiving doses of 0.1 and 0.5 mg/kg/day were necropsied. The five remaining males in the control and high dose group were assigned to a 14-day recovery group and then necropsied. Twelve females per dose group were administered PFDoDA from 14 days prior to mating (main group) through gestation and nursing periods until 5 days after delivery and then necropsied on day 6 of nursing. Pregnant females which did not deliver by day 25 of gestation and females which showed abnormal delivery (stillbirth) were necropsied on day 26 of gestation and on day 0 of nursing, respectively. Five females administered 0 or 2.5 mg/kg/day for 42 days without mating served as the 14-day recovery groups and were necropsied on the day after the 14-day recovery period. The female in the recovery group were not examined fully (reproductive/developmental outcome) at the end of administration period.

Animals in the repeated dose toxicity groups were evaluated for clinical observations, food consumption, body weight changes, functional observations (i. sensorimotor reactivity to visual, tactile, auditory, pain, proprioceptive stimuli, and air righting reflex; ii. Forelimb and hindlimb grip strength; and iii. Spontaneous motor activity), urinalysis, haematology, gross necropsy, and histopathology. Results indicated significant decreases in male and female body weights and food consumption at 2.5 mg/kg/day. Significant increases in relative liver weight were reported in male and female rats in 0.5 mg/kg/day and in males at 2.5 mg/kg/day (only one female was observed in the high dose group due to decreased survival and early delivery of pups). Significant increases in relative liver weights were also reported in male and female recovery groups administered 2.5 mg/kg/day. Histopathological evaluation indicated significant increases in the incidence of liver hypertrophy in male and females in the 2.5 mg/kg/day group; however, the incidence was not significantly increased in the recovery group. In females there was also a significant increase in the incidence of hepatocyte necrosis at the high dose group and an increase in the incidence of thymus atrophy at the high dose. The authors reported liver necrosis and inflammatory cholestasis at the lower dose of 0.5 mg/kg/day; however, the incidence of these changes was not significantly different from controls. Based on statistically significant changes in liver weights reported in male and female rats the NOAEL for repeated dose toxicity is 0.1 mg/kg/day.

9.4.1.1.2 Carcinogenicity

No relevant data was found

9.4.1.1.3 Mutagenicity

No relevant data was found

9.4.1.1.4 Toxicity to reproduction

(Kato et al., 2015) conducted a combined repeated dose toxicity test and reproductive/developmental test in accordance with OECD guideline 422. Male and female Crl:CD(SD) rats (12/sex/group) were administered PFDoDA at doses of 0, 0.1, 0.5, or 2.5 mg/kg/day via oral gavage for 42 to 47 days. Twelve male rats in each dose group were administered PFDoDA for 42 days beginning 14 days prior to mating. On day 42, seven males in the control and high dose group and all males receiving doses of 0.1 and 0.5 mg/kg/day were necropsied. The five remaining males in the control and high dose group were assigned to a 14-day recovery group and then necropsied. Twelve females per dose group were administered PFDoDA from 14 days prior to mating (main group) through gestation and nursing periods until 5 days after delivery and then necropsied on day 6 of nursing. Pregnant females which did not deliver by day 25 of gestation and females which showed abnormal delivery (stillbirth) were necropsied on day 26 of gestation and on day 0 of nursing, respectively. Five females administered 0 or 2.5 mg/kg/day for 42 days without mating served as the 14-day recovery groups and were necropsied on the day after the 14-day recovery period. The females in the recovery group were not examined fully (reproductive/developmental outcome) at the end of administration period.

Females and offspring included in the reproductive/developmental toxicity groups were evaluated for oestrous cycle, gestational length, copulation index, fertility index, gestation index, number of live and dead pups, live birth index, sex ratios, general pup appearance and behaviour, viability index on post-natal day (PND) 4, gross external and internal observations, corpora lutea and implantation in uterus, implantation index and delivery index. Results indicated non-significant decreases in spermatid and spermatozoa counts in male reproductive organs, and significantly decreased gestation index and significantly decreased oestrous cycle normality at 2.5 mg/kg/day. Seven of twelve females receiving 2.5 mg/kg/day died during late pregnancy. Four females did not deliver live pups at 2.5 mg/kg/day. No reproductive or developmental parameters were significantly changed at doses of 0.1 and 0.5 mg/kg/day. The NOAEL for reproductive/developmental toxicity is 0.5 mg/kg/day.

(Z. Shi, Zhang, et al., 2009) studied the effects of PFDoDA on puberty and endocrine status in pre-pubertal female rats. PFDoDA was administered orally at 0, 0.5, 1.5, or 3 mg/kg/day for 28 days. Following exposure, body weight, reproductive organ weight and morphology, endocrine hormones, pubertal indicators, total serum cholesterol levels and steroidogenic enzyme gene expression were evaluated. Results indicated a significant decrease in body weight at 3 mg/kg/day, along with a significant increase in total serum cholesterol and a significant decrease in oestradiol. No significant changes in FSH or LH were reported at any dose level. No abnormalities in ovarian or uterine structure were reported, and the ovaries of treated rats had a normal complement of growing follicles and corpus luteum. No significant differences in the number of primordial, primary, preantral or antral follicles in the ovary were observed. At 3 mg/kg/day altered ovarian expression of genes responsible for cholesterol transport and steroidogenesis, including steroidogenic acute regulatory protein, cholesterol side-chain cleavage enzyme and 17-beta-hydroxysteroid dehydrogenase were reported. The authors concluded that PFDoDA did not affect the endocrine status of pubertal rats but may impact oestradiol production and the expression of some key genes responsible for oestrogen synthesis at higher doses. However, the effects described in this study are not necessarily adverse effects.

(Z. Shi et al., 2013) compared testicular phosphorylation profiles between normal and PFDoDA-treated rats to determine the molecular mechanism of the toxic action of PFDoDA in testes at the protein phosphorylation level. Groups of 6 male rats were gavaged with doses of 0, 0.02, 0.2, or 0.5 mg/kg/day for 110 days. The authors combined efficient prefractionation of tryptic peptide

mixtures using self-packed reversed phase C18 columns with titanium dioxide (TiO_2) and immobilized metal affinity chromatography (IMAC) phosphopeptide enrichment techniques, along with two-dimensional liquid chromatography tandem mass spectrometry (2D-LC MS/MS), to analyse the phosphoproteome of normal rat testes and testes after 110 days of PFDoDA exposure. The results identified 4077 unique phosphopeptides from 1777 proteins, with 937 novel phosphorylation sites discovered in testicular proteins. Significant dose related increases were reported in the number of casein kinase 2 kinase-modified peptides. Pathway analysis suggested that the mitogen-activated protein kinase pathway and cell division cycle protein 2 (CDC2) may have contributed to sperm activity and testicular function. The authors suggest these data provide a unique and detailed insight into the roles of phosphorylation in testicular function.

(Z. Shi et al., 2010) evaluated the mechanism of action of effects of PFDoDA in testis of male rats administered 0, 0.02, 0.2, or 0.5 mg/kg/day for 110 days via oral gavage. Using a two-dimensional gel electrophoresis (2-DE) approach, the alteration of protein expression in the testes was investigated, along with changes in serum progesterone. Decreased serum progesterone levels were observed. Study results indicated serum progesterone levels were significantly decreased at 0.2 and 0.5 mg/kg/day. In addition, 40 expressed proteins were identified that were involved in mitochondrial respiration, sperm activity, oxidative stress, cytoskeleton and intracellular signal transduction. The authors concluded that PFDoDA treatment led to decreases in activities of proteins involved in mitochondrial respiratory and antioxidative responses that play important roles in the inhibition of testicular steroidogenesis.

9.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

(Ding et al., 2009) investigated the potential hepatoxicity of PFDoDA in male rats. Groups of 10 male rats were administered PFDoDA at doses of 0, 0.02, 0.05, 0.2, or 0.5 mg/kg/day via oral gavage for 110 days. All animals were sacrificed after 110 days of exposure and for each animal, half of the liver was fixed for histopathological evaluation and the other half along with serum were frozen and stored for nuclear magnetic resonance spectroscopy (NMR), RNA isolation and microarray analysis. Results indicated there was a significant body weight decrease (7.1%) in animals dosed at 0.5 mg/kg/day with no significant differences in body weights reported at the lower concentrations. Absolute and relative liver weights were significantly increased at 0.02, 0.05, 0.2 and 0.5 mg/kg/day. Histopathological examination indicated lipid droplets and widespread disintegrated cell systems at doses of 0.05, 0.2, and 0.5 mg/kg/day. The lipid droplets were reported to be larger in the higher-dosed animals compared to the animals in the lower dose groups. No histopathological changes were reported in the 0.02 mg/kg/day dosed group. Hydropic degeneration and steatosis was reported at doses of 0.2 and 0.5 mg/kg/day, as well as swollen and vacuolated hepatocytes. Clinical chemistry results indicated significantly increases in albumin (ALB) and glucose at all dose levels. Significant increases in the levels of total bile acids (TBA), alkaline phosphatase (ALP), urea nitrogen (BUN), and creatinine (CRE) were also detected in animals dosed at 0.2 and 0.5 mg/kg/day. Significantly elevated levels of total bilirubin (T-Bil) were observed at 0.5 mg/kg/day and concentrations of CK were significantly increased at 0.02 and 0.05 mg/kg/day. A significant decrease in concentrations of low-density lipid-cholesterol (LDL-C) was reported at 0.02- and 0.05 mg/kg/day while the levels of triglyceride (TG) were decreased significantly at 0.05-, 0.2- and 0.5 mg/kg/day. NMR-based metabonomics results demonstrated hepatic lipidosis characterized by a severe elevation in hepatic triglycerides and decreased serum lipoprotein levels; and transcriptomic changes supported these results with changes in gene transcript levels associated with fatty acid homeostasis. The authors concluded that treatment with PFDoDA induced hepatic steatosis via

perturbations to fatty acid uptake, lipogenesis, and fatty acid oxidation in male rats. Based on the results of this study a LOAEL of 0.02 mg/kg bw/d could be assumed.

In a study designed to identify the potential mechanism of hepatoxic effects associated with exposure to PFDoDA, two-dimensional differential gel electrophoresis (2-D DIGE) followed by mass spectrometric analyses of the livers of rats treated with PFDoDA was evaluated (H. Liu et al., 2016). Male rats (10/group) were administered 0, 0.05, 0.2, or 0.5 mg/kg/day PFDoDA via oral gavage for 110 days. At the end of treatment, six rats from each group were weighed and killed by decapitation; the other four rats from each group were used for another study. Results indicated PFDoDA bioaccumulation in the rat liver increased in a dose-related manner. Results of 2-D DIGE showed expression of 73 proteins involved in lipid metabolism, inflammation, stress response and other at doses of 0.2 and 0.5 mg/kg/day, with six significantly changed proteins (CTE1, MTE1, HADHA, ECH1, ALDH2 and CPS1) known to be regulated by peroxisome proliferator-activated receptor alpha (PPARα). Results implied induction of oxidative stress following treatment with PFDoDA, concluded from anti-oxidant enzyme activity assays of superoxide dismutase and glutathione peroxidase and the content of thiobarbituric acid-reactive substances in the liver. In the PPARα knockdown groups there was a significant increase in reactive oxygen species (ROS) content in rat hepatocytes, consistent with the PPARα antagonist GW6471- and agonist WY14643-treated groups. The authors concluded that the results of the study strongly suggest PPARα played an important role in suppressing ROS content in hepatocytes following PFDoDA exposure.

9.4.2 Relevant Human data

9.4.2.1 New data

Epidemiological studies of PFDoDA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFDoDA (ng/ml). Epidemiological studies that evaluated exposure-response relationships using untransformed PFDoDA are described below.

(W. Cao et al., 2018) performed a longitudinal analysis of PFAS in umbilical cord blood and possible associations with gestational and postnatal growth among 337 mother-child pairs recruited in China from 2013-2015. The median cord serum level of PFDoDA was 0.02 ng/ml. Adjusting for infant sex, maternal age, income, parity, and some paternal factors (depending on analysis), the authors reported no association between PFDoDA and neonatal growth. PFDoDA was associated with increased postnatal length in the highest exposure category (>0.04 ng/ml) compared to the lowest category (<0.02 ng/ml; β=2.03, 95% CI 0.21–3.85). However, there was no statistically significant trend of decreased birth weight; PFDoDA was associated with a small non-statistically significant increase in birth weight in the highest exposure category. This study assessed 11 PFAS, including PFDoDA.

(Gao et al., 2019) conducted a cross-sectional study of maternal PFAS exposure and birth outcomes. The study population included 132 pregnant women with a mean age of 31 years who visited the Affiliated Hospital of Capital Medical University in Beijing for delivery between 2015 and 2016. Maternal blood samples for exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. The median levels of PFDoDA in maternal and cord serum were 0.25 and 0.26 ng/ml, respectively. Models were adjusted for maternal age, height, weight, BMI, and gestational age, depending on the analysis. Cord serum PFDoDA was associated with increased birth length, with the middle exposure group (0.184–0.316 ng/ml) associated with an increase of 0.67 cm (95% CI 0.27–1.10 cm) and the highest exposure group (>0.316 ng/ml) associated with an increase of 0.50 cm

(95% CI 0.07–0.92 cm) compared to the lowest exposure group (<0.184 ng/ml). A total of 14 PFAS were measured in blood and cord serum, including PFDoDA.

(Goudarzi et al., 2016) conducted a longitudinal cohort study examining prenatal PFAS exposure and allergic diseases in early childhood. The study population included 1,558 mother-child pairs enrolled in the Hokkaido Study on Environment and Children's Health, recruited in Japan from 2003-2009. Maternal blood samples were collected at 23 to 32 gestational weeks; the median plasma concentration of PFDoDA was 0.186 ng/ml. Adjusting for maternal age and education, parental allergies, breastfeeding, older siblings, passive smoking, and day care attendance, the authors reported that PFDoDA was associated with decreased prevalence at age 4 of allergic diseases across increasing quartiles (OR = 0.621, 95% CI 0.454, 0.847 for the fourth quartile (>0.233 ng/ml) compared to the first quartile (<0.14 ng/ml)). When stratified by sex, this association persisted among male children (OR = 0.492, 95% CI 0.314–0.766 for fourth quartile compared to first quartile) but did not reach statistical significance among female children. This study examined 11 PFAS, including PFDoDA.

(H.-W. Lin et al., 2020) conducted a case-control study of PFAS exposure and paediatric germ cell tumours (GCT) among 42 cases and 42 sex- and age-matched controls recruited from a hospital in Shanghai, China, 2014-2017. Among the cases, who had a mean age of 29 months, the median serum PFDoDA concentration was 0.140 ng/ml; among the controls, who had a mean age of 22 months, median serum PFDoDA was 0.118 ng/ml. Adjusting for maternal factors during pregnancy such as cosmetics use and living near farmland, the authors reported no association between PFDoDA and increased odds of paediatric germ cell tumours. This study assessed 10 PFAS including PFDoDA.

(Okada et al., 2014) conducted a longitudinal study of prenatal PFAS exposure and childhood allergic diseases among 2,062 mother-child pairs originally enrolled in the Hokkaido Study on Environment and Children's Health from 2003–2009 in Japan. Median maternal plasma PFDoDA in this cohort was 0.182 ng/ml. Analyses were adjusted for maternal age and education, breastfeeding, siblings, passive smoking, day care attendance, and parental allergies. Among girls, the odds of total allergic diseases in the first 24 months were statistically significantly decreased for the top quartile of prenatal PFDoDA (>0.230 ng/ml) compared to the bottom quartile (<0.138 ng/ml) (OR 0.58, 95% CI 0.39–0.86) but no association was seen among boys. There was no association between PFDoDA, and eczema analysed independently from total allergic disease. No statistically significant associations were detected for PFDoDA and eczema at 12 months of age or wheezing at 12 or 24 months of age. This study evaluated 11 PFAS, including PFDoDA.

(Reardon et al., 2019) studied 25 different PFAS including PFDoDA (median 0.06 ng/ml) and thyroid hormones (free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), and thyroid peroxidase antibodies (TPOAb)) in 494 women from the Alberta Pregnancy Outcomes and Nutrition (APrON) longitudinal Canadian pregnancy cohort, 2009-2012. Women were followed at <13 weeks, 14-26 weeks, 27-40 weeks gestation and 3 months post-partum; thyroid hormones were measured at each time point and PFAS were analysed using blood samples taken in the second trimester. PFAS were measured in plasma for 86% of all samples, and in serum for the 14% of samples where plasma was not available. Adjusting for maternal age, ethnicity, smoking, and diagnosed thyroid condition, the authors found no association between PFDoDA and thyroid hormones in the main analysis or when effect modification by mercury (Hg) co-exposure was examined.

(B. Wang et al., 2017) conducted a case-control study of the association between exposure to 10 different PFAS and endometriosis-related infertility in Chinese women of reproductive age.

Cases included 157 women with surgically confirmed endometriosis; their median plasma PFDoDA was 0.22 ng/ml. Controls included 178 women seeking infertility treatment due to male reproductive dysfunction; their median plasma PFDoDA was 0.23 ng/ml. Adjusting for age, BMI, education, and income, the authors observed no association between PFDoDA (highest exposure tertile >0.27 – 1.02 ng/ml) and endometriosis-related infertility.

(Wang et al., 2014) conducted cross-sectional and longitudinal analyses of the association between maternal serum concentrations of nine PFAS and thyroid hormones among 285 pregnant women and 116 newborns. Pregnant women were recruited from 2000-2001 in Taiwan. Maternal serum PFAS were measured in the third trimester for 7 PFAS; median PFDoDA was 0.36 ng/ml. Analyses of maternal thyroid hormones were adjusted for maternal age, previous live births, and education; analyses of cord blood thyroid hormones were additionally adjusted for infant sex and type of delivery. In cross-sectional analyses, maternal PFDoDA was associated with decreased maternal free T4 (β=-0.132 ng/dL, 95% CI -0.204 to -0.059) and decreased total T4 (β=-1.742 µg/dL, 95% CI -2.785 to -0.700) per one ng/ml increase in maternal PFDoDA. PFDoDA was not associated with statistically significant changes in maternal total triiodothyronine (T3) or in thyroid-stimulating hormone (TSH). In longitudinal analyses, maternal PFDoDA was associated with decreased total T4 (β=-1.920 µg/dL, 95% CI -3.345 to -0.495) and decreased total T3 (β=-0.22 ng/dL, 95% CI -0.035 to -0.009) in cord blood, per one ng/ml increase in PFDoDA. PFDoDA was not associated with statistically significant changes in free T4 or TSH in cord blood. Wang et al. (2014) reported that they were unable to distinguish an independent association of PFDoDA on thyroid hormones, however, because PFDoDA was highly correlated with both PFNA and PFUnDA.

(Zhou et al., 2016) conducted a cross-sectional study of Taiwanese adolescents in 2009-2010 to determine whether there is an association between serum concentration of nine different PFAS and reproductive hormones. Median serum PFDoDA among the 225 participants aged 13-15 years was 2.7 ng/ml. Adjusting for age, BMI, passive smoking, exercise, parental education, and month of survey, the authors observed decreased testosterone (β=-0.0119, 95%CI -0.0227 to -0.0010 nmol/l of natural log-transformed testosterone per ng/ml increase of PFDoDA) among girls only; among boys, the association was not statistically significant. There was no association between PFDoDA and oestradiol in either boys or girls.

As can be seen from the results above, most of these epidemiological studies do not have data that demonstrate an exposure-response relationship exists between PFDoDA and an adverse health outcome. Although the study by Wang et al. (2014) described decreases in some health-relevant biomarkers (thyroid hormones in maternal and cord blood) in relation to maternal PFDoDA, the investigators were unable to separate the effects from those of PFNA and PFUnDA, which were highly correlated with PFDoDA. Based on these studies, there are no suitable data for identifying a POD for use in deriving a drinking water limit value for PFDoDA.

9.5 Proposal for a starting point for deriving a drinking water limit value

9.5.1 Selected study

Based on data from a combined repeated dose and reproductive/developmental toxicity study conducted in male and female rats (Kato et al., 2015), the starting point for a TWLW is the lowest NOAEL of 0.1 mg/kg/day.

9.5.2 Proposal of assessment factors/modification factors with justification

An assessment factor (ECHA 2010) of overall 12,600 is proposed with the following justifications for its derivation:

- AF of 6 to account for the time extrapolation (adjusting for subacute 42-day exposure to chronic exposure).
- AF of 84 based on an approximate half-life in humans of 4,380 days (ATSDR 2021) compared to approximately 52 days in rats (Dzierlenga et al. 2020) (4380 days/52 days) derived for PFDA (please refer to Section 7.5) for intraspecies toxicokinetic differences. No half-life data were identified specific for PFDoDA in animals or humans.
- AF of 2.5 for interspecies toxicodynamics differences.
- AF of 10 to account for intraspecies differences (general population).

This would result in a total factor of 12,600 and a human-related tolerable dose of 8 ng/kg·d.

10 Toxicological evaluation of perfluorotridecanoic acid (PFTrDA)

10.1 Chemical and physical information

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 31 and Table 32.

Table 31: Chemical identity of perfluorotridecanoic acid (PFTrDA, CAS 72629-94-8)

Name	Perfluortridecansäure
English Name	Perfluorotridecanoic acid
Acronym	PFTrDA
Mol. Formula	C13HF25O2
Mol. Weight (g/mol)	664.11
CAS	72629-94-8
EC	276-745-2

Table 32: Physicochemical properties of perfluorotridecanoic acid (PFTrDA, CAS 72629-94-8)

Properties	Value	Source
Density (g/cm^3)	1.92 (mod.)	(USEPA, 2020)
Melting point (°C)	112-123 (exp.)	(Sigma-aldrich, n.d.)
Boiling point (°C)	224-261 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	0.09-2.1 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	3.7 x 10^5 (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	5.2 (exp.)	(Munoz et al., 2015)
Water Solubility (mg/l)	28 - 3.4 x10^6 (mod.)	(USEPA, 2020)

(exp. = experimental, mod = modelled)

10.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFTrDA (Table 33: Drinking water limits determined for PFTrDA or sum of PFAS). Data was identified by general desk research. Only sum values for PFAS including PFTrDA were found. No quantitative toxicological assessments of PFTrDA on human data determined by other institutions were found.

Table 33: Drinking water limits determined for PFTrDA or sum of PFAS

Country/ institution	PFTrDA limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 20 PFAS including PFTrDA, drinking water guide value	(SFA, 2022)
Hawaii (USA)	0.013	Drinking water guideline level	(ECOS, 2022)

10.3 Toxicokinetics

10.3.1 Animal data

10.3.1.1 New data

The bioaccumulation potential of PFTrDA was investigated in eight brain regions of polar bears (Ursus maritimus, n=19) including whole-brain burden and evaluated distribution patterns, sex differences, correlations with lipid content, and correlations with age (Greaves et al., 2013).The transport of PFTrDA was located in all brain regions, with regions that are close to incoming flow of blood, such as pons/medulla, thalamus, and hypothalamus, consistently containing relatively high levels (ranging from 43 to 49 ng/g wet weight) of PFTrDA. While cerebellum, striatum, and frontal, occipital, and temporal cortices, which are comprised in outer brain regions contained relatively lower levels of PFTrDA. When normalised for lipid content PFTrDA concentrations were not significantly ($p>0.05$) different among brain regions. According to the study authors this study demonstrates that in the polar bear PFTrDA crosses the blood–brain barrier with varying wet-weight concentrations depending on the region of brain.

10.3.2 Human data

10.3.2.1 New data

Studies in humans have shown varying rates of placental and breast milk transfer between different C9-C14 PFCAs. The PFAS exposure of neonates from mother during gestation and lactation was analysed for twelve PFAS in matched maternal serum, cord serum and breast milk samples collected from 50 pairs of women and their newborns between June and July 2009 in Jinhu, China (J. Liu et al., 2011). The median partition ratio through the placenta was for PFTrDA 1.74:1. The results of the study show that the partition ratio is highly varied among different PFAS. Among the six detectable PFAS, only PFTrDA has a higher concentration in cord serum comparing to corresponding maternal serum. The high detected frequency and high value of CS:

MS (child serum:maternal serum) of PFTrDA have never been observed elsewhere. Therefore, to be prudential, the results for PFTrDA have to be discussed with caution.

10.4 Health effects in humans and/or animals

10.4.1 Relevant Animal data

10.4.1.1 New data

10.4.1.1.1 Repeated dose toxicity

No relevant data was found.

10.4.1.1.2 Carcinogenicity

No relevant data was found.

10.4.1.1.3 Mutagenicity

No relevant data was found.

10.4.1.1.4 Toxicity to reproduction

Pregnant Sprague–Dawley female rats were daily administered by oral gavage doses of 0, 1, 5, or 10 mg/kg bw PFTrDA from gestational day 14 to 21 to study the effect of in utero exposure on the differentiation of foetal Leydig cells and mechanisms (C. Li et al., 2021). There was no effect on maternal body weight of dams or overt signs of maternal toxicity at any dose tested. There was no change in the total number of pups and litter size across the dose groups. Body weight and anogenital distance (biomarker for androgen) of male pups was significantly reduced at birth at the top dose of 10 mg/kg bw. At 1 mg/kg bw dose the substance significantly decreased serum testosterone (T) levels. While foetal Leydig cell number was not affected the substance promoted abnormal aggregation of foetal Leydig cells at doses of 5 and 10 mg/kg bw. Down-regulation of the expression of a number of genes and the respective proteins was also reported at a dose of 1 and 5 mg/kg bw, respectively. Autophagy was induced as evidenced by increased LC3II and beclin1 levels and reduced phosphorylation of mTOR, both occurring at 5 and 10 mg/kg bw. The study authors concluded that the substance inhibits the differentiation of foetal Leydig cells in make pups following in utero exposure principally by induction of autophagy and increased oxidative stress.

10.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

10.4.2 Relevant Human data

10.4.2.1 New data

Epidemiological studies of PFTrDA in blood serum typically evaluated health outcomes or health-relevant biomarkers in relation to log-transformed continuous measures of PFTrDA (ng/ml). Two studies (W. Cao et al., 2018; Ou et al., 2021) reported birth outcomes in relation to quantitative measures of PFTrDA that were dichotomized; studies that dichotomize exposure are inadequate for use in dose-response assessments. Cao et al. (2018) found no association between neonatal and postnatal growth measurements in infants exposed to ≥0.02 ng/ml in cord serum when compared to infants exposed to <0.02 ng/ml PFTrDA in cord serum. Ou et al.

(2018) reported no association between PFTrDA and congenital heart defects in infants exposed to maternal blood plasma PFTrDA concentrations ≥0.325 ng/ml compared <0.325 ng/ml.

In a prospective cohort study of 2,603 infants whose mothers participated in the Hokkaido Study on Environment and Children's Health Study in Japan, (Okada et al., 2014) reported decreased risk of allergic diseases at age 2 years in relation to maternal plasma PFTrDA. Pregnant women were recruited during 2003-2009. Maternal mean and median plasma PFTrDA, measured between 28 and 32 weeks of gestation, was 0.347 ng/ml and 0.329 ng/ml, respectively. Allergic diseases were defined as one or more instances of eczema, wheezing or symptoms of allergic rhino conjunctivitis. When compared to the children exposed to maternal PFTrDA concentrations in the 1st quartile (< 0.244 ng/ml), Okada et al. (2014) reported statistically significantly decreased risk of allergic diseases for 2-year old children who were exposed to maternal PFTrDA in plasma concentrations in the 2nd (0.244 – <0.329 ng/ml), 3rd (0.329 - <0.424 ng/ml), and 4th quartiles of PFTrDA (≥0.424 ng/ml): the ORs were 0.74 (95% CI 0.57–0.95), OR 0.77 (95% CI 0.60–0.99) and 0.73 (95% CI 0.56–0.94), respectively. These results were adjusted for maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, number of siblings, day care attendance, and ETS exposure in infancy at 24 months.

(Goudarzi et al., 2016) followed the same cohort of children as (Okada et al., 2014). At age 4 years, (Goudarzi et al., 2016) reported significantly decreased risk of allergic disease among children who were exposed to the highest concentrations of maternal PFTrDA (≥0.424 ng/ml compared to <0.244 ng/ml): OR 0.712, 95% CI 0.524 - 0.966 (p for trend= 0.013). After stratifying by sex, the decreased risk of allergic diseases was seen in boys but not girls.

There were no dose-response data from epidemiological studies found that are suitable for identifying a point of departure to derive a drinking water limit for PFTrDA.

10.5 Proposal for a starting point for deriving a drinking water limit value

10.5.1 Selected study

Ramboll identified a LOEL of 1 mg/kg bw from the study of (C. Li et al. 2021) on the basis of significantly reduced testosterone levels in male pups at birth. While a reduction in testosterone is not considered to be adverse it is an effect and may be linked to reduced bodyweight and reduced anogenital distance effects that have also been reported.

10.5.2 Proposal of assessment factors/modification factors with justification

An assessment factor of overall 2,100 is proposed with the following justifications for its derivation:

- AF of 1 to account for using point of departure from a developmental study to calculate a chronic drinking water limit. Timescales are not applicable since developmental toxicity does not get worse with longer exposure but is relevant to a critical time window and the experimental exposure adequately covers the pregnancy of the species under investigation.
- AF of 84 based on an approximate half-life in humans of 4,380 days (ATSDR 2021) compared to approximately 52 days in rats (Dzierlenga et al. 2020) (4380 days/52 days) derived for PFDA (please refer to Section 7.5) for intraspecies toxicokinetic differences. No half-life data were identified specific for PFTrDA in animals or humans.
- AF of 2.5 for interspecies toxicodynamic differences.

- AF of 10 to account for intraspecies differences (general population).

11 Toxicological evaluation of perfluorobutanesulfonic acid (PFBS)

11.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 34 and Table 35.

Table 34: Chemical identity of Perfluorobutane sulfonic acid (PFBS, CAS 375-73-5)

Name	Perfluorbutansulfonsäure
English Name	Perfluorobutane sulfonic acid
Acronym	PFBS
Mol. Formula	C4HF9O3S
Mol. Weight (g/mol)	300.1
CAS	375-73-5
EC	206-793-1

Table 35: Physicochemical properties of Perfluorobutane sulfonic acid (PFBS, CAS 375-73-5)

Properties	Value	Source
Density (g/cm^3)	1.81-1.85 (mod.)	(USEPA, 2020)
Melting point (°C)	20.4-106 (mod.)	(USEPA, 2020)
Boiling point (°C)	205-214 (exp.)	(USEPA, 2020)
Vapour Pressure (Pa)	132 (ext.)	(M. Kim et al., 2015)
Henry's Constant (Pa m^3/mol)	2.6 x 10^4 (exp.)	(M. Kim et al., 2015)
pKa	<0.3 (exp.)	(Vierke et al., 2013)
Log Koc	1.79 (±0.10) (exp.)	(Guelfo & Higgins, 2013)
Water Solubility (mg/l)	6875 (ext. at 25 °C)	(M. Kim et al., 2015)

(exp. = experimental, ext. = extrapolated, mod. = modelled).

11.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews quantitative toxicological risk assessments of PFBS on human data determined by other institutions (Table 36) and drinking water limits for PFBS (Table 37). Data was identified by general desk research.

Table 36: Summary of quantitative toxicological assessments of PFBS and corresponding health endpoints by other institutions

Agency	Quantitative assessment	Health endpoint	Value	Reference
ANSES	Chronic indicative toxicity values	kidney effect	0.08 mg/kg-day	(ANSES, 2017)
US EPA	Subchronic Oral Reference Dose (RfD) for Noncancer Effects	perturbation of thyroid hormone levels	9×10^{-4} mg/kg-day	(US EPA, 2021d)
US EPA	Chronic Oral Reference Dose (RfD) for Noncancer Effects	perturbation of thyroid hormone levels	3×10^{-4} mg/kg-day	(US EPA, 2021d)
Department of Health Minnesota	Subchronic Non-Cancer Health-Based Value (nHBVSubchronic)	Decreased total T4, Thyroid (E)	0.1 µg/l	(MDH, 2022)
Department of Health Minnesota	Chronic Non-Cancer Health-Based Value ($nHRL_{Chronic}$) = $nHRL_{Short\text{-}term}$	Decreased total T4, Thyroid (E)	0.1 µg/l	(MDH, 2022)

Table 37: Drinking water limits determined for PFBS or sum of PFAS

Country/ Institution	PFBS limit value in drinking water (µg/l)	Comment	Source
Germany/ UBA	6	Drinking water guide value	(UBA, 2017)
California (USA)/ OEHHA	0.5	State drinking water guideline level	(ECOS, 2022)
Hawaii (USA)/ HDH	0.6	State drinking water guideline level	(ECOS, 2022)
Illinois (USA)/ Office of the Governor	2.1	State drinking water guideline level	(ECOS, 2022)
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFBS, drinking water guide value	(SFA, 2022)

11.3 Toxicokinetics

11.3.1 Animal data

11.3.2 Data/studies reported in previous UBA evaluation

A study on the pharmacokinetics of potassium PFBS (K-PFBS) in rats and monkeys investigated the elimination after oral and intravenous (iv) administration in rats and after iv administration in cynomolgus monkeys (Macacus cynomolgus, macaques; n = 3 per sex). The PFBS concentrations were also measured in the serum and urine, and in the liver and faeces of the rat. In rats, the mean elimination half-life after iv administration of 30 mg/kg K-PFBS was 4.51 ± 2.22 h in males and 3.96 ± 0.21 h in females; after oral administration of the same dose 4.68 ± 0.43 h (males) and 7.42 ± 0.79 h (females). With a lower single PFBS dose, the mean half-life also decreases (males 2.1 h, females 0.64 h due to an iv administration of 10 mg/kg, confidence intervals not given; (Chengelis, Kirkpatrick, Myers, et al., 2009)). In the monkeys, the mean elimination half-life after iv administration of 10 mg/kg PFBS were 95.2 ± 27.1 h in the males and 83.2 ± 41.9 h in the females. Although the differences in the elimination half-lives of male and female rats were not statistically significant, clearance was significantly higher in female rats (469 ± 40 ml/h) than in males (119 ± 34 ml/h) and the concentration-time product (area under the curve, AUC) for male rats significantly larger (294 ± 77 µg·h/ml) than for the females (65 ± 5 µg·h/ml). These sex-specific differences were not observed in the monkeys (Olsen et al., 2009).

11.3.2.1 New data

According to (ATSDR, 2021), PFBS is rapidly absorbed in animals from the gastrointestinal tract with absorption half-life of <2 hours. In mice, ^{35}S-PFBS was detected in all tissues reaching a plateau following dietary exposure for 3 days. The tissue:blood ratios for PFBS, excluding stomach and small intestine, were 1.6 for liver, 1.3 for kidney, 1.1 for whole bone, and 1.1 for cartilage. Approximately 90% of the ingested ^{35}S was recovered in combined blood, liver, bone, skin, and muscle, at all-time points.

The toxicokinetic parameters of PFBS following a single intravenous (4 mg/kg) and gavage (4, 20 or 100 mg/kg) administration in male and female Sprague Dawley rats were evaluated (M. C. Huang et al., 2019). Following administration, the concentration of PFBS in the liver, kidney, and brain were measure in the male and female rats receiving 20 mg/kg via gavage. The plasma half-life of PFBS following gavage exposure was 3.3 hours in males and 1.3 hours in females. A two-compartment model was the best fit for male rats (iv and gavage) and female rats (IV), and a one-compartment model was a better fit for the female gavage exposure groups. The toxicokinetic parameters for PFBS are presented in the table below. Concentrations of PFBS were higher in the liver compared to the kidney in both males and females. PFBS concentrations in all tissues decreased slightly over time with faster decreases noted in the females.

Table 38: Pharmacokinetic Properties of PFBS as reported by (M. C. Huang et al., 2019)

Parameter	Male				Female			
	iv	gavage			iv	gavage		
	4[a] mg/kg	4[a] mg/kg	20[a] mg/kg	100[a] mg/kg	4[a] mg/kg	4[b] mg/kg	20[b] mg/kg	100[b] mg/kg
C_{max}[c] (mM)	0.0118 ± 0.017	0.053 ± 0.008	0.250 ± 0.026	0.750 ± 0.070	0..104 ± 0.016	0.028 ± 0.001	0.129 ± 0.006	0.422 ± 0.035

Parameter	Male				Female			
	iv	gavage			iv	gavage		
	4[a] mg/kg	4[a] mg/kg	20[a] mg/kg	100[a] mg/kg	4[a] mg/kg	4[b] mg/kg	20[b] mg/kg	100[b] mg/kg
T_{max}[c] (h)	NA	2.37 ± 0.56	2.18 ± 0.24	1.42 ± 0.18	NA	0.99 ± 0.13	0.71 ± 0.16	1.42 ± 0.27
K_{10} $T_{1/2}$(h)	2.26 ± 0.33	4.37 ± 18.1	2.73 ± 0.84	2.86 ± 0.39	0.36 ± 0.03	1.50 ± 0.10	1.23 ± 0.12	1.11 ± 0.10
$\alpha T_{1/2}$ (h)	0.53 ± 0.25	1.37 ± 31.5	2.37 ± 1.07	2.60 ± 0.61	0.28 ± 0.03	NA	NA	NA
$\beta T_{1/2}$ (h)	4.22 ± 028	4.89 ± 1.67	5.36 ± 1.24	5.25 ± 1.19	0.95 ± 0.10	NA	NA	NA
AUC_{∞}[a] (mM/h)	0.387 ± 0.023	0.513 ± 0.050	1.776 ± 0.150	4.399 ± 0.332	0.053 ± 0.004	0.088 ± 0.011	0.364 ± 0.078	1.289 ± 0.165
AUC_{∞}/Dose (mM/h/mg/kg)	0.097 ± 0.006	0.128 ± 0.012	0.089 ± 0.007	0.044 ± 0.003	0.013 ± 0.001	0.022 ± 0.003	0.018 ± 0.004	0.013 ± 0.002
CL (ml/h/kg)	34 ± 2.0	26.0 ± 2.5	37.6 ± 3.1	75.5 ± 5.8	252 ± 18	152 ± 20	183 ± 39	259 ± 33
V_1[d] (ml/kg)	113 ± 16	164 ± 677	148 ± 52	311 ± 12	123 ± 12	328 ± 57	326 ± 95	415 ± 83
V_2[e] (ml/kg)	74.8 ± 18.8	13.3 ± 544	19.0 ± 17.7	23.9 ± 19.5	42 ± 7	NA	NA	NA
F[f] (%)	NA	133	92	46	NA	166	137	97

NA, not applicable; C_{max}, peak concentration; AUC, Area under the curve; CL, clearance; α $T_{1/2}$ initial half- life; β $T_{1/2}$, terminal half-life; K_{10} $T_{1/2}$ elimination half-life; V_1, apparent volume of central distribution; V_2, apparent volume of peripheral distribution; F, bioavailability

[a] Predicted using two compartment model

[b] Predicted using a one compartment model

[c] Predicted from model

[d] Volume of distribution for the central compartment

[e] Volume of distribution for the peripheral compartment

[f] Estimated by dividing dose-normalized gavage AUC by dose-normalized iv AUC

(Lau et al., 2020) evaluated the pharmacokinetic properties of PFBS in male and female CD-1 mice given PFBS via oral gavage at doses of 30 or 300 mg/kg. Blood was collected at 0.5, 1, 2, 4, 8, 16 and 24 hours post dosing. Liver and kidneys were harvested and serum and tissue concentrations of PFBS were determined. The half-life of PFBS was determined to be 5.8[4] hours

[4] Data are expressed as means and 95% confidence intervals: statistical analysis did not indicate a significant difference between doses (30 and 300 mg/kg), therefore, estimates of combined doses (30/300 mg/kg) are indicated in hours:
Males: serum dose 30/300 → half life serum: 5.8 (4.9, 6.8); half life kidney: 5.8 (4.9, 7.0); half life liver: (4.4 (3.3, 5.9)

in males and 4.5[5] hours in females, which is comparable to the half-lives reported in rats. The volume of distribution was similar between males and females and Tmax was reached within 1 to 2 hours. Clearance was linear with administered doses and serum PFBS declined to <5% of Cmax with 24 hours.

(Narizzano et al., 2021) administered PFBS to male and female mice in drinking water at concentrations of 0, 5, 10, or 12 mg/kg/day for 28 days. Blood samples were collected at 14, 21, and 28 days and analysed for PFBS. Serum levels of PFBS were similar at days 21 and 28 with higher serum levels reported in the higher dose groups. After 28 days of exposure the serum PFBS levels were 300, 500, and 700 ng/ml in both the males and females in the low-, mid- and high-dose groups, respectively.

During a three-week feeding study conducted in 24 fattening pigs, the transfer of a mixture of 12 PFAS (PFSAs: PFBS, PFHxS, PFHpS, PFOS, PFDS; PFCAs: PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA) from contaminated feed was evaluated (ECHA, 2017; Numata et al., 2014). Analyses of PFAS were performed in the feed, tissues, and urinary excretions. Results indicated a fast equilibrium between plasma and edible tissue with PFAS generally accumulating in the plasma, fat, muscle, liver, and kidney. The authors developed a toxicokinetic model to calculate elimination half-lives. The 95% variability elimination half-life interval for PFBS was 13 to 135 days.

(Feng et al., 2017) reported that oral administration of PFBS at doses of 0, 50, 200, or 500 mg/kg bw·d during gestation days 1 through 20 in pregnant mice resulted in a dose-dependent increase in serum PFBS levels. PFBS serum levels were 1.73±0.65 ng/ml; 74.01±22.52 ng/ml; 332.41±53.04 ng/ml; 720.86±98.4 ng/ml at doses of 0, 50, 200, or 500 mg/kg bw·d, respectively.

11.3.3 Human data

11.3.3.1 Data/studies reported in previous UBA evaluation

(Olsen et al., 2009) investigated the serum half-lives of potassium PFBS (K-PFBS) in humans in five men and one woman who were exposed in the workplace. The serum half-life was up to 180 days and the geometric mean was 25.8 days (95% confidence interval 16.6–40.2 days; Olsen et al., 2009).

11.3.3.2 New data

The concentration of PFBS was analysed in 132 paired samples of maternal and cord serum from residents in Beijing China (Gao et al., 2019). PFBS was detected in 87% of the maternal serum and 87% of the cord serum with median levels of 0.14 and 0.12 ng/ml in the maternal and cord serum, respectively. The calculated placental transfer efficiency for PFBS was 97%.

(Y. Xu et al., 2020) estimated the half-life of PFBS in serum in a group of 26 airport employees. Blood samples were collected from all 26 employees and 17 of them were followed up monthly for 5 months. The average half-life of PFBS was 44 days.

[5] Data are expressed as means and 95% confidence intervals: statistical analysis did not indicate a significant difference between doses (30 and 300 mg/kg), therefore, estimates of combined doses (30/300 mg/kg) are indicated in hours:
Females: serum dose 30/300 → half life serum: 4.5 (3.7, 5.4); half life kidney: 4.4 (3.5, 5.5); half life liver: 3.0 (1.9, 4.7)

11.4 Health effects in humans and/or animals

11.4.1 Relevant Animal data

11.4.1.1 Data/studies reported in previous UBA evaluation

11.4.1.1.1 Repeated dose toxicity

A 90-day gavage study was conducted in rats administered K-PFBS doses of 0, 60, 200, or 600 mg/kg bw·d. Clinical observations, food consumption and body weight were recorded, and macroscopic and microscopic pathology, clinical chemistry and haematology were carried out. In the groups administered 60 and 200 mg/kg·d, the nasal cavities and turbinates, stomach and kidneys were also examined histologically. The K-PFBS administration produced no effects on mortality, body weight or neurological parameters. Erythrocyte count (in the 600 mg/kg bw·d group), haemoglobin and haematocrit values were significantly reduced in the males in the 200 and 600 mg/kg bw ·d dose group (haemoglobin in g/dl: controls: 16.4 ± 0 .96, 60 mg/kg bw·d: 16.0 ± 0.41, 200 mg/kg bw ·d: 15.6 ± 0.48, $p \leq 0.05$; 600 mg/kg bw·d: 15.5 ± 0.78, $p \leq 0.05$; Haematocrit values in %: control: 44.2 ±2.32; 60 mg/kg bw·d: 42.7 ± 1.44; 200 mg/kg bw ·d: 41.9 ± 1.50, $p \leq 0.05$; 600 mg/kg bw ·d: 40.9 ± 2.24, $p \leq 0.01$). In this study, the female rats showed no significant effects up to the highest dose of 600 mg/kg bw ·d. For the male rats, the NOAEL was 60 mg/kg bw ·d due to the haematological effects at reported at 200 mg/kg bw·d (Lieder, Chang, et al., 2009).

Male mice were administered K-PFBS at 30 mg/kg bw d in the diet for 4 to 6 weeks. Decreases in plasma triglycerides (-37%) and non-HDL-cholesterol (non-high-density lipoprotein; - 28 %) were reported at 30 mg/kg bw d and an increased excretion of (radioactively labeled) triolein (-51 %) was reported (Bijland et al., 2011).

11.4.1.1.2 Carcinogenicity

No data was reported.

11.4.1.1.3 Mutagenicity

The *in vitro* investigation of the induction of oxidative DNA damage and the potential for the formation of reactive oxygen species (ROS) in human hepatoma cells Hep G2 of up to 2000 µM PFBS showed no effects compared to the controls (Eriksen et al., 2010).

11.4.1.1.4 Toxicity to reproduction

A two-generation study with rats was also carried out with K-PFBS. Males and females of the parental generation (P) were given gavage doses of 30, 100, 300 or 1000 mg/kg bw·d K-PFBS for ten weeks before and during mating and during pregnancy and lactation (females). The first branch generation (F1) was dosed comparably from weaning onwards. The second filial generation (F2) was no longer directly exposed, and the study ended three weeks after their birth. Body weight and food consumption were recorded, and clinical signs, oestrus cycle, sperm quality, pregnancy, parturition, litter size and developmental parameters were examined. An adverse effect was not detectable in either the P or the F1 generations at 100 mg/kg bw ·d (NOAEL). In the groups administered 300 and 1000 mg/kg bw ·d, the males had increased liver weights (absolute and relative) and, correspondingly, an increased occurrence of adaptive hepatocellular hypertrophy. In addition, an increased occurrence of minimal to slight microscopic findings in the kidney marrow in both males and females and renal papillae were reported. Neither in the P nor in the F1 generation were K-PFBS-related (biologically relevant) effects on fertility, reproduction or on the corresponding parameters reported. There were no K-PFBS-related effects on offspring survival across the two-generation study. Litter size and

average birth weight per litter were not statistically significantly different from the controls in any dose group. No adverse effects were observed in the F1 females, the F2 offspring had normal body weights. According to the authors (Lieder, York, et al., 2009), the reproductive NOAEL was > 1000 mg/kg bw·d in both generations.

11.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

In COS 1 cells into which mouse or human PPARα plasmids had been transferred, 1–250 µM PFBS activated the luciferase of both mouse and human plasmids in a concentration-dependent manner compared to controls. The human PPARα reacted more sensitively to PFBS (in contrast to the investigated perfluorinated carboxylic acids) than the mouse PPARα (Wolf et al., 2008, 2012).

(Slotkin et al., 2008) investigated the *in vitro* neurotoxicity of PFBS on neuronal PC12 cells at concentrations up to 250 µM, a standard *in vitro* model for neuronal development. The inhibition of DNA synthesis, deficits in cell number and growth, oxidative stress, reduced cell viability (functionality) and a shift in the differentiation of the neurotransmitters dopamine (DA) and acetylcholine (ACh) were examined. In undifferentiated cells, DNA synthesis, cell number, and lipid peroxidation were not significantly altered by PFBS. In differentiating cells, on the other hand, the number of cells was increased with the DNA content remaining the same, as was lipid peroxidation, but without impairment of functionality. The differentiation of both neurotransmitters DA and ACh was reduced by PFBS in a concentration-dependent manner (in contrast to the other tested substances PFOA, PFOS or PFOSA). The authors conclude that there is no common neurotoxicity mechanism of action of the perfluorinated substances.

In contrast to PFOS and PFOA, K-PFBS did not inhibit the activity of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in microsomes of human and rat testes at 250 µM *in vitro* (Zhao et al., 2010). The glucocorticoid metabolism (a steroid hormone from the adrenal cortex) in human and rat kidney microsomes is only slightly disturbed by PFBS (Zhao et al., 2011).

In JEG-3 choriocarcinoma cells of human placenta, PFBS inhibited the CYP19 aromatase activity in a dose-dependent manner (in vertebrates, the aromatase catalyses the conversion or the aromatization of testosterone to oestradiol and of androstenedione to estrone), an indication of an effect of PFBS on the hormonal system balance between androgens and oestrogens. The authors (Gorrochategui et al., 2014) found that PFBS was effective in these cells even at very low concentrations, although PFBS uptake was low.

11.4.1.2 New data

11.4.1.2.1 Repeated dose toxicity

(NTP, 2022) conducted a 28-day toxicity study in groups of 10 male and 10 female Sprague Dawley rats to compare the toxicity of seven PFAS (three sulfonic acids or salt: perfluorobutane sulfonic acid [PFBS], perfluorohexane sulfonate potassium salt [PFHxSK], and perfluorooctane sulfonic acid [PFOS], and four carboxylates). PFBS was administered via oral gavage for 28 days at doses of 0, 62.6, 125, 250, 500, or 1000 mg/kg bw·d (half of the dose administered twice a day). Results indicated statistically significant dose-dependent decreases in total triiodothyronine (T3), total thyroxine (T4), and free T4 in male and female rats at all doses tested (≥62.6 mg/kg-day) (NTP, 2022). The reported reductions in rat total T3 were up to 57% (male) and 43% (female), in free T4 up to 86% (male) and 77% (female), and in total T4 up to 97% (male) and 71% (female). After 28 days of PFBS exposure in male or female rats at doses up to 1,000 mg/kg-day no changes of thyroid gland weight, thyroid histopathology, and TSH levels were observed (NTP, 2022). At 500 mg/kg bw·d PFBS exposure resulted in significantly

increased absolute and relative right kidney weights in male rats (NTP, 2022). Only relative kidney weights were altered in female rats, but this effect was significant at all tested PFBS doses (≥62.6 mg/kg bw·d). PFBS exposure significantly increased relative and absolute liver weights in males at doses of 125 and 62.6 mg/kg bw·d and greater, respectively, and females at doses of 250 and 125 mg/kg bw·d and greater, respectively. A significantly increased incidence of hepatocellular hypertrophy was reported in male (≥125 mg/kg bw·d) and female (≥500 mg/kg bw·d) rats. In these rats (male and female at ≥500 mg/kg bw·d) significantly increased cytoplasmic alteration of hepatocytes was observed.

11.4.1.2.2 Carcinogenicity

No relevant data was found.

11.4.1.2.3 Mutagenicity

No relevant data was found.

11.4.1.2.4 Toxicity to reproduction

In (NTP, 2022) male and female rats were exposed to PFBS via oral exposure for 28-days at doses of 0, 62.2, 125, 250, 500, or 1000 mg/kg bw·d. At the time of necropsy, for male rats the results indicated a decreased trend in testicular spermatid count per mg testis. No significant effects on other sperm measures were reported, including sperm motility and caudal epididymal sperm count. In their review of the (NTP, 2022) study, (US EPA, 2021d) noted that a complete spermatogenesis cycle in male rats is typically 7 weeks in length, thus study designs of shorter duration could potentially miss effects of chemical exposure on some sperm parameters. Over all doses groups, exposure to PFBS for 28 days resulted in a significant trend for increased testosterone levels for the female rats in general, but not in males (NTP, 2022). However, the increase in testosterone was not statistically significant when compared to control at any dose by pairwise analysis. No significant histopathological changes in reproductive organs were reported and reproductive organ weights, including testes, ovaries, and uterus, were unchanged.

(Feng et al., 2017) exposed pregnant ICR mice to doses of 0, 50, 200 or 500 mg/kg bw·d PFBS via oral gavage throughout gestation (GDs 1−20). Statistically significant reductions of total T3, total T4, and free T4 (reduced 17, 21, and 12%, respectively, relative to control at 200 mg/kg-day and reduced 16, 20, and 11%, respectively, relative to control at 500 mg/kg bw·d) on GD 20 at doses of 200 and 500 mg/kg bw·d were observed, with no significant changes reported at 50 mg/kg bw·d (Feng et al., 2017). Decreased total T3 and total T4 were also reported in the female offspring on post-natal days (PNDs) 1, 30, and 60 in offspring gestationally exposed to PFBS at the same doses. In dams and pubertal (PND 30) offspring (21 and 14% relative to control at 200 mg/kg bw·d, respectively) exposed gestationally to PFBS significantly increased thyroid-stimulating hormone (TSH) was reported. The female offspring exposed to 200 and 500 mg/kg bw·d in utero exhibited significantly decreased perinatal body weight and delated eye opening compared to control offspring. Significantly delated vaginal opening and first oestrus, as well as significantly prolonged dioestrus were also reported in female offspring exposed to 200 and 500 mg/kg bw·d in utero. In pubertal and adult offspring exposed to 200 and 500 mg/kg bw·d in utero significant decreases in serum oestrogen (E2) and progesterone (P4) levels were reported with the elevation of luteinizing hormone levels. The authors concluded that prenatal PFBS exposure at doses of 200 mg/kg bw·d produces hypothyroxinemia accompanied by deficits in prenatal growth, pubertal onset, and reproductive organ development in female mice.

11.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

11.4.2 Relevant Human data

11.4.2.1 Data/studies reported in previous UBA evaluation

No data was reported.

11.4.2.2 New data

(Gao et al., 2019) conducted a cross-sectional study of maternal PFAS exposure and birth outcomes. The study population included 132 pregnant women with a mean age of 31 years who visited the Affiliated Hospital of Capital Medical University in Beijing for delivery between 2015 and 2016. Maternal blood samples for exposure assessment were collected one to two days before delivery and cord blood samples were collected immediately after birth. Median PFBS in maternal serum was 0.14 ng/ml; in cord serum, median PFBS was 0.12 ng/ml. Models were adjusted for maternal age, height, weight, BMI, and gestational age, depending on the analysis. The highest exposure category of PFBS in maternal serum (>0.170 ng/ml) was not statistically significantly associated with birth weight, birth length, gestational age, or ponderal index; similarly, the highest exposure category of PFBS in cord serum (>0.155 ng/ml) was not statistically significantly associated with any of these outcomes.

(T.-W. Lin et al., 2020) conducted a cross-sectional study of 397 adults between 55 and 75 years of age from PFAS-exposed communities in Taiwan from 2016-2017 to examine the association between nine serum PFAS and metabolic syndrome and related biomarkers (uric acid, fasting blood sugar, total cholesterol, and triglycerides). The median serum PFBS among participants was 6.33 ng/ml. In models stratified by sex and adjusted for age, smoking, and alcohol use, no statistically significant associations were detected between PFBS and metabolic syndrome status, uric acid, fasting blood sugar, total cholesterol, triglycerides, or LDL cholesterol. Among women only, the highest quartile of PFBS (>7.8 ng/ml) was associated with increased HDL cholesterol (β=5.05, 95% CI 0.09-10.01) compared to the lowest quartile (<4.3 ng/ml); the association did not reach statistical significance among men. In analyses not stratified by sex, the authors did not observe associations between PFBS and metabolic syndrome or related biomarkers.

11.5 Proposal for a (new) starting point for deriving a drinking water limit value

In the previous UBA assessment the drinking water limit for PFBS was based on a NOAEL of 60 mg/kg bw·d based on haematological effects at the next higher dose of 200 mg/kg bw d following 90 days of exposure in rats reported in (Lieder, Chang, et al., 2009). Based on the NOAEL from the study by Lieder et al. (2009a) in the amount of 60 mg/kg·d, after taking into account a time extrapolation (factor 10 for the transfer of the NOAEL from a 90-day study to a lifelong exposure), an interspecies extrapolation (for the toxicokinetics according to the elimination half-life human/rat [mean of ♀ and ♂]: 620 h (≈25.6 d) / 4.25 h = factor 146, and for toxicodynamics factor 2.5) and the intraspecies differences (factor 10, $10^{0.5}$ each or 3.16 for the toxicokinetic and toxicodynamic differences; WHO, 2005) the UBA derived a human-equivalent dose of 1.64 µg/kg·d. With the usual key data (70 kg body weight, 2 litres of drinking water consumption per day, 10 % allocation of the tolerable body dose for drinking water only) a TW_{LW} of (5.74 or rounded up) 6 µg/l.

Based on new data from a developmental study conducted in female mice and the female offspring, the starting point for a TW_{LW} is the lowest NOAEL of 50 mg/kg bw d in mice based on the results of the study conducted by (Feng et al., 2017), which is slightly lower than the previous recommended POD of 60 mg/kg bw·d (Lieder, Chang, et al., 2009).

The half-life of elimination (HWZ) in male mice is used for the toxicokinetics factor. However, female animals were exposed. The HWZ (males: 5.8 h, females: 4.5 h) hardly differ, but correctly the data of female animals should be used. (Y. Xu et al., 2020) calculated a HWZ for PFBS in humans of 44 d.

The effect seen is the reduced concentration of T3 and T4 in the blood, which was observed in the pregnant dams (at the end of exposure GD1-20) and in the (female) offspring (postnatally).

(In the subchronic and multi-generation rat studies, exposure was longer but unfortunately T3, T4 and TSH were not measured).

It is now an aspect for discussion:

If one sees the effect on the dams as an effect in its own right, independent of the effect on the offspring. In this case one would have to apply a time assessmentextrapolation factor of 6 (subacute to chronic).

If, on the other hand, one sees the reduced concentration of T3 and T4 in the maternal blood as the cause of the developmental delay in the offspring, then the time extrapolation factor would be 1, because the entire time seen as the critical time window (gestation) is covered by the exposure and thus a time extrapolation is not necessary.

The project team tends to the latter view, but this can certainly be discussed.

In that case, the "old" derivation (basis: rat study, subchronic, but with new time extrapolation factor) and the "new" derivation would yield an almost identical value as shown in the following table:

Table 39: Comparison of old and new assessment factors

"New":	"Old"
NOAEL 50 mg/kg x d	NOAEL: 60 mg/kg x d
Time extrapolation: 1	Time extrapolation: 2 (not 10 as originally)
Toxicokinetics: 44 d: 0.1875 d (=4.5 h) = 235 (rounded)	Toxicokinetics Intraspecies: 146 (where the HWT of elimination human/rat [mean of ♀ and ♂] was set to 620 h (≈25.6 d) / 4.25 h, i.e. shorter than in the "new").
Residual factor: 2.5	Residual factor: 2.5
Intraspecies: 10	Intraspecies: 10
Thus 50 : (235 x 2.5 x 10) = 0.0085 mg/kg bw x d	Thus 60 : (2 x 146 x 2.5 x 10) = 0.0082 mg/kg bw x d.

However, if in the "old" derivation the toxicokinetics factor was set at 44 d : 0.177 d (4.25 h) = 248, this would result in 60 (2 x 248 x 2.5 x 10) = 0.0048 mg/kg x d.

12 Toxicological evaluation of perfluoropentanesulfonic acid (PFPeS)

12.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 40 and Table 41.

Table 40: Chemical identity of Perfluoropentanesulfonic acid (PFPeS, CAS 2706-91-4)

Name	Perfluorpentansulfonsäure
English Name	Perfluoropentanesulfonic acid
Acronym	PFPeS
Mol. Formula	C5HF11O3S
Mol. Weight (g/mol)	350.1
CAS	2706-91-4
EC	220-301-2

Table 41: Physicochemical properties of Perfluoropentanesulfonic acid (PFPeS, CAS 2706-91-4)

Properties	Value	Source
Density (g/cm^3)	1.81-1.84 (mod.)	(USEPA, 2020)
Melting point (°C)	10,7-135 (mod.)	(USEPA, 2020)
Boiling point (°C)	198-225 (exp.)	(USEPA, 2020)
Vapour Pressure (Pa)	3.8 x 10^{-5} (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	2.2 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	8.1-823 (mod.)[a]	(USEPA, 2020)

(mod = modelled); [a] EPISUITE: 2.31 x 10^{-5} mol/l = 8.1 mg/l, TEST Model: 2.79 x 10^{-4} mol/l = 98 mg/l, OPERA Model: 2.0 x 10^{-3} mol/l = 823 mg/l.

12.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFPeS (Table 42). Data was identified by general desk research.

Table 42: Drinking water limits determined for PFPeS or sum of PFAS

Country/ Institution	PFPeS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0,1	Sum of 21 PFAS including PFPeS, drinking water guide value	(SFA, 2022)

12.3 Toxicokinetics

12.3.1 Animal data

12.3.1.1 New data

No relevant data was found.

12.3.2 Human data

12.3.2.1 New data

An overview of the data on PFAS in general can be found in (Bull et al., 2014); no information for PFPeS was found. No information about PFPeS is available in the Agency for Toxic Substances and Disease Registry (ATSDR) report (ATSDR, 2021).

The serum half-life of the short-chain PFAS including PFPeS were measured in 26 airport employees after accidental expose to high levels of short-chain PFAS through drinking water in 2018. Average serum half-live of PFPeS was found to be 0.63 year (~230 days) (Y. Xu et al., 2020).

The serum half-lives for different PFAS (including PFPeS) and their determinants were estimated in the population of Ronneby, Sweden after long-term high exposure to contaminated drinking water. The mean estimated half-live (in years) for PFPeS was 0.94 (95 %CI 0.86–1.02) based on the result of this study (Y. Li et al., 2022).

12.4 Health effects in humans and/or animals

12.4.1 Relevant Animal data

12.4.1.1 New data

12.4.1.1.1 Repeated dose toxicity

No relevant data was found.

12.4.1.1.2 Carcinogenicity

No relevant data was found.

12.4.1.1.3 Mutagenicity

No relevant data was found.

12.4.1.1.4 Toxicity to reproduction

The zebrafish is a widely used *in vivo* model for toxicity testing. Developmental toxicity (DevTox) and developmental neurotoxicity (DNT) of PFPeS were investigated in assays conducted on zebrafish. In DevTox assay zebrafish were exposed to 1.7, 3.1, 5.5, 9.8, 17.6, 31.4, 56.0, 100.0 µM PFPeS, or 0.4% DMSO (vehicle control). Chlorpyrifos was used as positive control for malformations and lethality. PFPeS was found to be a more potent developmental toxicant (EC_{50} = 48.8 µM; LOEC = 56.0 µM) than other tested sulfonic acid aliphatic PFAS (e.g., PFHxS and PFHpS). PFPeS was nearly as potent as PFOS, the most potent chemical evaluated in the study. In DNT assay zebrafish were exposed to 3.1, 5.5, 9.8, 17.6, 31.4, or 56.0 µM PFPeS, or 0.4% DMSO. PFPeS showed to provoke hyperactivity in DNT assay. It was concluded in the study that data suggest that developmental neurotoxicity is an important end point to consider for the PFAS (including PFPeS) (Gaballah et al., 2020).

12.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

12.4.2 Relevant Human data

12.4.2.1 New data

No relevant data was found.

12.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

13 Toxicological evaluation of perfluoroheptanesulfonic acid (PFHpS)

13.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 43 and Table 44.

Table 43: Chemical identity of Perfluoroheptanesulfonic acid (PFHpS, CAS 375-92-8)

Name	Perfluorheptansulfonsäure
English Name	Perfluoroheptanesulfonic acid
Acronym	PFHpS
Mol. Formula	C7HF15O3S
Mol. Weight (g/mol)	450.1
CAS	375-92-8
EC	206-800-8

Table 44: Physicochemical properties of Perfluoroheptanesulfonic acid (PFHpS, CAS 375-92-8)

Properties	Value	Source
Density (g/cm^3)	1.84-1.89 (mod.)	(USEPA, 2020)
Melting point (°C)	24.9-182 (mod.)	(USEPA, 2020)
Boiling point (°C)	206-226 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	4.4 x 10^{-5} (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	1.8 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	3.7 (± 0.3) - 4.1 (exp.)	(Munoz et al., 2015)
Water Solubility (mg/l)	0.04-464 (mod.)[a]	(USEPA, 2020)

(exp. = experimental, mod = modelled); [a] EPISUITE: 9.66 x 10^{-8} mol/l = 0.04 mg/l, TEST Model: 1.17 x 10^{-5} mol/l = 5.3 mg/l, OPERA Model: 1.03 x 10^{-3} mol/l = 464 mg/l.

13.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFHpS (Table 45). Data was identified by general desk research.

Table 45: Drinking water limits determined for PFHpS or sum of PFAS

Country/ Institution	PFHpS limit value in drinking water (µg/l)	Comment	Source
Germany/ UBA	0.3	Health related indicator value (GOW)	(UBA, 2017)
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFHpS, drinking water guide value	(SFA, 2022)

13.3 Toxicokinetics

13.3.1 Animal data

13.3.1.1 Data/studies reported in previous UBA evaluation

No data was reported.

13.3.1.2 New data

PFHpS was detected in the serum of cattle that had been exposed for several years to water contaminated by residues of Aqueous Film-Forming (AFFF) firefighting foam (Drew et al., 2022). The elimination serum half-life was determined in five heifers from serial blood sampling over 215 days. In this regard days means the number of days after elimination stated (i.e. end of exposure). Eleven additional animals that had blood sampled on day 19 were euthanised on day 63. PFAS half-life estimates from the serial blood sampling and from day 19/day 63 data were not significantly different. The combined (n = 16) serum half-life (in days) was 45.7 ± 9.4. Neither the age of animal (1.4–12.3 years old) nor serum concentration at the start of depuration influence the half-life, and there was no difference between steers and heifers. Depuration of PFHpS was essentially the same in serum, kidney and liver, and the study authors expected depletion from muscle to be comparable.

During a three week feeding study conducted in 24 fattening pigs, the transfer of a mixture of 7 PFAS (PFSAs: 132 ± 11 µg/kg PFBS, 91.3 ± 8 µg/kg PFHxS, 4 ± 0.5 µg/kg PFHpS, 137 ± 13 µg/kg PFOS; PFCAs: 47.8 ± 4,4 PFHxA, 10.2 ± 1.7 PFHpA, 22.4 ± 2.6 PFOA)) from contaminated feed was evaluated (EFSA, 2020; Numata et al., 2014). Analyses of PFAS were performed in the feed, tissues, and urinary excretions. Results indicated a fast equilibrium between plasma and edible tissue with PFAS generally accumulating in the plasma, fat, muscle, liver, and kidney. Over 80% of the total mass of ingested PFHpS together with PFBS, PFHxS, PFOS, PFOA was found in blood and other tissues. For all investigated PFAS faecal excretion occurred at a limited extent with less than 8% of the ingested dose and urinary excretion was <5% of the dose for PFHxS, PFHpS, PFOS and PFOA, ranging from 10 to 20% for PFHpA and PFBS and higher than 60% for PFHxA. Significant differences between males and females could not be found. The authors developed a toxicokinetic model to calculate elimination half-lives. The plasma half-life was estimated to be 2 years for PFHxS and 411 days for PFHpS.

13.3.2 Human data

13.3.2.1 Data/studies reported in previous UBA evaluation

PFHpS has been detected in the serum of pregnant women (0.09 ng/ml; n = 29 of 44 above the limit of quantification) and in the serum from umbilical cord blood (0.06 ng/ml; n = 14 of 42 above the limit of quantification) of the general population of Seoul (S. Kim et al., 2011).

13.3.2.2 New data

Cord:maternal serum (transplacental transfer) and serum:whole cord blood (blood partitioning) ratios were estimated for PFHpS in 151 mother-newborn pairs from two successive Faroese birth cohorts (Eryasa et al., 2019). Serum: whole blood ratios reflecting blood partitioning of PFHpS of median ratios of 2.64 (IQR: 2.31–3.42). This study reports the transplacental transfer of PFHpS.

The serum half-lives of PFHpS were determined in 100 inhabitants of varying ages in southern Sweden, Ronneby, following a high volume PFAS contamination event of a drinking water source at a waterworks (Y. Li et al., 2022). The serum PFHpS at 6 months following the contamination event was substantially higher than that found in the unexposed reference population. In a four-year period (June 2014 to May 2018), the geometric mean serum level of PFHpS decreased by 38%. PFHpS exhibited a half-life of 4.6 years (4.55 years, 95 % CI 4.14–5.06) and a longer half-life was associated with a higher initial exposure.

The PFAS profile in drinking water and biological samples (paired serum and urine) was determined to estimate serum half-lives of the short-chain PFAS together with legacy PFAS after cessation of exposure from a drinking water contaminated with firefighting foam at a municipal airport in northern Sweden (Y. Xu et al., 2020). Blood samples of 26 airport workers were collected. From 17 study participants blood and urine samples were collected on a monthly basis for 5 months. PFHpS showed an average half-life of 1.46 years, which according to the study authors reflected the lower serum/water ratio and larger urine/serum ratio thus indicating higher rate of renal clearance. The authors note that the half-life of PFHpS is much lower in this study compared to the study by (Y. Li et al., 2022) described above and attributed this difference to the difference in study population and the difference in the follow up period.

13.4 Health effects in humans and/or animals

13.4.1 Relevant Animal data

13.4.1.1 Data/studies reported in previous UBA evaluation

13.4.1.1.1 Repeated dose toxicity

No data was reported.

13.4.1.1.2 Carcinogenicity

No data was reported.

13.4.1.1.3 Mutagenicity

No data was reported.

13.4.1.1.4 Toxicity to reproduction

No data was reported.

13.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No data was reported.

13.4.1.2 New data

13.4.1.2.1 Repeated dose toxicity

No relevant data was found.

13.4.1.2.2 Carcinogenicity

No relevant data was found.

13.4.1.2.3 Mutagenicity

No relevant data was found.

13.4.1.2.4 Toxicity to reproduction

No relevant data was found.

13.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

13.4.2 Relevant Human data

13.4.2.1 Data/studies reported in previous UBA evaluation

PFHpS has been detected in the serum of pregnant women (0.09 ng/ml; n = 29 of 44 above the limit of quantification) and in the serum from the umbilical cord blood (0.06 ng/ml; n = 14 of 42 above the limit of quantification) of the general population of Seoul (S. Kim et al., 2011).

13.4.2.2 New data

(Bach et al., 2018) studied a subset of 1,251 women from the longitudinal Danish National Birth Cohort, recruited during 1996-2002. Median serum PFHpS in this cohort was 0.4 ng/ml among nulliparous women and 0.3 ng/ml among parous women. After adjusting for age, pre-pregnancy BMI, socioeconomic status, and interpregnancy interval (among parous women only), the authors reported no association between quartiles of plasma PFHpS and fecundability ratio among either nulliparous or parous women (highest quartile of PFHpS 0.55-1.73 ng/ml among nulliparous women; 0.43-2.01 ng/ml among parous women).

(Meng et al., 2018) used data from 2,120 women and infants from the Danish National Birth Cohort, originally recruited during 1996-2002, to examine the relationship between maternal plasma PFAS and birth outcomes. Median maternal PFHpS measured in the first trimester was 0.4 ng/ml. In analyses adjusted for child sex, birth year, week of blood draw, maternal age, parity, socio-occupational status, pre-pregnancy BMI, and smoking and alcohol consumption during pregnancy, decreased birth weight was reported in the two highest exposure quartiles (0.4 to <0.5 ng/ml and ≥0.5 ng/ml) when compared to the lowest quartile (<0.3 ng/ml) (β= -110.8 g, 95% CI -177.7 to -43.8 and β= -102.6 g, 95% CI -169.0 to -36.2, respectively). Decreased gestational age was also observed in the three highest exposure quartiles when compared to the lowest quartile: β= -1.7 days, 95% CI -3.0 to -0.4 for maternal plasma PFHpS concentrations 0.3 to <0.4 ng/ml, β= -2.6 days, 95% CI -4.0 to -1.3 for maternal plasma PFHpS concentrations 0.4 ng/ml to <0.5 ng/ml, and β= -2.0 days, 95% CI -3.3 to -0.7 for maternal plasma PFHpS ≥5 ng/ml. PFHpS was not statistically significantly associated with low birth weight (<2500 g) or with preterm birth (<37 weeks).

(Inoue et al., 2019) conducted a cross-sectional study of PFAS exposure and thyroid hormones among 1,366 women from the Danish National Birth Cohort, which enrolled pregnant women at their first antenatal care visit between 1996 to 2002. Maternal blood samples were collected at a median of 8 gestational weeks; the median PFHpS in maternal plasma was 0.37 ng/ml. In analyses adjusted for age, socio-economic status, parity, smoking, pre-pregnancy body mass index, and birth year, the authors found no association between PFHpS and thyroid-stimulating hormone or free total thyroxine.

(Ernst et al., 2019) examined the association between prenatal PFAS exposure and pubertal development in 445 Danish children born between 2000-2003 who were part of the Puberty Cohort or the Danish National Birth Cohort. Maternal blood samples were collected between 5 and 25 gestational weeks (median 9 weeks); the median maternal plasma PFHpS concentration was 0.4 ng/ml. After adjusting for parental social class, mother's age at menarche and delivery, parity, pre-pregnancy body mass index, and maternal smoking in the first trimester, the authors reported no statistically significant associations between tertiles of PFHpS and the age at attaining a sex-specific combined indicator of puberty (described as the Tanner rating scale). In additional analyses which compared average differences in the age of onset of specific pubertal milestones by tertiles of PFHpS, the authors reported that breasts developed in girls on average at earlier age (9.05 months) for the second (0.30 – 0.42 ng/ml), but not third (0.43 – 1.52 ng/ml), tertiles of exposure.

(Averina et al., 2021) performed a cross-sectional study of 940 adolescents in Norway during 2010-2011. The geometric mean PFHpS was 0.14 ng/ml and 0.16 ng/ml among girls and boys, respectively. After adjusting for age, sex, and dietary factors, the authors reported that serum PFHpS was associated with obesity, but the odds ratios did not increase with increasing quartiles of exposure. The authors reported no statistically significant association between PFHpS and serum lipids or hypertension.

(Itoh et al., 2021) conducted a case control study of PFAS exposure, including PFHpS, and breast cancer. The study population included 401 cases and 401 controls recruited from four hospitals in Nagano Prefecture, Japan during 2001-2005. Controls were matched on age (within 3 years) to cases. PFAS was measured in serum samples. PFHpS exposure was categorized as low (<0.09 ng/ml), medium (0.09 – 0.14 ng/ml), and high (0.15 – 2.62 ng/ml). Among cases, 200, 148, and 53 subjects were in the low, medium, and high groups, respectively, and among controls 153, 138, and 110 subjects were in the low, medium, and high groups, respectively. After adjusting for age, area of residence, BMI, height, menopausal status, age at menopause, age at first childbirth, family history of breast cancer, smoking, physical activity, age at menarche, number of births, duration of breastfeeding, alcohol use, education, serum PCBs, dietary factors, and year of blood sampling, the authors reported risk of breast cancer decreased with increasing PFHpS exposure (p for trend 0.029). The odds ratio for breast cancer was significantly decreased when cases and controls with high PFHpS exposure were compared to those with low PFHpS exposure (OR 0.36, 95% CI 0.16-0.84).

13.5 Proposal for a (new) starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

14 Toxicological evaluation of perfluorononanesulfonic acid (PFNS)

14.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 46 and Table 47.

Table 46: Chemical identity of perfluorononanesulfonic acid (PFNS, CAS 68259-12-1)

Name	Perfluornonansulfonsäure
English Name	Perfluorononanesulfonic acid
Acronym	PFNS
Mol. Formula	C9HF19O3S
Mol. Weight (g/mol)	550.1
CAS	68259-12-1
EC	No data

Table 47: Physicochemical properties of perfluorononanesulfonic acid (PFNS, CAS 68259-12-1)

Properties	Value	Source
Density (g/cm^3)	1.84-1.88 (mod.)	(USEPA, 2020)
Melting point (°C)	13.4-183 (mod.)	(USEPA, 2020)
Boiling point (°C)	224-251 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	2.0 x 10^{-4} (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	1.9 x 10^{-6} (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	2.0 x 10^{-4} -384 (mod.)[a]	(USEPA, 2020)

(mod = modelled); [a] EPISUITE: 4.04 x 10^{-10} mol/l = 2.0 x 10^{-4} mg/l, OPERA Model: 6.98 x 10^{-4} mol/l = 384 mg/l.

14.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFNS (Table 48). Data was identified by general desk research.

Table 48: Drinking water limits determined for PFNS or sum of PFAS

Country/ Institution	PFNS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFNS, drinking water guide value	(SFA, 2022)

14.3 Toxicokinetics

14.3.1 Animal data

14.3.1.1 New data

No relevant data was found.

14.3.2 Human data

14.3.2.1 New data

No relevant data was found.

14.4 Health effects in humans and/or animals

14.4.1 Relevant Animal data

14.4.1.1 New data

14.4.1.1.1 Repeated dose toxicity

No relevant data was found.

14.4.1.1.2 Carcinogenicity

No relevant data was found.

14.4.1.1.3 Mutagenicity

No relevant data was found.

14.4.1.1.4 Toxicity to reproduction

No relevant data was found.

14.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

14.4.2 Relevant Human data

14.4.2.1 New data

No relevant data was found.

14.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

15 Toxicological evaluation of perfluorodecanesulfonic acid (PFDS)

15.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 49 and Table 50.

Table 49: Chemical identity of perfluorodecanesulfonic acid (PFDS, CAS 335-77-3)

Name	Perfluordecansulfonsäure
English Name	Perfluorodecanesulfonic acid
Acronym	PFDS
Mol. Formula	C10HF21O3S
Mol. Weight (g/mol)	600.1
CAS	335-77-3
EC	206-401-9

Table 50: Physicochemical properties of perfluorodecanesulfonic acid (PFDS, CAS 335-77-3)

Properties	Value	Source
Density (g/cm^3)	1.83-1.93 (mod.)	(USEPA, 2020)
Melting point (°C)	11.6-186 (mod.)	(USEPA, 2020)
Boiling point (°C)	224-255 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	0.001 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	3.4 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	0.14 (mod.)	(Steinle-Darling & Reinhard, 2008)
Log Koc	4.03 (exp.)	(Hunter Anderson et al., 2019)
Water Solubility (mg/l)	1.6 x 10^{-5}-194 (mod.)[a]	(USEPA, 2020)

(exp. = experimental, mod. = modelled); a EPISUITE: 2.61 x 10-11 mol/l = 1.6 x 10-5 mg/l, COSMOtherm: -5.39 log mol/l = 2.4 mg/l, OPERA Model: 3.23 x 10-4 mol/l = 194 mg/l.

15.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFDS (Table 51). Data was identified by general desk research.

Table 51: Drinking water limits determined for PFDS or sum of PFAS

Country/ Institution	PFDS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFDS, drinking water guide value	(SFA, 2022)

15.3 Toxicokinetics

15.3.1 Animal data

15.3.1.1 New data

No relevant data was found.

15.3.2 Human data

15.3.2.1 New data

No relevant data was found.

15.4 Health effects in humans and/or animals

15.4.1 Relevant Animal data

15.4.1.1 New data

15.4.1.1.1 Repeated dose toxicity

No relevant data was found.

15.4.1.1.2 Carcinogenicity

No relevant data was found.

15.4.1.1.3 Mutagenicity

No relevant data was found.

15.4.1.1.4 Toxicity to reproduction

No relevant data was found.

15.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

15.4.2 Relevant Human data

15.4.2.1 New data

(Spratlen et al., 2020) conducted a cross-sectional study of PFAS and lipids in cord blood in 222 mother-child pairs from the Columbia University World Trade Center Birth Cohort, recruited in 2001-2002. The median cord blood PFDS concentration was 0.11 ng/ml. PFDS exposure was divided into quartiles, with cut-points at 0.09, 0.11, and 0.16 ng/ml. After adjusting for maternal age, education, race/ethnicity, parity, family smoking, pre-pregnancy BMI, marital status, gestational age, and child sex, a statistically significant trend of decreasing triglycerides with increasing quartiles of PFDS exposure was reported (p for trend=0.04). Decreased triglycerides were observed among those with PFDS exposure between 0.11 and 0.16 ng/ml (OR 0.77, 95% CI 0.65-0.91) compared to PFDS<0.11 ng/ml. However, triglycerides were not statistically significantly decreased among those with the highest PFDS exposure (≥0.16 ng/ml) (OR 0.89, 95% CI 0.74–1.08). PFDS showed no statistically significant associations with total lipids or total cholesterol.

15.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

16 Toxicological evaluation of perfluoroundecanesulfonic acid (PFUnDS)

16.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 52 and Table 53.

Table 52: Chemical identity of perfluoroundecanesulfonic acid (PFUnDS, CAS 749786-16-1)

Name	Perfluorundecansulfonsäure
English Name	Perfluoroundecanesulfonic acid
Acronym	PFUnDS
Mol. Formula	C11HF23O3S
Mol. Weight (g/mol)	650.15
CAS	749786-16-1
EC	No data

Table 53: Physicochemical properties of perfluoroundecanesulfonic acid (PFUnDS, CAS 749786-16-1)

Properties	Value	Source
Density (g/cm3)	1.83 (?)	(USEPA, 2020)
Melting point (°C)	168 (mod.)	(USEPA, 2020)
Boiling point (°C)	223 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	1.4×10^{3} (mod.)	(USEPA, 2020)
Henry's Constant (Pa m3/mol)	3.4×10^{5} (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	35.0 (mod.)[a]	(USEPA, 2020)

(mod = modelled), [a] OPERA Model: 5.38×10^{-5} mol/l = 35.0 mg/l.

16.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFUnDS (Table 54). Data was identified by general desk research.

Table 54: Drinking water limits determined for PFUnDS or sum of PFAS

Country/ Institution	PFUnDS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFUnDS, drinking water guide value	(SFA, 2022)

16.3 Toxicokinetics

16.3.1 Animal data

16.3.1.1 New data

No relevant data was found.

16.3.2 Human data

16.3.2.1 New data

No relevant data was found.

16.4 Health effects in humans and/or animals

16.4.1 Relevant Animal data

16.4.1.1 New data

No relevant data was found.

16.4.1.1.1 Carcinogenicity

No relevant data was found.

16.4.1.1.2 Mutagenicity

No relevant data was found.

16.4.1.1.3 Toxicity to reproduction

No relevant data was found.

16.4.1.1.4 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

16.4.2 Relevant Human data

16.4.2.1 New data

No relevant data was found.

16.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

17 Toxicological evaluation of perfluorododecanesulfonic acid (PFDoDS)

17.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 55 and Table 56.

Table 55: Chemical identity of perfluorododecanesulfonic acid (PFDoDS, CAS 79780-39-5)

Name	Perfluordodecansulfonsäure
English Name	Perfluorododecanesulfonic acid
Acronym	PFDoDS
Mol. Formula	C12HF25O3S
Mol. Weight (g/mol)	700.16
CAS	79780-39-5
EC	279-259-9

Table 56: Physicochemical properties of perfluorododecanesulfonic acid (PFDoDS, CAS 79780-39-5)

Properties	Value	Source
Density (g/cm^3)	1.83 (?)	(USEPA, 2020)
Melting point (°C)	164 (mod.)	(USEPA, 2020)
Boiling point (°C)	224 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	1.6 x 10^3 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	3.5 x 10^5 (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	23.9 (mod.)[a]	(USEPA, 2020)

(mod = modelled), [a] OPERA Model: 3.41 x 10^{-5} mol/l = 23.9 mg/l.

17.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFDoDS (Table 57). Data was identified by general desk research.

Table 57: Drinking water limits determined for PFDoDS or sum of PFAS

Country/ Institution	PFDoDS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFDoDS, drinking water guide value	(SFA, 2022)

17.3 Toxicokinetics

17.3.1 Animal data

17.3.1.1 New data

No relevant data was found.

17.3.2 Human data

17.3.2.1 New data

No relevant data was found.

17.4 Health effects in humans and/or animals

17.4.1 Relevant Animal data

17.4.1.1 New data

17.4.1.2 Repeated dose toxicity

No relevant data was found.

17.4.1.2.1 Carcinogenicity

No relevant data was found.

17.4.1.2.2 Mutagenicity

No relevant data was found.

17.4.1.2.3 Toxicity to reproduction

No relevant data was found.

17.4.1.2.4 Other (endocrine, neurotoxicity, immunotoxicity…)

No relevant data was found.

17.4.2 Relevant Human data

17.4.2.1 New data

No relevant data was found.

17.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

18 Toxicological evaluation of perfluorotridecanesulfonic acid (PFTrDS)

18.1 Chemical and physical information

This section provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 58 and Table 59.

Table 58: Chemical identity of perfluorotridecanesulfonic acid (PFTrDS, CAS 791563-89-8)

Name	Perfluortridecansulfonsäure
English Name	Perfluorotridecanesulfonic acid
Acronym	PFTrDS
Mol. Formula	C13HF27O3S
Mol. Weight (g/mol)	750.17
CAS	791563-89-8
EC	No data

Table 59: Physicochemical properties of perfluorotridecanesulfonic acid (PFTrDS, CAS 791563-89-8)

Properties	Value	Source
Density (g/cm^3)	1.83	(USEPA, 2020)
Melting point (°C)	161 (mod.)	(USEPA, 2020)
Boiling point (°C)	220 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	3.08 x 10^3 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	3.6 x 10^5 (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	54.8 (mod.)[a]	(USEPA, 2020)

(mod = modelled), [a] OPERA Model: 7.31 x 10^{-5} mol/l = 54.8 mg/l.

18.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews drinking water limits for PFTrDS (Table 60). Data was identified by general desk research.

Table 60: Drinking water limits determined for PFTrDS or sum of PFAS

Country/ Institution	PFTrDS limit value in drinking water (µg/l)	Comment	Source
Sweden/ Swedish Food Agency	≤ 0.1	Sum of 21 PFAS including PFTrDS, drinking water guide value	(SFA, 2022)

18.3 Toxicokinetics

18.3.1 Animal data

18.3.1.1 New data

No relevant data was found.

18.3.2 Human data

18.3.2.1 New data

No relevant data was found.

18.4 Health effects in humans and/or animals

18.4.1 Relevant Animal data

18.4.1.1 New data

18.4.1.1.1 Repeated dose toxicity

No relevant data was found.

18.4.1.1.2 Carcinogenicity

No relevant data was found.

18.4.1.1.3 Mutagenicity

No relevant data was found.

18.4.1.1.4 Toxicity to reproduction

No relevant data was found.

18.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

18.4.2 Relevant Human data

18.4.2.1 New data

No relevant data was found.

18.5 Proposal for a starting point for deriving a drinking water limit value

There is no POD available to derive a drinking water limit.

19 Recent toxicological findings that might influence the EFSA assessment for PFOA, PFNA, PFHxS and PFOS

19.1 Background of EFSA evaluation

In 2020, the European Food Standards Agency (EFSA) Panel on Contaminants in the Food Chain (CONTAM) published a scientific evaluation of the human health risks of PFAS in food. In this evaluation, an assessment for the sum of four PFAS (PFOA, PFNA, PFHxS and PFOS) was done since these four PFAS made up half of the lower bound exposure to PFAS with the remaining contribution from PFAS with short half-lives and similar effects have been observed for these substances (EFSA, 2020). Thus, a Health Based Guidance Value (HBGV) for all four PFAS in sum has been derived.

It was concluded that effects on the immune system are regarded as the most critical effect for risk assessment, since these findings were observed at the lowest serum PFAS levels. EFSA considers this critical effect as robust, as it has been constantly observed in both animals and humans for PFOS and PFOA. Equal potencies for effects of these four PFAS on immune outcomes were assumed by the Panel as a pragmatic approach since current data does not allow the derivation of potency factors. For this effect, a tolerable weekly intake (TWI) of 4.4×10^{-6} mg/kg bw/week for the sum of four PFAS was established by the CONTAM Panel, considering accumulation over time.

The point of departure (POD) for the derivation of the TWI as a HBGV was based on human epidemiological studies, which found an inverse correlation between PFAS serum levels in children and reduced antibody titres. Two critical studies were considered for the deviation. In the first study carried out on 5-year-old children on the Faroe Islands, several correlations between the serum levels of individual PFAS as well as the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against diphtheria and tetanus. The researchers identified a NOAEC of 27.0 ng/ml for the combined levels of these four PFAS in 5 year olds as well as for the antibody titres against diphtheria in 7 year olds (Grandjean et al., 2012). The second more recent study, conducted on 1-year-old children in Germany (mainly breastfed), showed an inverted correlation between serum levels of PFOA, as well as the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against three vaccines (haemophilus influenzae type b (Hib), diphtheria and tetanus). The researchers identified a BMDL10 of 17.5 ng/ml for the sum of these four PFAS at 1 year of age (Abraham et al., 2020). This BMDL10 value was identified as the most sensitive POD.

Starting from this value, a Reference Point of 0.63 ng/kg bw per day for the intake of the mother was calculated using a PBPK model and assuming 12 months of breastfeeding for the sum of all four PFAS. This intake is equal to a serum level of 6.9 ng/ml, considering the body burden of the four PFAS for a 35-year-old mother. The Panel used the daily intake of 0.63 ng/kg bw per day to establish a group tolerable weekly intake (TWI) of 7 days x 0.63 ng/kg bw per day = 4.4 ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS. The derived HBGV value is considered protective, since a weakened response to vaccination is regarded as a risk factor for disease rather than a disease in itself. Furthermore the study focused on infants, who are a considered vulnerable population group, hence it was not considered necessary to incorporate additional uncertainty factors (UFs) for potential intraindividual differences. The Panel stated that this TWI is also protective for the other potential critical endpoints (increase in serum cholesterol, reduced birth weight and elevated serum levels of ALT) that were previously evaluated in the Opinion on PFOS and PFOA (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2018).

Two animal studies on the effects of PFAS on the immune system (G.-H. Dong et al., 2009; Peden-Adams et al., 2008) were also reported; however, the Panel concluded that the use of uncertainty factors for animal data is too conservative, since the age of the mice in these studies corresponds more to an adult in humans and therefore the epidemiological data on human children leads to more realistic values.

19.2 Perfluorooctanoic acid (PFOA)

19.2.1 Considerations of US EPA

As part of its rulemaking for National Primary Drinking Water Regulation for PFOA and PFOS, the U.S. Environmental Protection Agency (EPA) has published a draft report on proposed approaches to the derivation of a draft maximum contaminant level goal (MCLG) for PFOA in drinking water in 2021 (US EPA, 2021b). For the derivation of the draft noncancer reference dose (RfD) for PFOA, USEPA considered studies conducted in both animals and humans reporting immune effects, developmental effects, changes in serum lipids, hepatic effects, endocrine effects, and reproductive effects. Candidate PODs were considered from each of the studies and RfD values were calculated for each by applying uncertainty factors to the PODs. The uncertainty factors used by USEPA were:

- An interspecies UF (UFA) of 1 applied to developmental and immunological effects observed in epidemiological studies due to the direct relevance of dose response information from these studies to humans.
- A UF of 10 for intraspecies (UFH) to consider the variability in responses among the human populations due to both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (lifestyle) factors that can affect the response to a given dose.
- A LOAEL-to-NOAEL extrapolation UF (UFL) of 1 because a BMDL is used as the basis for the POD_{HED} derivation.
- A UF of 1 for extrapolation from subchronic to a chronic exposure duration (UFS) for developmental endpoints as the developmental period is considered a vulnerable stage of life where exposure during a specific period of development is more significant to the induction of developmental effects than the exposure over the entire lifespan. Similarly a UFS of 1 was also applied to immune endpoints in children as the developing immune system is considered a susceptible lifestage; and exposure during this period can be considered more relevant than exposure over a lifetime.
- A database UF (UFD) of one to account for deficiencies in the database for PFOA

The draft RfD selected by (US EPA, 2021b) for PFOA was 1.5×10^{-9} mg/kg-day based on the critical effect of decreased serum anti-tetanus antibody concentration in children (Budtz-Jørgensen & Grandjean, 2018; Grandjean et al., 2012; Grandjean, Heilmann, et al., 2017; Grandjean, Nielsen Flemming, et al., 2017, 2017), which resulted in the lowest POD_{HED} and potentially may lead to severe clinical outcomes in a sensitive life stage (children). Therefore, the draft RfD is expected to be protective of all other health effects in humans. This draft RfD was used to develop an interim health advisory level of 0.004 ng/l (parts per trillion, ppt) for PFOA in drinking water (US EPA, 2022b). Since the interim health advisories were issued in June 2022, the Scientific Advisory Board (SAB) to the EPA issued a final report on its review of the draft reports on proposed approaches to deriving MCLGs. The EPA has indicated that the RfD may change, based on recommendations by the SAB to consider different critical effects, a

combination of results for the same critical effect, or an RfD based on multiple endpoints. Regardless of the RfD selected, the EPA expects the health-based drinking water values to be below 4 ng/l.

In comparison, the EFSA tolerable daily intake of 0.63 ng/kg/day is based on a BMDL10 of 17.5 ng/ml in the serum of infants and children with reduced antibodies to diphtheria, tetanus and haemophilus influenza type b; similar to the basis for the RfD proposed by EPA. However, the BMDL10 of 17.5 ng/ml is lower than the NOAEC of 27 ng/ml from the Grandjean study used by EPA as the basis for the RfD. Using PBPK modelling, the serum level of 17.5 ng/ml in children was estimated to correspond to long-term maternal exposure of 0.63 ng/kg bw/day. Converting the EPA health advisory level of 0.004 ng/l to similar units assuming a daily water consumption for humans of 2 L/day and a human body weight of 70 kg the resulting value would be 0.0001 ng/kg/day, which is 6000 times lower than the EFSA tolerable daily intake of 0.63 ng/kg/day. Overall, the EFSA tolerable daily intake is based on more recent data and incorporates both benchmark modelling and PBPK modelling leading to more accurate estimates for the POD.

Table 61: Summary of studies and endpoints identified for POD derivation of PFOA by (US EPA, 2021b) (Page 317ff)

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	Notes
Immune	Reduced Antibody Concentrations for Diphtheria and Tetanus	(Budtz-Jørgensen & Grandjean, 2018; Grandjean et al., 2012; Grandjean, Heilmann, et al., 2017; Grandjean, Nielsen Flemming, et al., 2017); Medium confidence	Human (male and female children)	The impact was significant in scale and generally consistent with epidemiological data observed for other antibody effects. BMD modelling performed by study authors.
	Reduced immunoglobulin M (IgM) Response	(DeWitt Jamie C. et al., 2008; Loveless et al., 2008); Medium confidence	C57BL/6N mice (females), Crl:CD-1(ICR)BR mice (males)	Functional assessment showed signs of immune system suppression. Multiple studies consistently showed immune related effects , such as reduced spleen and thymus weights, changes in immune cell populations, and reduced cell count in the spleen and thymus.
Developmental	Decreased Birth Weight	(Chu et al., 2020; Govarts et al., 2016; Sagiv et al., 2018; Starling et	Human (male and female infants)	The impact was significantly large and consistent with epidemiological

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	Notes
		al., 2017; Wikström et al., 2020); High confidence		evidence for other biologically related effects.
	Decreased Offspring Survival	(Song et al., 2018); Medium confidence	Kunming mice (F1 males and females)	Effect was consistently observed across multiple studies and species. Supported by the prenatal loss observed in (Lau et al., 2006; Wolf et al., 2007).
	Decreased Foetal Body Weight	(Li et al., 2018); Medium confidence	Kunming mice (F1 males and females)	Effect was consistently observed across multiple studies and species: (Lau et al., 2006; Wolf et al., 2007) (J. L. Butenhoff, Kennedy, Frame, et al., 2004). Note that decreases in maternal body weight were not further considered because the decreased foetal body weight could be a potential confounder and was a more sensitive effect.
	Developmental Scores for the Mammary Gland	(Macon et al., 2011); Medium confidence	CD-1 mice (F1 females)	Effect observed at low doses with no study NOAEL.
	Delayed Time to Eye Opening	(Lau et al., 2006); Medium confidence	CD-1 mice (F1 males and females)	Effect also observed in (Wolf et al., 2007); however, those data were not amenable to BMD modelling.
	Increased Placental Lesions	(Blake et al., 2020); Medium confidence	CD-1 mice (parental females)	Histopathological evidence of placental damage was selected over changes in placental weight

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	Notes
				observed in (Blake et al., 2020; W. Jiang et al., 2020).
Serum Lipid	Increased Total Cholesterol	(Z. Dong et al., 2019); Medium confidence	Human (male and female)	BMD modelling performed by study authors.
Hepatic	Necrosis (focal, individual cell, both) in the Liver	(Loveless et al., 2008); Medium confidence, (NTP, 2020a); High confidence	Crl:CD-1(ICR)BR mice (males), Sprague-Dawley rats (males)	Effect was accompanied by other liver lesions including cytoplasmic alteration and apoptosis. Females appear to be less sensitive. Necrotic liver cells were also observed in male mice in (Crebelli et al., 2019) and pregnant dams in (Blake et al., 2020). Effect is further supported by changes in serum ALT levels in animals and humans.
Endocrine	Increased TSH	(NTP, 2019); High confidence	Sprague-Dawley rat (females)	Effect observed at low doses in the female rat and is supported by suggestive evidence of increased TSH in the epidemiological literature. Female rats exhibited decreases in free T4 and total T4 but only in the high dose group. This effect was not considered for the male animals since the effect decreased in rats (NTP, 2019) and increased in monkeys (J. Butenhoff et al.,

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	Notes
				2002). The changes in FT4 were more sensitive than TSH in males and are further considered below.
	Decreased Free T4	(NTP, 2019); High confidence	Sprague-Dawley rats (male)	Effect was generally large in magnitude and consistently observed across multiple studies and species in the male. Decreases in free and total T4 are consistent with hypothyroxinemia in that a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology. Decreased FT4 was also observed in the female rat, but it was limited to a high dose effect and TSH was more sensitive.
Reproductive	Reduced Number of Leydig Cells	(Song et al., 2018); Medium confidence	Kunming mice (male)	Effect observed at a later time point (PND70) and accompanied by decreased testosterone. Supported by evidence of decreased testosterone and altered sperm parameters observed in animals and humans.
	Increased Length of Diestrus	(Y. Zhang et al., 2020); Medium confidence	ICR mice (female)	Effect also observed in female rats (NTP, 2019), though at higher dose levels.

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	Notes
				Supported by evidence of altered ovarian physiology in mice and mixed evidence of reduced female fertility in humans.

19.2.2 Identified studies that could change EFSA evaluation

19.2.2.1 Recent toxicological studies

The project team has identified 12 relevant *in vivo* studies that were published after the EFSA assessment (2020 onwards) which are summarized in Table 62. Nevertheless, none of the *in vivo* studies will change the TWI developed by EFSA because the TWI developed by EFSA is based on human data and the exposure to PFOA in these animal studies is much higher than that expected in human population.

Table 62: Summary of Recent Toxicological Studies Following Exposure to PFOA

Study Name	Species	Study Design	Life Stage/ Sex	Dose	Result
(Blake et al., 2020)	CD-1 mice (parental females)	Gestational CD-1 mouse gavage dosing from GD1.5–GD11.5 or 17.5 (dams)	Pregnant/female	0, 1, 5 mg/kg bw/day	Increased placental lesions at 5 mg/kg/day
(NTP, 2020a, 2020b)	Sprague Dawley rats	16-week and 2-year Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure	Time-mated female; F_1 postweaning/male and female	Postweaning males: 0, 20, 40, or 80 ppm Postweaning females: 0, 300, or 1000 ppm Perinatal/postweaning males: 0/0, 0/150, 150/150, 0/300, or 300/300 ppm Perinatal/postweaning females: 0/0, 0/300, 150/300, 1/1000, or 300/1000 ppm	Male: Increased incidence of hepatocellular neoplasms and acinar cell neoplasms of the pancreas. Female: increased incidences of pancreatic acinar cell adenoma or adenoma (combined,
(Cope et al., 2021)	CD-1 mice	Pregnant mice exposed via gavage on GD1.5 to 17.5; offspring fed High- or low-fat diet from weaning to necropsy at 6 or 18 weeks.	Pregnant/female	0, 0.1, 1.0 mg/kg bw/day	Adverse metabolic outcomes

Study Name	Species	Study Design	Life Stage/ Sex	Dose	Result
(Endirlik et al., 2022)	Balb/c mice	Daily gavage administration for 10 days	10-week-old/male	0, 15, or 30 mg/kg bw/day	Decreased body weight; increase in relative brain and liver weights; lipid peroxidation and reduced glutathione peroxidase (GPx) activity in the brain; changes in GSH levels, GPx, superoxide dismutase (Cu-Zn SOD), and catalase (CAT) activities in the liver tissue
(Guo, Zhang, et al., 2021)	BALB/c mice	Daily gavage administration for 28 days	6- to 8-week-old/male	0, 0.4, 2, or 10 mg/kg bw/day	Decreased spleen weight (2 and 10 mg/kg bw/day)
(W. Jiang et al., 2020)	Kunming mice	Daily gavage dose from GD1 through GD 13	Pregnant/female	0, 2.5, 5, or 10 mg/kg bw/day	Decreased placental weight and interstitial oedema of placenta.
(NTP, 2020b)	Sprague Dawley rat	Administered in diet for 2 years	F_1 postweaning /male	0, 15.6, 15.8, 31.7 or 32.1 mg/kg bw/day	Significantly increased incidence of hepatic and pancreatic tumours
(NTP, 2020b)	Sprague Dawley rat	Administered in diet for 2 years	F_1 postweaning/female	0, 29.6, 98.6, or 104.4 mg/kg bw/day	No significant increase in tumours
(Peng et al., 2022)	BALB/c mice	Daily gavage dose for 28 days	6-week-old/male	0, 1, 5, 10, or 20 mg/kg bw/day	Decreased testosterone at all doses.
(L. Shi et al., 2020)	C57BL/6J mice	Daily gavage dose for 5 weeks	8-week-old/male	0, 0.5, 1, or 3 mg/kg bw/day	Decreased body weight; inflammation in gut and brain
(G. Wang et al., 2021)	C57BL/6J mice	Daily gavage for 14 days or 30 days	6-week-old/male	0, 3, or 30 mg/kg bw/day (14 days) 0, 2.5, or 5 mg/kg bw/day (30 days)	Subacute and subchronic exposure produced liver inflammation, disrupted antioxidative homeostasis and

Study Name	Species	Study Design	Life Stage/ Sex	Dose	Result
					liver histological abnormalities with hepatomegaly
(Yang et al., 2022)	CD-1 Mice	Daily gavage for 10 days	Young adult/female	0, 5, 10, or 20 mg/kg bw/day	Significant decrease in liver weight at 5, 10, and 20 mg/kg bw/day; Significant increase in antral follicle counts at 10 mg/kg bw/day; Significant decrease in primordial follicle counts at 5 mg/kg bw/day; Significant increase in preantral and antral follicle counts at 5 mg/kg bw/day; Significant increase in testosterone at 1 mg/kg bw/day; Significant decrease in progesterone and pregnenolone at 5 mg/kg

19.2.2.2 Recent epidemiological studies

Epidemiological studies of PFOA that could potentially change the TWI developed by EFSA include studies which (1) reported a dose-response association for an adverse effect and (2) the adverse effect was observed at PFOA concentrations below approximately 30 ng/ml. This value is conservative because 30 ng/ml is the approximate concentration of the NOAEC associated with decreased vaccine antibodies against Hib for the sum of four PFAS: PFOA, PFOS, PFNA, and PFHxS (presented in Appendix K of EFSA 2020 analysing data by (Abraham et al., 2020)) and is similar to the NOAEC of 27 ng/ml for the sum of four PFAS based on information from (Grandjean et al., 2012). The results from epidemiological studies that meet these criteria are summarized below and in Table 63. However, further modelling similar to the EFSA approach would be necessary to check how these study results might influence the TWI developed by EFSA. Separately, epidemiological studies that log-transformed PFOA values and presented risk estimates per unit of log-transformed PFOA were not considered here. These studies require back transformation of log values and additional analyses to evaluate the shape of the dose-response function in order to identify a POD using a NOAEC or LOAEC or to otherwise calculate a benchmark dose.

(Shearer et al., 2021) conducted a nested case-control study to examine serum PFAS and risk of renal cell carcinoma. A total of 324 cases and 324 controls matched on age at enrolment, sex, race/ethnicity, study centre and year of blood draw were identified from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, a study of 55–74-year-old adults from 10 US cities recruited between 1993 and 2001. In controls, the adjusted geometric mean serum PFOA was 4.0 ng/ml in women and 4.5 ng/ml in men; geometric means were adjusted for age, centre, sex, race, body mass index, hypertension, smoking status, previous freeze-thaw cycle, and year of blood draw. In logistic regression models, PFOA was categorized as <4.0, ≥4.0-5.5, >5.5-7.3, and >7.3-27.2 ng/ml. After adjusting for body mass index, smoking, hypertension history, estimated glomerular filtration rate, previous freeze-thaw cycle, and year of blood draw, the authors reported higher odds of renal cell carcinoma in the second, third, and fourth quartiles of PFOA exposure compared to the first quartile. This association did not reach statistical significance in the second or third quartiles; in the fourth quartile, the odds ratio was 2.63 (95% CI 1.33-5.20). The p-trend for increased risk across quartiles was statistically significant (p=0.007).

(Souza et al., 2020) conducted a nested case-control study within the Brazilian Ribeirao Preto and Sao Luiz Birth Cohort Study cohort, in which pregnant women were recruited from 2010 to 2011 between 22 and 26 weeks of gestational age. From this larger cohort, the authors identified 63 cases of children with foetal growth ratio <0.85 and 189 matched controls. The median whole blood PFOA concentration measured in the second trimester was 0.20 ng/ml, and the exposure cut-points used in analyses were based on exposure in cases (0.07, 0.16, and 0.22 ng/ml). After adjusting for preterm birth, parity, maternal education, socioeconomic status, pre-pregnancy BMI, and smoking during pregnancy, the authors reported higher odds of intrauterine growth restriction associated with the highest quartile of PFOA exposure compared to the lowest (OR 2.81, 95% CI 1.12-7.05). Although the associations seen in the second and third exposure quartiles did not reach statistical significance, the p for trend across exposure quartiles was statistically significant (p=0.0134).

(Spratlen et al., 2020) conducted a cross-sectional study of PFAS and lipids in cord blood in 222 mother-child pairs from the Columbia University WTC Birth Cohort, recruited in 2001-2002. The median cord blood PFOA concentration was 2.46 ng/ml; exposure was divided into quartiles, with cut-points at 1.77, 2.46, and 3.24 ng/ml. After adjusting for maternal age, education, race/ethnicity, parity, family smoking, pre-pregnancy BMI, marital status, gestational age, and child sex, the third quartile of PFOA exposure showed an association with increased total lipids in cord blood (geometric mean ratio 1.10, 95% CI 1.01-1.19) compared to the first quartile, with a statistically significant trend of increased total lipids with increased quartiles of PFOA exposure (p-trend=0.04). Similarly, the fourth quartile of PFOA showed an association with increased triglycerides in cord blood compared to the first quartile (geometric mean ratio 1.33, 95% CI 1.13-1.57); associations in the second and third quartiles did not reach statistical significance, but the p for trend across increasing exposure quartiles was statistically significant (p=0.001).

(Liao et al., 2020) conducted a cross-sectional analysis of PFAS exposure and hypertension among 6,967 adult participants in the US NHANES study between 2003 and 2012. The median serum PFOA in this population was 3.33 ng/ml; exposure categories were ≤2.5 ng/ml, >2.5 ng/ml to ≤4.4 ng/ml, and >4.4 ng/ml. In a model adjusted for age, sex, education, race/ethnicity, diabetes, alcohol use, smoking status, BMI, waist circumference, haemoglobin, total cholesterol, estimated glomerular filtration rate, sodium intake, potassium intake, and calcium intake, the highest PFOA exposure group was associated with increased risk of hypertension (OR 1.32, 95%

CI 1.13-1.54). Although the association in the middle exposure group did not reach statistical significance, the p for trend across increasing categories was statistically significant (p≤0.001).

(Chang et al., 2022) conducted a cohort study of maternal PFAS exposure and foetal growth measurements among 426 pregnant women enrolled in the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, which recruited participants aged 18 to 40 in Atlanta, GA from 2014 to 2018. Median serum PFOA, measured between 8 and 14 weeks of gestation, was 0.71 ng/ml; exposure quartiles in analyses were <0.45, 0.45–0.71, 0.71–1.07, and 1.07–4.42 ng/ml. Infants below the 10th percentile for their gestational age were considered to be SGA (small for gestational age) births. After adjusting for infant sex and maternal age, education, BMI, parity, tobacco and marijuana use, the authors reported that the second, third, and fourth quartiles of PFOA were all associated with increased odds of SGA compared to the first quartile (second quartile OR 2.22, 95% CI 1.10-4.50; third quartile OR 2.44, 95% CI 1.21-4.92; fourth quartile OR 2.23, 95% CI 1.10-4.54). The p for trend across exposure quartiles was not statistically significant at the 0.05 level (p=0.06).

(Jensen et al., 2022) conducted a cross-sectional analysis of PFAS exposure and thyroid hormones among 1,007 pregnant women participating in the Odense Child Cohort study. Participants were originally recruited from 2010-2012 in Denmark during their early pregnancy. Serum PFOA and thyroid hormones were measured at a median of 12 gestational weeks; median serum PFOA in this population was 1.67 ng/ml, and the exposure categories used in analyses were 0.26–1.11, 1.11-1.67, 1.67–2.35, and 2.35–10.12 ng/ml. After adjusting for age, parity, and education, the authors reported an association between the highest category of serum PFOA and a 2.74% increase in serum free thyroxine concentration compared to the first category (β=2.74, 95% CI 0.26-5.28). Although the associations in the second and third categories did not reach statistical significance, the p for trend across categories was statistically significant (p≤0.01).

Table 63: Summary of Recent Epidemiological Studies Following Exposure to PFOA

Reference	Finding	Conc. in serum (ng/ml)
(Shearer et al., 2021)	648 adults, 1993-2001, United States Increased odds of renal cell carcinoma OR 2.63 (95% CI 1.33-5.20)	Adjusted geometric mean, 4.0 (women), 4.5 (men) >7.3-27.2 (4th quartile) compared to <4.0 (1st quartile)
(Souza et al., 2020)	252 mother-child pairs, 2010-2011, Brazil Increased odds of intrauterine growth restriction OR 2.81 (95% CI 1.12-7.05)	Maternal PFOA, median, 0.2 (second trimester) >0.22 (4th quartile) compared to <0.07 (1st quartile)
(Spratlen et al., 2020)	222 mother-child pairs, 2001-2002, United States Increased total lipids and triglycerides in cord blood 10% increase of total lipids, 95% CI 1% to 19 %; 33% increase in triglycerides, 95% CI 13% to 57%	Cord blood, median, 2.46 >3.24 (4th quartile) compared to <1.77 (1st quartile)

Reference	Finding	Conc. in serum (ng/ml)
(Liao et al., 2020)	6,967 adults, NHANES, 2003-2012, United States Increased odds of hypertension OR 1.32 (95% CI 1.13-1.54)	Median, 3.33 >4.4 (4th quartile) compared to ≤2.5 (1st quartile)
(Chang et al., 2022)	426 pregnant women, 2014-2018, United States Increased odds of small for gestational age (SGA) infants OR 2.23 (95% 1.10-4.54)	Maternal PFOA, median, 0.71 (8-14 weeks gestation) 1.07-4.42 (4th quartile) compared to <0.45 (1st quartile)
(Jensen et al., 2022)	1007 pregnant women, recruited 2010-2012, Denmark 2.74% increase in serum free thyroxine (FT4) concentration, 95% CI 0.26% to 5.28%	Median, 1.67 2.35-10.12 (4th quartile) compared to 0.26-1.11 (1st quartile)

19.3 Perfluorononanoic acid (PFNA)

19.3.1 Identified studies that could change EFSA evaluation

19.3.1.1 Recent toxicological studies

In this project, one relevant *in vivo* study was identified that was published after the EFSA assessment in 2020, and the results are summarized in Table 64. The developmental toxicity study found (Y. Zhang et al., 2021), does not add new information to the EFSA safety assessment, therefore, this study would not change the TWI developed by Panel. Developmental toxicity studies with similar doses, and lower doses, were reported in the EFSA assessment. The current EFSA TWI of 4.4 ng/kg bw/week would also be protective of the developmental toxicity effects observed. Additionally, based on the EFSA safety assessment, human epidemiological data is preferred for deriving HBGVs for PFAS.

Table 64: Summary of Recent Toxicological Studies Following Exposure to PFNA

Study Name	Species	Study Design	Life Stage/ Sex	Dose	Result
(Y. Zhang et al., 2021)	Mice, ICR	Developmental toxicity study. Exposure from gestational day 1 to 18.	Adult female mice	0.5 or 3 mg/kg bw/day	Dams dosed at 3 mg/kg bw/day showed an increase in liver weight and hepatic FGF21 synthesis via PPARa activation, and their female offspring (PFNA mice) showed an increase in liver weight and hepatic FGF21 synthesis from postnatal day (PND) 1 to PND21, which were corrected by the administration of the PPARa antagonist GW6471 from PND1-14.

19.3.1.2 Recent epidemiological studies

Epidemiological studies of PFNA that could potentially change the TWI developed by EFSA include studies which (1) reported a dose-response association for an adverse effect and (2) the adverse effect was observed at PFNA concentrations below approximately 30 ng/ml. This value is conservative because 30 ng/ml is the approximate concentration of the NOAEC associated with decreased vaccine antibodies against Hib for the sum of four PFAS: PFOA, PFOS, PFNA, and PFHxS (presented in Appendix K of EFSA 2020 analysing data by (Abraham et al., 2020)) and is similar to the NOAEC of 27 ng/ml for the sum of four PFAS based on information from (Grandjean et al., 2012). The results from epidemiological studies that meet these criteria are summarized below and in Table 65. However, further modelling similar to the EFSA approach would be necessary to check how these study results might influence the TWI developed by EFSA. Separately, epidemiological studies that log-transformed PFNA values and presented risk estimates per unit of log-transformed PFNA were not considered here. These studies require back transformation of log values and additional analyses to evaluate the shape of the dose-response function in order to identify a POD using a NOAEC or LOAEC or to otherwise calculate a benchmark dose.

(Liao et al., 2020) conducted a cross-sectional analysis of PFAS exposure and hypertension among 6,967 adult participants in the US National Health and Nutrition Examination Survey (NHANES) between 2003 and 2012. The median serum PFNA in this population was 1.10 ng/ml; exposure categories were ≤0.85 ng/ml, >0.85 ng/ml to ≤1.40 ng/ml, and >1.40 ng/ml. In a model adjusted for age, sex, education, race/ethnicity, diabetes, alcohol use, smoking status, BMI, waist circumference, haemoglobin, total cholesterol, estimated glomerular filtration rate, sodium intake, potassium intake, and calcium intake, the highest PFNA exposure group was associated with increased risk of hypertension (OR 1.18, 95% CI 1.01-1.37). Although the association in the middle exposure group did not reach statistical significance, the *p* for trend across increasing categories was statistically significant (p≤0.034).

(Chang et al., 2022) conducted a cohort study of maternal PFAS exposure and foetal growth measurements among infants of 426 pregnant women enrolled in the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, which recruited participants aged 18 to 40 in Atlanta, GA from 2014 to 2018. Median serum PFNA, measured between 8 and 14 weeks of gestation, was 0.27 ng/ml; exposure quartiles in analyses were <LOD–0.16, 0.16–0.27, 0.27–0.42, and 0.42–2.27 ng/ml. Infants below the 10th percentile for their gestational age were considered to be SGA (small for gestational age) births. After adjusting for maternal age, education, BMI, parity, tobacco and marijuana use, and infant sex, the authors reported that the second, third, and fourth quartiles of PFNA were all associated with increased odds of SGA compared to the first quartile. The associations in the second and third quartiles did not reach statistical significance; in the fourth quartile, the OR was 2.22 (95% CI 1.12-4.38). The p for trend across exposure categories was statistically significant (p=0.04).

(Jensen et al., 2022) conducted a cross-sectional analysis of PFAS exposure and thyroid hormones among 1,007 pregnant women participating in the Odense Child Cohort study. Participants were originally recruited from 2010-2012 in Denmark during their early pregnancy. Serum PFNA and thyroid hormones were measured at a median of 12 gestational weeks; median serum PFNA in this population was 0.64 ng/ml, and the exposure categories used in analyses were 0.26–1.11, 1.11-1.67, 1.67–2.35, and 2.35–10.12 ng/ml. [Note that these categories are the same as reported for PFOA, and do not match the median for PFNA; these cut-points may be a typo in Table 3.] After adjusting for age, parity, and education, the authors reported an association between the highest category of serum PFNA and a 2.42% increase in serum free thyroxine concentration compared to the first category (β=2.42, 95% CI 0.13-4.77).

Although the associations in the second and third categories did not reach statistical significance, the p for trend across categories was statistically significant (p≤0.01).

(Zeeshan et al., 2021) conducted a cross-sectional analysis of 1,045 Chinese adults, aged ≥35 years, who were recruited during 2015-2016 as part of the Isomers of C8 Health Project. The median serum PFNA was 1.44 ng/ml; exposure quartile cut-points were 0.98, 1.44, and 2.16 ng/ml. After adjusting for age, sex, ethnicity, income, education, smoking, alcohol, and seafood consumption, the authors reported increased odds of diabetes in those exposed to ≥2.16 ng/ml PFNA compared to those exposed to <0.98 ng/ml (OR 5.78, 95% CI 3.13-10.69). Although the associations in the second and third exposure quartiles did not reach statistical significance, the p for trend across quartiles was statistically significant (p≤0.001).

Table 65: Summary of Recent Epidemiological Studies Following Exposure to PFNA

Reference	Finding	Conc. in serum (ng/ml)
(Liao et al., 2020)	6,967 adults, NHANES, 2003-2012, United States Increased odds of hypertension OR 1.18 (95% CI 1.01-1.37)	Median, 1.10 >1.40 (4th quartile) compared to ≤0.85 (1st quartile)
(Chang et al., 2022)	Infants of 426 pregnant women, 2014-2018, United States Increased odds of small for gestational age (SGA) OR 2.22 (95% CI 1.12-4.38)	Maternal PFNA, median, 0.27 (8-14 weeks gestation) 0.42–2.27 (4th quartile) compared to <LOD-0.16 (1st quartile)
(Jensen et al., 2022)	1007 pregnant women, recruited 2010-2012, Denmark 2.42% increase in serum free thyroxine (FT4) concentration, 95% CI 0.13% to 4.77%	Median, 0.64 (measured at median of 12 weeks gestation) 2.35–10.12 (highest exposure category) compared to 0.26–1.11 (lowest exposure category)
(Zeeshan et al., 2021)	1,045 adults, recruited 2015-2016, China Increased odds of diabetes OR 5.78, 95% CI 3.13-10.69	Median, 1.44 >2.16 (4th quartile) compared to <0.98 (1st quartile)

19.4 Perfluorohexanesulfonic acid (PFHxS)

19.4.1 Identified studies that could change EFSA evaluation

19.4.1.1 Recent toxicological studies

Overall, seven recent *in vivo* studies were identified (Table 66) that were published after the EFSA assessment in 2020. None of these studies would influence the TWI derived by EFSA because the TWI developed by EFSA is based on human data and the exposure to PFHxS in these animal studies is much higher than that expected in human population.

Table 66: Summary of Recent Toxicological Studies Following Exposure to PFHxS

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
(He et al., 2022)	Mice, C57BL/6	Repeated dose oral (drinking water) toxicity study 12 weeks exposure	Adult male mice	0 and 450 µg/l	Exposure to PFHxS significantly promoted obesity and metabolic syndrome in male C57 mice fed a high-fat diet.
(Yin et al., 2021)	Mice, ICR	Repeated dose oral (gavage) toxicity study, investigated reproductive parameters 42 days exposure	Adult female mice	0, 0.5, 5, and 50 mg/kg/day	Exposure to 5 mg/kg/day of PFHxS altered reproductive functions in mice by inhibiting kisspeptin expression in the ARC and AVPV regions, leading to deficits in follicular development and ovulation.
(Sim & Lee, 2022)	Mice, C57Bl/6	Developmental neurotoxicity study (oral, gavage) Exposure on PND 10	Neonatal mice, PND 10	0, 6.1 and 9.1 mg/kg/bw	PFHxS impairs learning and memory in mice and induced neuronal death and GAP-43 and CaMKII (biomarkers of neuronal development) downregulation.
(Ramskov Tetzlaff et al., 2021)	Rats, Wistar	Developmental toxicity study (oral, gavage) from GD 7 to GD 22 and again after birth from PD 1 to PD 22.	Time-mated adult	0 (corn oil), 0.05, 5, and 25 mg/kg bw/day	Both male and female offspring have lower birth weight following PFHxS exposure. PFHxS exposure did not affect leptin levels in blood.
(Pfohl et al., 2020)	Mice, C57BL/6J	Repeated dose oral (feed) toxicity 29 weeks exposure	Adult male mice	PFHxS in feed (0.0003% wt/wt)	PFHxS increased hepatic expression of targets involved in lipid metabolism and oxidative stress. In the blood, PFHxS altered serum phosphatidylcholines, phosphatidylethanolamines, plasmogens, sphingomyelins, and triglycerides. PFHxS increase the risk of metabolic and inflammatory disease induced by diet, possibly by inducing dysregulated lipid metabolism and oxidative stress.
(Gilbert et al., 2021)	Rats, Long-Evans	Developmental neurotoxicity study (oral, gavage) Exposure from gestation day (GD) 6 until	Pregnant female rats	0 (2% Tween-20 in deionized water) and 50 mg/kg bw/day	Reduction of T4 level upon treatment with PFHxS has no effects on brain morphology or neurobehavior.

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
		postnatal day (PN) 21.			
(Ramhøj et al., 2020)		Developmental neurotoxicity study Exposure from gestation day 7 through to postnatal day 22.	Time-mated rat dams	0.05, 5 or 25 mg/kg/day	PFHxS reduced T3 and T4 in pregnant dams and they offspring but did not activate the HPT axis at doses up to 25mg/kg bw/day. The thyroid hormone disruptions were not correlated with effects on motor activity or learning and memory. PFHxS induced hypothyroxinemia and effects on the developing brain. Based on results it was suggested that the primary effect of low doses of PFHxS is to disrupt sexual differentiation of the brain.

19.4.1.2 Recent epidemiological studies

Epidemiological studies of PFHxS that could potentially change the TWI developed by EFSA include studies which (1) reported a dose-response association for an adverse effect and (2) the adverse effect was observed at PFHxS concentrations below approximately 30 ng/ml. This value is conservative because 30 ng/ml is the approximate concentration of the NOAEC associated with decreased vaccine antibodies against Hib for the sum of four PFAS: PFOA, PFOS, PFNA, and PFHxS (presented in Appendix K of EFSA 2020 analysing data by (Abraham et al., 2020)) and is similar to the NOAEC of 27 ng/ml for the sum of four PFAS based on information from (Grandjean et al., 2012). The results from epidemiological studies that meet these criteria are summarized below and in Table 67. However, further modelling similar to the EFSA approach would be necessary to check how these study results might influence the TWI developed by EFSA. Separately, epidemiological studies that log-transformed PFHxS values and presented risk estimates per unit of log-transformed PFHxS were not considered here. These studies require back transformation of log values and additional analyses to evaluate the shape of the dose-response function in order to identify a POD using a NOAEC or LOAEC or to otherwise calculate a benchmark dose.

(Shearer et al., 2021) conducted a nested case-control study to examine serum PFAS and risk of renal cell carcinoma. A total of 324 cases and 324 controls matched on age at enrolment, sex, race/ethnicity, study centre and year of blood draw were identified from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, a study of 55- to 74-year-old adults from 10 US cities recruited between 1993 and 2001. In controls, the adjusted geometric mean serum PFHxS was 2.3 ng/ml in women and 3.1 ng/ml in men; geometric means were adjusted for age, centre, sex, race, body mass index, hypertension, smoking status, previous freeze-thaw cycle, and year of blood draw. In logistic regression models, PFHxS was categorized as ≤2.2, >2.2-3.4, >3.4-5.5, and >5.5-37.4 ng/ml. After adjusting for body mass index, smoking, hypertension history, estimated glomerular filtration rate, previous freeze-thaw cycle, and year of blood draw, the authors reported higher odds of renal cell carcinoma in the second, third, and fourth quartiles of PFHxS exposure compared to the first quartile. This association did not reach statistical significance in the second or third quartiles; in the fourth quartile, the odds ratio was

2.07 (95% CI 1.06-4.04). The *p*-trend for increased risk across quartiles was statistically significant (p=0.04).

(Spratlen et al., 2020) conducted a cross-sectional study of PFAS and lipids in cord blood in 222 mother-child pairs from the World Trade Center (WTC) Birth Cohort, recruited during 2001-2002. The median cord blood PFHxS concentration was 0.66 ng/ml; exposure was divided into quartiles, with cut-points at 0.48, 0.66, and 0.95 ng/ml. After adjusting for maternal age, education, race/ethnicity, parity, family smoking, pre-pregnancy BMI, marital status, gestational age, and child sex, the third and fourth quartiles of PFHxS exposure showed an association with increased triglycerides in cord blood (third quartile geometric mean ratio 1.22, 95% CI 1.04-1.45; fourth quartile geometric mean ratio 1.26, 95% CI 1.07-1.49) compared to the first quartile, with a statistically significant trend of increased triglycerides with increased quartiles of PFHxS exposure (*p*-trend=0.002).

Table 67: Summary of Recent Epidemiological Studies Following Exposure to PFHxS

Reference	Finding	Conc. in serum (ng/ml)
(Shearer et al., 2021)	324 cases of renal cell carcinoma and 324 controls selected from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, recruited 1993-2001, United States Increased odds of renal cell carcinoma OR 2.07 (95% CI 1.06-4.04)	Geometric mean (adjusted), 2.3 (women) and 3.1 (men) >5.5-37.4 (4th quartile) compared to ≤2.2 (1st quartile)
(Spratlen et al., 2020)	222 mother-child pairs, World Trade Center birth cohort, recruited 2001-2002, United States Increased triglycerides in cord blood 22%, 95% CI 4% to 45%	Median, 0.66 >0.95 (3rd quartile) compared to <0.48 (1st quartile)

19.4.1.3 Discussion of the EFSA assessment in review papers

In the publication of Bernstein *et al.* (Bernstein et al., 2021) an error in one of the blood flow rates in the models for PFHxS, PFNA, and PFDA which were cited the EFSA assessment was identified. The model template published by the authors allows for faster evaluation and review of published PBPK models and provides a proof of concept for using this approach.

Relative potency factor (compared to perfluorooctanoic acid (PFOA)) for PFAS (including PFHxS) were calculated based on the induced liver effects. Relative potency factor for PFHxS was calculated as 0.6 (Bil et al., 2021).

19.5 Perfluorooctanesulfonic acid (PFOS)

19.5.1 Considerations of US EPA

As part of its rulemaking for National Primary Drinking Water Regulation for PFOA and PFOS, the US EPA has published a draft report on proposed approaches to the derivation of a draft Maximum Contaminant Level Goal (MCLG) for PFOS in drinking water in 2021. For the derivation of the draft noncancer reference dose (RfD) for PFOS, (US EPA, 2021a) considered studies conducted in both animals and humans reporting immune effects, developmental effects,

changes in serum lipids, hepatic effects, endocrine effects, and nervous system effects. Candidate PODs were considered from each of the studies and RfD values were calculated for each by applying uncertainty factors to the PODs.

The draft RfD selected by EPA (2021) for PFOS was 7.9 x 10^{-9} mg/kg-day based on the critical effect of decreased serum anti-diphtheria antibody concentration in children (Budtz-Jørgensen & Grandjean, 2018; Grandjean et al., 2012). This draft RfD was used to develop an interim health advisory level of 0.02 ng/l (ppt) for PFOS in drinking water (US EPA, 2022a). Since the interim health advisories for PFOA and PFOS were issued in June 2022, the Scientific Advisory Board to the EPA issued a final report on its review of the draft reports on proposed approaches to deriving MCLGs. The EPA has indicated that the final RfD may change, based on recommendations by the SAB to consider different critical effects, a combination of results for the same critical effect, or base the RfD on multiple endpoints. Regardless of the RfD selected, the EPA expects the health-based drinking water values to be below 4 ng/l.

The RfD by EPA was derived by selecting relevant animal and/or human studies. For human epidemiological studies, a benchmark dose modelling was used which results in internal serum concentrations. These values were converted into a point of departure human equivalent dose (POD_{HED}) using a life-stage modelling. The chronic RfD was calculated by dividing the POD_{HED} by a selected uncertainty factor. For the selected candidate values an intraspecies uncertainty factor of 10 was applied to the POD_{HEDs} from immune and developmental epidemiological studies with the argument of variability in the responses within the human population, resulting in the above mentioned RfD for PFOS. This value is based on the critical effect of decreased serum anti-diphtheria antibody concentration in children, investigated by (Grandjean et al., 2012).

In comparison to this value, the EFSA tolerable daily intake of 0.63 ng/kg was based on a $BMDL_{10}$ of 17.5 ng/ml in the serum of infants and children with reduced antibodies to diphtheria, tetanus and haemophilus influenza type b, which is 32-fold higher than the $BMDL_{5RD}$ of 0.54 ng/ml from (Grandjean et al., 2012). Using PBPK modelling, the serum level of 17.5 ng/ml in children was estimated to correspond to long-term maternal exposure of 0.63 ng/kg bw/day. EFSA did not consider uncertainty factors, since infants were expected to be a sensitive population group and a decreased vaccination response was considered a risk factor for a disease rather than a disease. Furthermore, EFSA did also take into account the study by (Grandjean et al., 2012), mentioning that findings on the vaccination response in this study could be confounded by other seafood contaminants. EFSA judged this study as observational as the timing of exposure was not standardized and assessed in relation to administration of a booster vaccination. Overall, in comparison to EPA, EFSA used a more recent epidemiological study for the derivation of the HBGV.

Table 68: POD_{HEDs} Considered for the Derivation of Candidate Reference Dose (RfD) Values by (US EPA, 2021a). (Page 305ff)

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/l)/Internal Dose Metric	PODHED (mg/kg-day)
Immune	Decreased serum anti-tetanus antibody	(Budtz-Jørgensen & Grandjea	Human, male and female	$BMDL_{5RD}$, piecewise	7.2×10^{-4}	1.05×10^{-7} [a]	

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/l)/Internal Dose Metric	PODHED (mg/kg-day)
	concentration in children	n, 2018; Grandjean et al., 2012; Grandjean, Heilmann, et al., 2017; Grandjean, Nielsen Flemming, et al., 2017); Medium confidence					
	Decreased serum anti-diphtheria antibody concentration in children	(Budtz-Jørgensen & Grandjean, 2018; Grandjean et al., 2012; Grandjean, Heilmann, et al., 2017; Grandjean, Nielsen Flemming, et al., 2017); Medium confidence	Human, male and female	$BMDL_{5RD}$, piecewise	5.4×10^{-4}	7.91×10^{-8} [a]	
	Decreased Plaque Forming Cell (PFC) Response to SRBC	(Zhong et al., 2016); Medium confidence	C57BL/6 Mice, F1 males	$BMDL_{1SD}$, Hill	1.27	2.01×10^{-4}	
	Extramedullary Haematopoie	(NTP, 2019); High	Sprague-Dawley Rats, female	$BMDL_{10RD}$, Multistage Degree 2	3.61	4.63×10^{-4}	

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/l)/Internal Dose Metric	PODHED (mg/kg-day)
	sis in the Spleen	confidence					
	Extramedullary Haematopoiesis in the Spleen	(NTP, 2019); High confidence	Sprague-Dawley Rats, male	$BMDL_{10RD}$, Logistic	9.42	1.21×10^{-3}	
Developmental	Decreased Birth Weight	(Chu et al., 2020); High confidence	Human, male and female	$BMDL_{5RD}$, Hybrid	7.6×10^{-3}	1.65×10^{-6}	
		(Sagiv et al., 2018); High confidence	Human, male and female	$BMDL_{5RD}$, Hybrid	41.2×10^{-3}	8.95×10^{-6}	
		(Starling et al., 2017); High confidence	Human, male and female	$BMDL_{5RD}$, Hybrid	5.8×10^{-3}	1.26×10^{-6}	
		(Wikström et al., 2020); High confidence	Human, male and female	$BMDL_{5RD}$, Hybrid	7.9×10^{-3}	1.72×10^{-6}	
	Decreased Foetal Body Weight	(C. K. Lee et al., 2015); Medium confidence	CD-1 Mice, F1 males and females	$BMDL_{5RD}$, Exponential 5	2.8×10^{-1}	1.05×10^{-4}	
	Decreased Pup Body Weight	(Luebker et al., 2005); Medium confidence	Sprague-Dawley Rats, F1 male and female	$BMDL_{0.5SD}$, Exponential 4	2.37	8.74×10^{-4}	
	Increased Number of Dead Foetuses	(C. K. Lee et al., 2015); Medium	CD-1 Mice, females	LOAEL	0.5 mg/kg/day	2.13	3.32×10^{-4}

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/l)/Internal Dose Metric	PODHED (mg/kg-day)
		confidence					
Alterations in Serum Lipids	Increased Total Cholesterol	(Z. Dong et al., 2019); Medium confidence	Human, male and female	$BMDL_{10RD}$, Hybrid	2.41×10^{-2}	3.08×10^{-6} [b]	
Hepatic	Individual Cell Necrosis in the Liver	(J. L. Butenhoff, Chang, et al., 2012); High confidence	Sprague-Dawley rats, females	$BMDL_{10RD}$, Multistage 3	24.5	3.13×10^{-3}	
Endocrine	Decreased Free T4	(NTP, 2019); High confidence	Sprague-Dawley rats, male	LOAEL	0.312 mg/kg/day	10.0	3.75×10^{-3}
	Decreased Free T4	(NTP, 2019); High confidence	Sprague-Dawley rats, female	$BMDL_{1SD}$, Exponential 4	3.89	4.98×10^{-4}	
	Decreased Total T4	(NTP, 2019); High confidence	Sprague-Dawley rats, female	$BMDL_{1SD}$, Exponential 4	2.65	3.39×10^{-4}	
	Decreased Total T3	(NTP, 2019); High confidence	Sprague-Dawley rats, male	$BMDL_{1SD}$, Hill	6.81	8.72×10^{-4}	
	Decreased Total T3	(NTP, 2019); High confidence	Sprague-Dawley rats, female	$BMDL_{1SD}$, Hill	16.1	2.06×10^{-3}	
	Decreased Total T3	(Seacat et al., 2002); Medium	Cynomolgus Monkeys, male	LOAEL	0.03 mg/kg/day	8.13	1.04×10^{-3}

Effect	Endpoint	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD (mg/kg-day)	POD Internal Dose (mg/l)/Internal Dose Metric	PODHED (mg/kg-day)
		confidence					
	Decreased Total T3	(Seacat et al., 2002); Medium confidence	Cynomolgus Monkeys, female	$BMDL_{1SD}$, Exponential 4	7.09	9.07×10^{-4}	
Nervous System	Decreased Performance on the Object Location Recognition Memory Test	(Mshaty et al., 2020); Medium confidence	C57BL/6J, F1 males	NOAEL	0.5 mg/kg/day	8.96×10^{-1}	9.97×10^{-5}

[a] Calculated as the dose to mothers & children that results in the same serum concentration at 5 years of age. Note that the model predicted slightly different serum concentrations for male and female children, so the lower HED was selected to be more health protective.

[b] Calculated as the dose that would result in the serum concentration POD at steady state.

19.5.2 Identified studies that could change EFSA evaluation

19.5.2.1 Recent toxicological studies

There was no recent *in vivo* study identified which would lead to a lower TWI than to the one derived by EFSA. As described above, EFSA also considered animal studies, but preferred to use human data to derive a TWI.

Several animal studies were identified determining the effects of PFOS in animals, which support the adverse effects identified in the EFSA assessment. Most of these studies referred to toxicity to reproduction, developmental toxicity and hepatotoxicity using similar doses as the animal studies reported by EFSA. These studies are summarized in Table 69. Investigated endpoints in these studies are toxicity to reproduction, developmental toxicity, cardiotoxicity, hepatotoxicity, lipid metabolism and neurotoxicity. All these endpoints are also addressed in the EFSA assessment. *In vivo* studies considering these endpoints with similar doses were reported in the EFSA assessment. EFSA did not consider these endpoints as critical since epidemiological studies provide insufficient evidence of these effects in humans. Furthermore, the EFSA assessment preferred to use human epidemiological data over animal data for deriving HBGVs, since they judged the use of uncertainty factors as too conservative when comparing the results from animal and human studies.

Table 69: Summary of Recent Toxicological Studies Following Exposure to PFOS

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
(Conley et al., 2022)	Sprague Dawley rats	Oral gavage, GD14 to GD18	Pregnant female,	0, 0.1, 0.3, 1, 3,	Potential NOAEL of 3 mg/kg bw/day; higher doses

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
		Developmental toxicity endpoints	90 days old	10 and 30 mg/kg bw/day	resulted in decreases in dam body weight gain, Total T3, Total T4, cholesterol and triglyceride levels
(Dangudubiyyam et al., 2022)	Sprague Dawley rats	Drinking water study, GD4 to GD20	Pregnant female, 12 weeks old	0, 0.005, 0.05, 0.5, 5,10 and 50 µg/ml	Increase of maternal blood pressure and decrease of foetal weights from 0.5 µg/ml. Left ventricular hypertrophy and fibrosis were observed in dams at 50 µg/ml.
(Hamilton et al., 2021)	Cyp2b-null mice and humanized CYP2B6/ 2A13/2F1-transgenic mice	Oral gavage, 21 days treatment Hepatotoxicity biomarkers, CYP2B6 induction	Male and female mice, 10-12 weeks old	1 and 10 mg/kg bw/day	Potential NOAEL of 1 mg/kg bw/day (based on mortality in female hCYP2B6-Tg mice at 10 mg/kg bw/day and a decrease in body weight and white adipose tissue, as well as increased liver weights, which were most pronounced in the 10 mg/kg bw/day group)
(J. Huang et al., 2022)	ICR mice	Oral gavage, 4 weeks treatment Male reproductive toxicity	Male, 8 weeks old	0.5, 5 and 10 mg/kg bw/day	Potential NOAEL of 0.5 mg/kg bw/day (based on significantly reduced sperm counts and structural and morphological alterations in testis (Leydig cell vacuolation) and reduced serum and testicular testosterone levels at dose levels ≥5 mg/kg bw/day)
(L. Jiang et al., 2022)	ICR mice	Oral gavage, 14 days treatment Hepatotoxicity	Male and female, 4 to 5 weeks old	1, 5 and 10 mg/kg bw	Potential NOAEL of 1 mg/kg bw/day (based on elevated enzyme levels of ALT and AST, concomitant with dose-dependent hepatic steatosis and necrosis at dose levels ≥5 mg/kg bw/day)
(X. Li et al., 2021)	BALB/c mice	Oral gavage, 2 months treatment Lipid metabolism	Female, 4 weeks old	0, 100 and 1000 µg/kg bw/day	Histopathological alterations in several organs were detected after PFOS exposure, which were most severe in the 1000 µg/kg bw/day group in the liver (steatosis, focal infiltration with inflammatory cells), heart (calcareous masses at

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
					the epicardial membrane surrounded by hyperplastic connective tissue) and brain (some neuronal cells were contracted and stained deeply, the cytoplasm and nucleus were not clearly defined, and a small amount of neuron phagocytosis was noted). Since the relevance of findings for the low dose group was not clear, a NOAEL was not defined.
(Z. Li et al., 2022)	CD-1 mice	Oral administration, 21 days treatment Male reproductive toxicity	Male, 6 weeks old	0, 1 and 5 mg/kg bw/day	Exposure to PFAS decreased the function of the Luteinizing hormone and decreased epididymal sperm motility. No changes in body weight were observed.
(Wan et al., 2020)	CD-1 mice	Oral gavage, GD 4.5 to GD 17.5 Developmental toxicity	Pregnant female mice	0, 1 and 3 mg/kg bw/day	At 3 mg/kg bw, a significant reduced foetal body weight and a significant increase in maternal liver weight was observed. No changes in maternal body weights were observed. At 3 mg/kg bw/day placenta and foetal livers were found to have significantly higher corticosterone levels.
(G. Wang et al., 2020)	C57BL/6J mice	Oral gavage, 16 days treatment Tissue damage	Male, 6 weeks old	0.3, 3 and 30 µg/kg bw/day	After PFOS exposure, active mice became more prone to lying down after PFOS exposure with reduced activity, most obvious in the highest dose. At the high dose group, body weight decreased by 9.54% compared to the control. Food intakes decreased in all treatment groups. Hepatocytic oedema and degeneration, increased liver weight and disruption of hepatocyte structure was observed in all treatment groups. All treatment groups

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
					exhibited significantly elevated serum concentrations of ALT and ALP. NOAEL could not be determined.
(L. Wang et al., 2020)	BALB/c mice	Oral gavage, 14 days treatment Hepatoxicity, lipid metabolism	Male	0, 10 and 20 mg/kg bw/day	Potential NOAEL of 10 mg/kg bw based on decreased body weight without changes in food and water intake and hypolipidemia observed in PFOS-exposed mice on days 7 and 14 (statistically different in the 20 mg/kg group).
(Z. Wang et al., 2022)	BALB/c mice	Oral gavage, 28 days treatment Hepatotoxicity	Male, 6 to 8 weeks old	0, 0.2, 1 and 5 mg/kg bw/day	Significant increase of relative liver weights in all PFAS groups. No change in body weight was observed. At 5 mg/kg bw/day ALT was significantly higher compared to the control. Karyolysis (from 0.2 mg/kg bw), cytoplasmic vacuolation (at 5 mg/kg bw) and necrosis (at 1 and 5 mg/kg bw) was observed
(D. Xu et al., 2022)	Sprague-Dawley rats	Oral gavage, 14 days treatment Cardiotoxicity	Male, 8 weeks old	0, 1 and 10 mg/kg bw/day	Potential NOAEL of 1 mg/kg bw/day based on significant increase of the percentage of heart to body weight, significant increase of expression levels of myocardial injury markers, cardiac fibrosis and myocadiac hypertrophy, inflammatory infiltration, upregulation of p53 and Bax
(Yu et al., 2020)	ICR mice	Oral gavage, 28 days Neurotoxicity	Male, 8 weeks old	0,0.25, 2.5, 25 and 50 mg/kg bw/day	Morphological and ultrastructural changes of blood-brain barrier was observed after PFAS exposure. PFOS significantly decreased the expression of tight junction related proteins in endothelial cells and disrupted the blood brain barrier.

Study	Species	Study Design	Life Stage/ Sex	Dose	Result
(H. Zhang et al., 2021)	Sprague-Dawley rats	Oral gavage, GD12 to GD18 treatment Developmental toxicity	Pregnant female	0, 1 and 5 mg/kg bw/day	Potential LOAEL of 1 mg/kg bw/day based on reduction of alveolar numbers, thickened alveolar septa, increased lung inflammation and upregulation of inflammasome associated proteins and inflammation cytokines, downregulation of HIF-1α and VEGFA
(H. Zhang et al., 2020)	Sprague-Dawley rats	Oral gavage, GD5 to GD20 treatment Developmental toxicity	Pregnant female	0, 1 and 5 mg/kg bw/day	Potential LOAEL of 1 mg/kg bw/day based on reduction of relative testis weight, serum testosterone and aldosterone, reduction of Leydig cells and different Leydig cell genes in F1 males

19.5.2.2 Recent epidemiological studies

Epidemiological studies of PFOS that could potentially change the TWI developed by EFSA include studies which (1) reported a dose-response association for an adverse effect and (2) the adverse effect was observed at PFOS concentrations below approximately 30 ng/ml. This value is conservative because 30 ng/ml is the approximate concentration of the NOAEC associated with decreased vaccine antibodies against Hib for the sum of four PFAS: PFOA, PFOS, PFNA, and PFHxS (presented in Appendix K of EFSA 2020 analysing data by (Abraham et al., 2020) and is similar to the NOAEC of 27 ng/ml for the sum of four PFAS based on information from (Grandjean et al., 2012). The results from epidemiological studies that meet these criteria are summarized below and in Table 70. However, further modelling similar to the EFSA approach would be necessary to check how these study results might influence the TWI developed by EFSA. Separately, epidemiological studies that log-transformed PFOS values and presented risk estimates per unit of log-transformed PFOS were not considered here. These studies require back transformation of log values and additional analyses to evaluate the shape of the dose-response function in order to identify a POD using a NOAEC or LOAEC or to otherwise calculate a benchmark dose.

(T.-W. Lin et al., 2020) conducted a cross-sectional study in 2016-2017 of 397 adults aged 55-75 who lived in PFAS-exposed communities in Taiwan to examine the association between nine serum PFAS and metabolic syndrome and related biomarkers (uric acid, fasting blood sugar, total cholesterol, and triglycerides). The median serum PFOS among participants was 16.3 ng/ml. In models adjusted for age, sex, smoking, and drinking, PFOS was associated with elevated LDL cholesterol in the second, third, and fourth exposure quartiles compared to the first (β=13.97, 95% CI 4.96, 22.99 for 10.1-16.2 ng/ml; β=11.06, 95% CI 2.01, 20.12 for 16.2-24.1 ng/ml; β=11.68, 95% CI 2.41, 20.95 for >24.1 ng/ml). Although the β coefficient did not consistently increase across increased exposure groups, the p for trend was statistically significant (p=0.03). Results were similar when lipid-lowering drug users were excluded. (Souza et al., 2020) conducted a nested case-control study within the Brazilian Ribeirao Preto and Sao Luiz Birth Cohort Study cohort, in which pregnant women were recruited from 2010 to 2011 between 22 and 26 weeks of gestational age. From this larger cohort, the authors identified 63

cases of children with foetal growth ratio <0.85 and 189 matched controls. The median whole blood PFOS concentration measured in the second trimester was 3.41 ng/ml, and the exposure quartiles (based on exposure in cases) used in analyses were <2.37, 2.37-3.34, 3.35-5.73, and >5.73 ng/ml. After adjusting for preterm birth, parity, maternal education, socioeconomic status, pre-pregnancy BMI, and smoking during pregnancy, the authors reported higher odds of intrauterine growth restriction associated with the highest quartile of PFOS exposure compared to the lowest (OR 3.67, 95% CI 1.38, 9.74). Although there was no association seen in the second and third exposure quartiles, the p for trend across exposure quartiles was statistically significant (p=0.0014).

(Jensen et al., 2022) conducted a cross-sectional analysis of PFAS exposure and thyroid hormones among 1,007 pregnant women participating in the Odense Child Cohort study. Participants were originally recruited from 2010-2012 in Denmark during their early pregnancy. Serum PFOS and thyroid hormones were measured at a median of 12 gestational weeks; median serum PFOS in this population was 7.72 ng/ml, and the exposure categories used in analyses were 1.03–5.57, 5.57-7.72, 7.72–10.53, and 10.53–27.47 ng/ml. After adjusting for age, parity, and education, the authors reported an association between the highest category of serum PFOS and a 2.82% increase in serum free thyroxine concentration compared to the first category (β=2.82, 95% CI 0.53, 5.16). Although the associations in the second and third categories did not reach statistical significance, the p for trend across categories was statistically significant (p=<0.01).

(Zeeshan et al., 2021) conducted a cross-sectional analysis of 1,045 Chinese adults, aged ≥35 years, who were recruited during 2015-2016 as part of the Isomers of C8 Health Project. The median serum br-PFOS (sum of all branched PFOS isomers) was 14.25 ng/ml; exposure quartile cut-points were 9.09, 14.25, and 21.81 ng/ml. After adjusting for age, sex, ethnicity, income, education, smoking, alcohol, and seafood consumption, the authors reported increased odds of diabetes in the fourth exposure quartile of br-PFOS compared to the first (OR 9.02, 95% CI 4.37-18.61). Although the associations in the second and third exposure quartiles did not reach statistical significance, the p for trend across quartiles was statistically significant (p=<0.001).

Table 70: Summary of Recent Epidemiological Studies Following Exposure to PFOS

Reference	Finding	Conc. in serum (ng/ml)
(T.-W. Lin et al., 2020)	397 adults aged 55-57, 2016-2017, Taiwan Increased LDL cholesterol 11.68 mg/dL increase, 95% CI 2.41 to 20.95 mg/dL	Median, 16.3 >24.1 (4th quartile) compared to <10.1 (1st quartile)
(Souza et al., 2020)	252 mother-child pairs, 2010-2011, Brazil Increased odds of intrauterine growth restriction in OR 3.67 (95% CI 1.38-9.74)	Maternal PFOS, median, 3.41 (second trimester) >5.73 (4th quartile) compared to <2.37 (1st quartile)
(Jensen et al., 2022)	1007 pregnant women, recruited 2010-2012, Denmark Increased serum free thyroxine (FT4) concentration	Median, 7.72 (measured at median of 12 weeks gestation) 10.53-27.47 (4th quartile) compared to 1.03-5.57 (1st quartile)

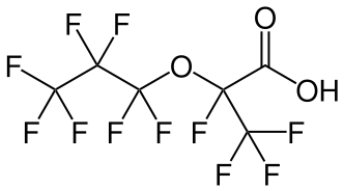
Reference	Finding	Conc. in serum (ng/ml)
	2.82% increase in serum FT4, 95% CI 0.53% to 5.16%	
(Zeeshan et al., 2021)	1,045 adults, 2015-2016, China Increased odds of diabetes OR 9.02, 95% CI 4.37-18.61	Median br-PFOS, 14.25 ≥21.81 (4th quartile) compared to <9.09 (1st quartile)

20 Toxicological evaluation of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid (HPFO-DA)

20.1 Chemical and physical information

HFPO-DA (also marketed as FRD-903) is a perfluoroalkyl ether carboxylic acid. The molecular structure of HPFO-DA is shown in Figure 2. The ammonium salt of HFPO-DA is typically known as GenX (also marketed as FRD-902). Please note that both substances are chiral. However, no information on the isolated enantiomers is available. For all following physicochemical properties, toxicokinetic and toxicological properties usually racemates were used.

Figure 2: Molecular structure of HPFO-DA



Source: Wikipedia

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 71 and Table 72.

Table 71: Chemical identity of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid (HFPO-DA, CAS 13252-13-6)

Name	Tetrafluor-2-(heptafluor-propoxy)propansäure
English Name	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid
Acronym	HFPO-DA (ammonium salt is called GenX)
Mol. Formula	C6HF11O3
Mol. Weight (g/mol)	330.19
CAS	13252-13-6 (acid) 62037-80-3 (ammonium salt)
EC	236-236-8 (acid)

Table 72: Physicochemical properties of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid (HFPO-DA, CAS 13252-13-6)

Properties	Value	Source
Density (g/cm^3)	HFPO-DA: 1.69 (exp. at 20 °C), GenX: 1.7 (exp. at 20 °C)	(Hoke et al., 2016)
Melting point (°C)	27.8 (mod.)	(USEPA, 2020)
Boiling point (°C)	143-145 (exp.)	Apollo Scientific website[a]
Vapour Pressure (Pa)	HFPO-DA: 306±13.7 (exp. at 20 °C)[b]; GenX: 1.17 x 10^{-2} (dried solid, exp. at 20 °C), 2910 (liquid, exp. at 20 °C)	(ECHA, 2019)
Henry's Constant (Pa m^3/mol)	2.4 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	2.84-3.82 (exp.)	(ECHA, 2019)
Log Koc	No data	
Water Solubility (mg/l)	367 (mod.)[c]	(USEPA, 2020)

(exp = experimental, mod = modelled); [a]http://www.apolloscientific.co.uk/index.php; [b]Results for HFPO-DA (product H-28307) with a purity of 98%, further containing 0.61% water and 8.6 ppm PFOA which might influence the result; [c]OPERA Model: 1.11 x 10^{-3} mol/l = 367 mg/l.

20.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

This chapter reviews quantitative toxicological risk assessments of HFPO-DA and GenX on human data determined by other institutions (Table 73) and drinking water limits for HFPO-DA and GenX (Table 74). Data was identified by general desk research.

Table 73: Summary of quantitative toxicological assessments of HFPO-DA/GenX and corresponding health endpoints by other institutions

Agency	Quantitative assessment	Health endpoint	Value	Reference
RIVM	Tentative TDI	increased albumin/globulin ratio in serum	21 ng/kg bw/day	(RIVM and RIKILT, 2019)
US EPA	Subchronic RfD for Noncancer Effects	liver effects	3×10^{-5} mg/kg/day	(US EPA, 2021c)
US EPA	Chronic RfD for Noncancer Effects	liver effects	3×10^{-6} mg/kg/day	(US EPA, 2021c)
ECHA	DNEL oral general population	liver and blood effects	0.01 mg/kg bw/day	ECHA (reg. Dossier)[a]

[a]ECHA Registration dossier of GenX (CAS 62037-80-3): https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2679/1/2 (last updated 2022).

Table 74: Drinking water limits determined for HFPO-DA/GenX or sum of PFAS

Country/ Institution	HFPO-DA/GenX limit value in drinking water (µg/l)	Comment	Source
Netherlands/RIVM	0.15	Provisional guideline value drinking water general population	(RIVM, 2017b)
North Carolina (USA)/Department of Health and Human Services	0.14	Provisional drinking water equivalent level (DWEL) general population	(ECOS, 2022; NC DHHS, 2017)
Michigan (USA)	0.37	drinking water maximum contaminant level	(ASDWA, 2021; ECOS, 2022)
Illinois (USA)	0.021	drinking water guideline value	(ECOS, 2022)
Wisconsin (USA)	0.3	drinking water guideline value	(ECOS, 2022)

20.3 Toxicokinetics

20.3.1 Animal data

20.3.1.1 Data/studies reported in previous government and general reviews

It should be highlighted that in the following information is extracted from the support document for identification of 2,3,3,3-TETRAFLUORO-2- (HEPTAFLUOROPROPOXY)PROPIONIC ACID, ITS SALTS AND ITS ACYL HALIDES (COVERING ANY OF THEIR INDIVIDUAL ISOMERS AND COMBINATIONS THEREOF) as substance of very high concern which is cites as (ECHA, 2019). In this report many references are made to unpublished studies to which no access is available. Therefore, the relevant studies are cited in the following as secondary literature.

20.3.1.1.1 Absorption

(ECHA, 2019) summarized the available toxicokinetic information for GenX following oral exposure. As reported in (ECHA, 2019) data indicate that HFPO-DA is readily absorbed in the gastrointestinal tract following oral exposure ((Fasano, 2011a, 2011b) as cited in (ECHA, 2019)). Groups of five Crl:CD-1(ICR) mice of each sex were administered with a single dose of 3 mg/kg/bw GenX (84% purity) by gavage (water) ((Fasano, 2011a) as cited in (ECHA, 2019)). In the study urine was collected and pooled for the first 12 hours as well as for 12-168 hours. During the first 12 hours post exposure, a mean percentage of 31% and 39% of the given dose was excreted in the urine for male and female mice. By 168 hours, 90% and 92% of the administered dose was retrieved in the urine respectively. ECHA reports on a similar study, five male and five female Crl:CD(SD) rats were administered with a single dose of 30 mg/kg/bw FRD-902 (84% purity) by gavage (water) ((Fasano, 2011b) as cited in (ECHA, 2019)). Urine collection followed the same time scheme as above (collected and pooled for the first 12 hours and 12-168 hours). A mean urinary excretion of 95% or 97% of the given dose was found during the first twelve hours post-exposure for female and male rats respectively. ECHA concluded based on these two studies that the absorbance of almost the entire given dose of

FRD-902 proceeds in the gastrointestinal tract for mice as well as rats. Mice however either absorb or excrete HFPO-DA at a slower rate than rats.

Male and female (C57BL/6) mice (6/sex/group) were administered FRD-903 (HPFO-DA) by gavage at doses of 1, 10, or 100 mg/kg bw/day for 28 days. Serum and Urine concentrations were measured at days 1, 5, 14, and 28 as well as 1, 2, 3, 5, 10, and 14 respectively. For the groups receiving 10 and 100 mg/kg bw/day the concentrations observed were significantly different from the control group. This was not the case for the group receiving 1 mg/kg bw/day though. ((Rushing et al., 2017) as cited in (ECHA, 2019)). Serum concentrations showed maximum levels after 5 days for all groups, after which a decrease was observed with the exception of the male group receiving 100 mg/kg bw/day, in which case the maximum concentration remained until day 14. Furthermore higher serum concentrations could be found in males receiving 10 or 100 mg/kg bw /day in serum as well as urine samples compared to females at all points in time, suggesting a higher absorption rate in male mice.

In a 90-day oral study (OECD test guideline 408) conducted in mice ((MacKenzie, 2010) as cited in (ECHA, 2019)), groups of ten Crl:CD-1(ICR) mice per dose and sex were exposed to FRD-902 (GenX, 84% purity) by gavage at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day. Additional mice were examined to evaluate the plasma concentration of the substance at 2 hours after exposure on day 0, 28 and 95. LOD and LOQ were indicated as 5 ng/ml and 20 ng/ml respectively. In Table 75 the serum concentration 2 hours after the first exposure can be found. It can be seen that serum concentrations correlate in a dose-dependent manner and, as indicated by large standard deviations, differences between individual animals are recognised. (ECHA, 2019) notes that the sex difference in absorption observed by (Rushing et al., 2017) is not reflected by these data.

Table 75: Plasma concentration in mice 2 hours after exposure (gavage) to FRD-902 ((MacKenzie, 2010) as cited in (ECHA, 2019))

Dose mg/kg bw/day	Males		Females	
	ng/ml	SD	ng/ml	SD
0	<LOD	NA	<LOD	NA
0.1	736	99	824	72
0.5	3806	1175	3608	1308
5	42580	5214	35340	9362

20.3.1.1.2 Distribution

(ECHA, 2019) concluded from the data, that GenX distributes mainly to the serum/plasma and the liver in rats and mice, with higher concentrations being found in males than in females. Three male and three female (Crl:CD(SD)) rats were given a single dose of GenX (FRD-902) (84% purity) at 10 or 30 mg/kg bw by gavage (water) ((Gannon, 2008b) as cited in (ECHA, 2019)). In the following plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours post-exposure. After sacrificing adipose and liver tissue samples were taken. LOD in plasma and tissue were identified to be approximately 20 ng/ml and 20 ng/g respectively. The data showed that the mean concentration found in liver tissue was below the LOQ for female specimen at both dosage levels and that at a single dose of 10 mg/kg bw a mean concentration of 73 ng/g and at a single dose of 30 mg/kg bw a mean concentration of 38 ng/g could be found. In females mean plasma concentrations were also below the LOQ and found to be 36 ng/ml when a single dose of 10 mg/kg bw is given and 57 ng/ml when a single dose of 30 mg/kg bw is given for males. From this data liver tissue to plasma concentration ratios for males

in the low and high dose groups of 2.2 ± 1.1 and 0.8 ± 0.3 could be obtained. As the samples for female specimen were below the LOQ no ratios were calculated in this case. Also in adipose tissue, no HFPO-DA could be found at concentrations above limits of quantification.

In a similar study conducted in (Crl:CD(SD)) rats, the animals were administered a single dose of FRD-903, the GenX dimer acid, at doses of 10 or 30 mg/kg bw ((Gannon, 2008c) as cited in (ECHA, 2019)). The concentrations in liver tissue and plasma in female rats were below the LOQ. In males, which were administered 10 mg/kg bw of the substance, a concentration of 24 ng/g could be found in liver tissue and 41 ng/ml could be found in plasma. Males which were administered a dose of 30 mg/kg bw, showed concentrations of 89 ng/g in liver tissue and 128 ng/ml in plasma. Limits of quantification could be determined as approximately 20 ng/g in liver tissue and 20 ng/ml in plasma. Using this data liver tissue to plasma concentration ratios of 0.6 ± 0.3 and 0.7 ± 0.2 for males in the low and high dose groups could be determined respectively.

Three Crl:CD-1(ICR) mice of each sex were given a single dose of GenX (84% purity) by gavage at 10 or 30 mg/kg bw respectively ((Gannon, 2008a) as cited in (ECHA, 2019)). Post-exposure plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. After sacrifice liver and adipose tissue were collected. Limits of quantification could be determined as approximately 20 ng/ml and 20 ng/g plasma and liver tissue respectively. All liver tissue samples collected from female mice were below the LOQ. Liver tissue collected from males administered doses of 10 mg/kg bw and 30 mg/kg bw, showed mean concentrations of 384 ng/g and 457 ng/g respectively. From these results liver tissue to plasma concentration ratios of 0.5 ± 0 and 0.6 ± 0.1 could be determined for males in the low and high dose groups respectively.

20.3.1.1.3 Gestational transfer and transfer via lactation

As cited in (ECHA, 2019), (Edwards, 2010b) exposed, Crl:CD-1(ICR) mice (N = 25) in a reproductive/developmental toxicity screening study to GenX through oral gavage at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day (purity 84%). The F0 males were administered GenX from day 0 to 84 (70 days prior to pairing until 1 day prior to euthanasia), leading to a total of 84 to 85 doses. The females that were selected for toxicokinetic evaluation were administered GenX until the day of euthanasia (lactation day 21) leading to a total of 54 to 65 doses. A limit of detection for HFPO-DA in plasma was determined to be 3 ng/ml. Results indicated that the concentration of GenX in plasma in pups on post-natal day 4 were 2- to 4-fold below the maternal plasma concentrations on post-natal day 21 and on post-natal day 21 the concentration of GenX in plasma was 40-60 fold lower. The authors concluded that, based on these results, GenX is transferred to the pups, through either gestation, lactation, or both. Furthermore, the study showed 10-32 times lower plasma levels in pups (retrieved on post-natal day 21) than concentrations observed in pups on post-natal day 4. Thus a negligible transfer via lactation can be concluded. On post-natal day 40, following direct administration of GenX to F1 through gavage for 20 consecutive doses at 0, 0.1, 0.5 or 5 mg/kg bw/day, the concentration measured in plasma were comparable between the dams and offspring. Further F1 males showed relatively higher mean concentrations than females .

ECHA reports on a developmental toxicity study (developmental toxicity / teratogenicity) ((Edwards, 2010a; Munley, 2011) as cited in (ECHA, 2019)) in which pregnant Crl:CD(SD) rats were exposed to GenX (84% purity) during gestation days (GD) 6-20 at 0, 5, 10, 100, or 1000 mg/kg bw/day by oral gavage. On day 21 the dams were sacrificed. Concentrations of GenX in plasma was measured in the foetuses on GD 20, and in dams on GD 20 as well as on GD 6 for the group that was administered the highest dose (1000 mg/kg bw/day)., Results indicated plasma

concentrations in foetuses (pooled concentration) were approximately one-third of the plasma concentration in the dam at GD20.

20.3.1.1.4 Metabolism

(ECHA, 2019) reported an *in vitro* study in which potential formation of metabolites was screened using male and female rat hepatocytes ((Nabb, 2007) as cited in (ECHA, 2019)). Incubated hepatocytes (1 x 10^6 cells/ml) were administered GenX at a concentration of 10 µL/ml, and samples were taken at 0, 30, 45, 60, 90, and 120 minutes and evaluated. The samples did not show any metabolites . A similar *in vitro* study was carried out using trout hepatocytes ((Dupont, 2007) as cited in (ECHA, 2019)) and showed no indication of metabolism.

(RIVM, 2016) presents the study results of (Gannon et al., 2016) in which the absorption, distribution, metabolism and excretion (ADME) and kinetics of GenX was evaluated in rats, mice and cynomolgus monkeys. Pharmacokinetics were determined by measuring in blood samples from rats and mice at multiple time points after a single oral dosing at 10 or 30 mg/kg. In addition, pharmacokinetics after single intravenous exposure (10 mg/kg) were measured at multiple time points up to 7 days in rats and up to 21 days in cynomolgus monkeys. Parameters were measured in tissue and excreta up to 168 hours after oral dosing in mice (dose: 3 mg/kg) and in rats (dose: 30 mg/kg). A hepatocyte metabolism test indicated that GenX is not metabolized by rat hepatocytes, which was supported by the absence of metabolites and the complete recovery of the dosed GenX in rat and mouse urine.

20.3.1.1.5 Excretion

(ECHA, 2019) reported that according to studies in rats and mice ((Fasano, 2011a, 2011b), as cited in (ECHA, 2019)), which were administered a single dose of FRD-902 with doses ranging from 3 – 30 mg/kg bw, GenX was mainly excreted via urine. 97% and 95% of the dose were retrieved through the urine of male and female rats respectively after 12 hours. At the end of the study (168 hours after admission) 103% and 100% of the given dose were recovered in male and female rats respectively. A negligible amount of GenX was found in the faeces (≤ 1%). However, a contamination with urine in this regard is expected. Cleaning of the cages accounted for another 1% and 5% retrieval of the given dose for males and females respectively. In mice, 31% (males) and 39% (females) of the administered GenX could be retrieved 12 hours after dosing. After 168 hours, at the end of the study, 90% and 92% of the given dose was excreted through urine in males and females respectively. A negligible amount in the faeces of male and female mice (2%) and another 10% and 6% were recovered through cleaning the cages.

The clearance rates following single oral exposure to GenX and the GenX dimer acid (FRD-902 and FRD-903) were investigated in two studies in rats (N = 3 per dose group) ((Gannon, 2008b, 2008c) as cited in (ECHA, 2019)) receiving 0, 10 or 30 mg/kg/bw. The definition of clearance time was set as the time needed for the removal of 98.4% of GenX from the plasma. In male rats clearance times of 12h for a dosage of 10 mg/kg bw and 22h for 30 mg/kg bw could be found and female rats showed clearance times of 4h and 8h following administration of GenX at doses of 10 or 30 mg/kg bw respectively. Exposure to either 10 or 30 mg/kg bw FRD-903 lead to clearance times of 28 h and 22 h in males and 8 h and 4 h in females. In mice a given single oral dose of 10 or 30 mg/kg bw GenX, lead to plasma clearance times of 143 h and 139 h for male and 57 h and 62 h for female mice ((Gannon, 2008a) as cited in (ECHA, 2019)).

(RIVM, 2016) presents the study results of (Gannon et al., 2016) in which the absorption, distribution, metabolism and excretion (ADME) and kinetics of GenX was evaluated in rats, mice and cynomolgus monkeys. Pharmacokinetics were determined by measuring in blood samples from rats and mice at multiple time points after a single oral dosing at 10 or 30 mg/kg. In addition, pharmacokinetics after single intravenous exposure (10 mg/kg) were measured at

multiple time points up to 7 days in rats and up to 21 days in cynomolgus monkeys. Parameters were measured in tissue and excreta up to 168 hours after oral dosing in mice (dose: 3 mg/kg) and in rats (dose: 30 mg/kg). Results indicated GenX is rapidly absorbed after oral exposure and shows biphasic elimination with a very rapid alpha-phase and a slower beta-phase elimination (Table 76). The alpha-phase elimination half-life was faster in female rats compared to male rats. Half-lives determined in male rats and mice following oral administration were 2.8 and 5.8 hours, respectively; while the half-lives in female rats and mice were 0.2 and 4.6 hours, respectively. Because the urinary elimination rate was very rapid (nearly the entire dose was eliminated within 12-24 hours), the sex difference observed in the plasma kinetics was not readily apparent in the urine kinetics. No test substance was detected in the blood of monkeys 16 days after dosing probably due to the much slower elimination in the beta-phase. For both rats and monkeys the alpha phase was very rapid and the contribution of the beta-phase was considered negligible. The authors concluded that the beta phase elimination did not contribute to potential accumulation after multiple dosing in rats or monkeys. Nearly the whole administered dose was eliminated in the urine in rats and mice. A small amount of the test substance was recovered in the faeces, but this was likely due to contamination of the faeces with urine.

Table 76: Pharmacokinetic parameters from (Gannon et al., 2016)

Constant	Rat, intravenous		Rat, oral		Mouse, oral		Cynomolgus monkey, intravenous	
	Male	Female	Male	Female	Male	Female	Male	Female
Absorption: Rate constant (1/h); Time (h)	NA	NA	3.30; 0.21	1.52; 0.46	3.83; 0.18	3.11; 0.22	NA	NA
Alpha phase Elimination rate constant (1/h); Half-life	0.20; 3.6	1.72; 0.4	0.25; 2.8	2.78; 0.2	0.12; 5.8	0.15; 4.6	0.30; 2.3	0.37; 1.9
Beta phase Rate (1/h); Half-life (h)	7.8E-03; 89.1	3.1E-02; 22.6	9.6E-03; 72.2	1.0E-02; 67.4	1.9E-02; 36.9	2.9E-02; 24.2	1.1E-02; 64.1	8.7E-03; 79.6
Volume of distribution Central (L/kg); Peripheral (L/kg)	0.168; 0.155	0.178; 1.508	0.142; 0.161	0.057; 2.462	0.117; 0.130	0.148; 0.078	0.068; 0.029	0.056; 0.021

20.3.1.2 New data

20.3.1.2.1 Absorption

No relevant data was found.

20.3.1.2.2 Distribution

No relevant data was found.

20.3.1.2.3 Metabolism

No relevant data was found.

20.3.1.2.4 Gestational transfer and transfer via lactation

Pregnant CD-1 mice were divided into two experimental blocks to achieve a total of 11 to 13 litters per treatment group (Blake et al., 2020). Dams were administered PFOA (0, 1, or 5 mg/kg/day) or GenX (0, 2, or 10 mg/kg/day) via oral gavage from embryonic day (E) 1.5 to E11.5 or from E1.5 to E17.5, in order to evaluate the potential effects GenX has on gestational weight gain (GWG), embryo growth, liver pathology, and placental development/morphology. Dams were euthanized by decapitation on E11.5 or E17.5 and evaluated for internal dosimetry analyses (Block 1), clinical chemistry analyses (Blocks 2), gross observation of the uterus for total implantation sites, viable embryos, nonviable embryos, and resorptions (Blocks 1 and 2), and histological analyses (Block 2).

Dosimetry analysis showed that the concentration of PFOA and GenX in the serum of dams in the high dose treatment groups was similar at E11.5 and no statistically significant differences at E17.5. Statistically significantly increased GenX accumulation in maternal serum was reported in the 10 mg/kg/day group as compared to the 2 mg/kg/day group at E11.5 and E17.5. Accumulation in maternal serum across all treatment groups was lower at E17.5 than at E11.5. At all-time points and in all treatment groups, PFOA accumulation in the maternal liver was statistically significantly greater than GenX accumulation. PFOA and GenX concentrations in amniotic fluid were comparable between high dose and low dose treatment groups at E11.5. GenX accumulation in whole embryo at E11.5 was statistically significantly greater than PFOA accumulation. However, at E17.5, GenX accumulation in whole embryo, male embryo, and female embryo was statistically significantly lower than PFOA accumulation.

In a study conducted by (Cope et al., 2021), pregnant CD-1 mice (target of n=10/group) were treated daily via oral gavage with 0, 0.2, 1.0, or 2.0 mg/kg/day GenX beginning at gestational day (GD) 1.5 to GD17.5 in order to evaluate the effects of gestational *in utero* exposure to GenX. Offspring were weaned at PND22 and litters were standardized to 10 pups (5 male and 5 female when possible) and fed either a high fat diet (HFD, 60% kcal fat diet; 5.21 kcal/g diet) or control low fat diet (LFD, 10% kcal fat diet; 3.8 kcal/g diet) from 3 weeks old (PND 22) to 18 weeks old. Results indicated that GenX concentrations in pup serum at PND 5.5 showed no significant differences between treated and control groups and at PND22 there was no detectable GenX concentration in the serum of any treatment group. However, pup urine analyses showed a statistically significant increased GenX concentration in the 1.0 and 2.0 mg/kg treatment groups, when compared to controls. The study authors suggested transplacental and lactational transfer of GenX occurs from dam to pup due to the persistence of GenX in the offspring after birth.

20.3.1.2.5 Excretion

No relevant data was found.

20.3.2 Human data

According to (ECHA, 2019), no information on the half-life of GenX in humans is known. Human biomonitoring data shows that detectable HFPO-DA concentrations in plasma/serum could be found in workers and the general population. Workers from a fluorochemical production site in the Netherlands were found to have plasma/serum concentrations of up to 169 ng/ml. Using this data a half-life for HFPO-DA cannot be derived with reasonable certainty. (ECHA, 2019) notes that the approximation of half-lives of perfluorinated substances in humans using animal data should proceed with caution, as experience with PFOA have shown. Half-lives of PFOA and

other PFAS (e.g. PFOS and PFHxS) in humans which are reported (Pizzurro et al., 2019; Z. Wang et al., 2015), show values much higher than what would be expected using allometric scaling. In sum the experimental animal data in (Gannon et al., 2016) indicate half-lives for GenX to vary between one and several days in animals. However lacking human data for GenX, it is not possible to draw a solid conclusion on the half-life in humans.

20.3.2.1 New data

No relevant data was found.

20.4 Health effects in humans and/or animals

20.4.1 Relevant Animal data

20.4.1.1 Data/studies reported in previous government and general reviews

It should be highlighted that in the following information is extracted from the support document for identification of 2,3,3,3-TETRAFLUORO-2- (HEPTAFLUOROPROPOXY)PROPIONIC ACID, ITS SALTS AND ITS ACYL HALIDES (COVERING ANY OF THEIR INDIVIDUAL ISOMERS AND COMBINATIONS THEREOF) as substance of very high concern which is cites as (ECHA, 2019). In this report many references are made to unpublished studies to which no access is available. Therefore, the relevant studies are cited in the following as secondary literature.

20.4.1.1.1 Repeated dose toxicity

As cited in (ECHA, 2019), (Haas, 2008a) administered GenX (purity 88%) by gavage to Crl:CD(SD) rats (10/sex/group) in the scope of an 28-day repeated dose toxicity study according to OECD TG 407. Male rats were administered doses of 0.3, 3 or 30 mg/kg bw/day, and females were administered doses of 3, 30 or 300 mg/kg bw/day. To determine the recovery after dosage, additional animals (number not specified) were used. Results indicated, significantly decreased red blood cell (RBC) count, haemoglobin, and haematocrit in males exposed to 3 and 30 mg/kg bw/day. After four weeks recovery period investigated values in blood returned to control levels. No significant changes in haematological effects were observed in females. Males which were administered 3 and 30 mg/kg bw/day and females which were administered 300 mg/kg bw/day showed alterations in serum clinical chemistry. This included significantly decreased total globulin levels and an increased A/G (albumin/globulin).A significant increase in albumin was also reported in males at 30 mg/kg bw/day. A significant decrease in cholesterol was also observed in males at all doses, as well as a significant decrease in triglycerides (22%) at 3 mg/kg bw/day. Further observations made in males receiving 30 mg/kg bw/day included a significant increase in blood urea nitrogen (BUN) and glucose levels. After a four-week recovery period the investigated values returned to control levels.

The 30 mg/kg bw/day dosed males showed an increased relative kidney weight of 15%. Further 1 out of 10 males showed minimal mineralization of the kidneys. In females no changes in kidney weight could be observed at a dosage of 30 mg/kg bw/day, but at a dosage of 300 mg/kg bw/day females showed a relative liver increase of 12.1%. Males further showed a relative liver weight increase of 18.6% and 55.5% at dosage levels of 3 and 30 mg/kg bw/day respectively. The liver of 4 out of 10 males dosed at 3 mg/kg bw/day, of 7 out of 10 males dosed at 30 mg/kg bw/day and of 4 out of 10 females dosed at 300 mg/kg bw/day showed test substance-related changes of multifocal centrilobular hypertrophy. Males dosed 30 mg/kg bw/day showed single cell necrosis (multifocal) and hepatocellular necrosis in 1/10 and 3/10 cases respectively and females dosed 30 and 300 mg/kg bw/day showed hepatocellular necrosis 1/10 and 1/10 cases

respectively. These effects were not statistically analysed. Lastly, β-oxidation activity could be observed at the middle and high doses in both male and female rats.

As changes in blood parameters, reduction in cholesterol and globulin, increases in albumin and the A/G ratio as well as incidences of liver hypertrophy could be observed in male rats at dosage levels of 3 mg/kg bw/day, the NOAEL of this study was set to 0.3 mg/kg bw/day.

In the 28-day study with mice according to OECD test guideline 407, Crl:CD-1(ICR) mice 10 to 20/sex/group) were administered GenX (88% purity) by gavage at dose levels of 0, 0.1, 3 and 30 mg/kg bw/day ((Haas, 2008b) as cited in (ECHA, 2019)). Determination of reversibility of the effects in mice administered the high dose was determined after a 4-week recovery period. Results indicated significant decreases in haemoglobin and haematocrit in the male mice at 3 and 30 mg/kg bw/day in male mice, accompanied by a significant decrease in RBC count at 30 mg/kg bw/day. No effects were observed in female mice.

In both male and female mice, the A/G ratio was significantly increased at dose levels of 3 and 30 mg/kg bw/day. In both sexes, dosage levels of 30 mg/kg bw/day lead to increased Albumin and dosage levels of 3 mg/kg bw /day or higher lead to a significant globulin decrease. Males which were administered doses of 3 and 30 mg/kg bw/day showed significantly increased serum liver enzymes (AST, ALT, ALP and SDH) and females which were administered doses of 30 mg/kg bw/day showed a significant increase in serum liver enzymes as well (ALP and SDH). These changes in liver enzyme level were consistent with hepatocellular injury. At the end of exposure, males which were administered 30 mg/kg bw/day also showed significantly increased BUN levels. Effects were shown reversible after the recovery period.

During gross necropsy enlarged livers could be found in males dosed at 30 mg/kg bw/day. At dosage levels of 3 and 30 mg/kg bw/day increased liver weights of 78% and 163% for males and of 32% and 103% for females could be found. The increase in liver weights were not completely reversible for animals of both sexes dosed at 30 mg/kg bw/day. Males which were administered 3 and 30 mg/kg bw/day and females which were administered 300 mg/kg bw/day showed test substance-related changes of multifocal centrilobular hypertrophy in the liver. Further, males dosed at 3 and 30 mg/kg bw/day were found to have an increase in adrenal gland weights (absolute and relative to body and brain weights. Multifocal single cell liver necrosis (minimal) in males administered 3 (4/10) and 30 (10/10) mg/kg bw/day and in females administered 30 (4/10) mg/kg/day group was increased, which was not present following the 4-week recovery period. In 1 out of 10 males and 2 out of 10 females dosed at 30 mg/kg bw/day single cell hepatocellular necrosis could be observed. Further β-oxidation activity could be found at all administered dosage levels in males and at middle ang high dosage levels in females.

As an increase in A/G ratio and a decrease in globulin in both sexes, as well as reduced haemoglobin, haematocrit, liver cell necrosis (during primary necropsy) and an increase of markers for liver damage (AST, ALT, ALP, SDH) in males administered dosage levels of 3 mg/kg bw/day were exhibited, the NOAEL for this study was set at 0.1 mg/kg bw/day.

Furthermore, groups of 10 Crl:CD(SD) rats per dose and sex were exposed to GenX (purity 84%) by gavage in the scope of a 90-day repeated dose toxicity study conducted according to OECD test guideline 408 ((Haas, 2009) as cited in (ECHA, 2019)). Both sexes were exposed to 0, 0.1, 10 and 100 mg/kg bw/day and a group of females were additionally exposed to 1,000 mg/kg

bw/day. Recovery within a four-week period was determined using additional animals. Three females administered 1000 mg/kg bw/day died prior to the end of the study and two of these deaths were found to be treatment related. Results indicated that in both - males and females - significant decreases were observed in haemoglobin, hematocrit, and RBC count at 10 and 100 mg/kg bw/day (males) and 1,000 mg/kg bw/day (females) respectively. Compared to the control group this would account to approximately 7-13% lower parameter levels in males and 18-28% in females. Males administered doses of 10 and 100 mg/kg bw/day showed a significantly decreased basophil count and at 100 mg/kg bw/day reticulocytes and platelets count were significantly increased. The group of females administered a dose of 1000 mg/kg bw/day were found to have a significant increase in reticulocytes, mean corpuscular haemoglobin (MCH), platelet count and mean corpuscular volume (MCV) and a decrease in basophil count and mean corpuscular hemoglobulin concentration (MCHC). Males dosed at 100 mg/kg bw/day still showed significant differences in parameters such as haemoglobin, RBC count, reticulocytes and haematocrit from parameters of the control group after the recovery period. Significant differences in parameters such as haemoglobin, haematocrit, reticulocytes and MCV after the recovery period could further be found in females administered 1000 mg/kg bw/day.

In males, which were administered 10 and 100 mg/kg bw/day, significant decreases in globulin and significant increases in albumin and A/G ratio were found. In females administered 1000 mg/kg bw/day, decreased globulin and increased A/G ratios could be found. Males dosed at 100 mg/kg bw/day further showed significantly decreased serum cholesterol and significantly increased BUN values. Females dosed at 100 and 1000 mg/kg bw/day also showed significantly decreased serum cholesterol. In males and females dosed at 10 and 100 ng/kg bw/day and 1000 mg/kg bw/day respectively, significantly increased serum phosphorous levels and APL levels were observed. A significant decrease in bilirubin could be observed in females dosed with 100 and 1000 mg/kg bw/day and a significant decrease in γ-glutamyl transferase (GGT) as well as total protein was observed in females at dosage levels of 1000 mg/kg bw/day. Females from the high dosage group also exhibited a significant increase in total urine volume and a significant decrease in urine pH.

An increase in absolute kidney weight in both males and females, was observed in the highest dose group (11% in males and 18% in females). The increase of relative kidney weight could be found as 12% and 16% in males dosed at 10 and 100 mg/kg bw/day respectively as well as 9% to 23% in females at all dosage levels. One female at the highest dose group, along with one female in the high dose group that died prior to the end of the study, showed tubular and papillary necrosis of the kidney. In one of the 10 males administered doses of 10 mg/kg bw/day mild acute inflammation of the kidney and transitional hyperplasia could be found. Even after the recovery period males dosed at 100 mg/kg bw/day still showed increased absolute and relative kidney weights.

Absolute liver weights and relative liver weights were increased in males at 10 and 100 mg/kg bw/day (23% and 59%) and at 1000 mg/kg bw/day for females (77%). Males dosed at 100 mg/kg bw/day mostly showed reversibility of increases in relative liver weight. However females that were administered doses of 1000 mg/kg bw/day only showed partial but incomplete recovery of liver weight changes after the recovery time of 4 weeks. Hepatocellular hypertrophy was reported in 3 out of 10 and 10 out of 10 males in the 10 and 100 mg/kg bw/day, respectively as well as 10 out of 10 females dosed at 1000 mg/kg bw/day. Hypertrophy

could not be found at the recovery necropsy and was not associated with liver injury indicative changes in serum chemistry or with microscopic changes indicative of liver injury (such as degeneration or necrosis).

Based on males, which were administered doses of 10 mg/kg bw/day, showing increases in albumin, A/G ratio and liver weight as well as changes in blood parameters and a decrease in globulin and cholesterol and both sexes showing an increase in relative kidney weight, the NOAEL for this study was set at 0.1 mg/kg bw/day as indicated in (ECHA, 2019).

((MacKenzie, 2010) as cited in (ECHA, 2019)) conducted an oral mouse 90-day study according to OECD test guideline 408, in groups of 10 Crl:CD-1(ICR) mice per dose and sex were exposed to GenX (84% purity) by gavage (water) at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day. The concentration of the substance in plasma was further monitored at 2 hours after exposure on day 0, 28 and 95 using additional animals. In comparison to the control group, the group of males dosed at 5 mg/kg bw/day showed an overall gain in bodyweight (136% compared to control) and a statistically increased mean final body weight (108% compared to control), which was primarily attributed to an increase in liver weight. Males administered doses of 0.5 and 5 mg/kg bw/day showed a significantly increased platelet count and males in the higher dosed group further showed significantly decreased cholesterol and significantly increased total serum protein. At a dose of 5 mg/kg bw/day significantly increased serum liver enzymes could be found in both sexes (males: ALT,AST, and ALP; females: ALP and ALT) and albumin and SDH were found to be significantly increased ion both sexes. Further at the highest dose a significant increase in total bile acid could be observed. .

The observed changes in serum liver enzymes were consistent with hepatocellular damage and/or cholestasis. As stated in (ECHA, 2019), an increase in mean liver weight parameters was observed in mice exposed to ≥ 0.5 mg/kg bw/day (males) and 5 mg/kg bw/day (females) test substance. Increased liver weight parameters in males and females were in correlation with microscopic hepatic changes and treatment-related enlarged livers. Males which were administered doses of 5 mg/kg bw/day showed an increase in mean relative (to brain) kidney weight, which could not be correlated to an increase in relative (% body weight) or absolute kidney weight. Further, compared to the control group, in male mice dosed at 5 mg/kg bw/day lower mean weights of epididymides and brain relative to body weight and a higher mean weight of heart (in relation to brain weight) could be observed. Female mice dosed at 0.5 and 5 mg/kg bw/day showed a decreased mean spleen wight in relation to brain and body weight.

(ECHA, 2019) listed histopathological findings in the liver. In female mice which were administered doses of 5 mg/kg bw/day 10 out of 10 mice showed mild hypertrophy, 3 out of 10 showed minimal focal necrosis and single-cell necrosis was observed in 1 out of 10 mice. In male mice administered the same dosage, 10 out of 10 mice showed Kupffer cell pigments, minimal hypertrophy and increased single-cell necrosis, and in 9 out of 10 mice mitotic figures and minimal renal tubular epithelial hypertrophy could be observed. At a dose of 0.5 mg/kg bw/day minimal hypertrophy could be observed in male mice. Further, one male mouse dosed at 5 mg/kg bw/day showed minimal bile duct hyperplasia.

As an increase in liver weight and liver hypertrophy could be observed in both sexes and as male mice dosed at 5 mg/kg bw/day showed changes in liver serum enzymes and single-cell necrosis, the NOAEL in this study was set at 0.5 mg/kg bw/day.

20.4.1.1.2 Carcinogenicity

(Caverly Rae et al., 2015) conducted a 2-year oral rat study according to OECD test guideline. In the study 80 Crl:CD(SD) rats (per dose and sex), were exposed to GenX (84% purity) by gavage at 0, 0.1, 1 and 50 mg/kg bw/day (males) or 0, 1, 50 and 500 mg/kg bw/day (females). After one year 10 animals were investigated via interim necropsy. The other animals were necropsied at the end of the study (females: 101 weeks; males: 104 weeks).

Inflammation/necrosis of the kidneys produced test substance-associated deaths and occurred in seven females in the 500 mg/kg bw/day dose group and was characterized by papillary necrosis. The early termination of females in week 101 resulted from a low survival in female groups of all dosage levels and the control group. It was stated though, that as the exposure time was close to 2 years, no impact on the study was expected. Even though the mortality among all female groups was high, a significant difference between the groups could not be found and survival was similar among all groups. .

In males dosed at 50 mg/kg bw/day a statistically significant lower mean body weight compared to the control group could be observed during most of the first 12 months, and females which were administered dosage levels of 500 mg/kg bw/day showed adverse reductions in body weight and body weight gain at 3, 6 and 12 months. Further in females in the high dosage group a significant increase in MCV and a significant decrease in MCHC could be observed at the 12 month time interval. Females dosed at 50 mg/kg bw/day showed a decrease in RBC at the 1-year time interval. In male rats a decrease in haemoglobin, haematocrit and RBC was observed at the 3- and 6-months' time interval but not at the 1 year mark.

At the 12-month time interval males dosed 1 mg/kg bw/day showed significantly increased albumin levels in serum and in females dosed 50 mg/kg bw/day significantly increased globulin levels were observed at the 6-month time interval. Thus except for the 1 mg/kg bw/day group at the 6-month time interval, a significantly increased A/G ratio was observed in all groups at all intervals due to the changes in albumin and globulin levels. Females of the mid- and high dose group further showed a statistically significant reduction of Bilirubin levels at almost all time intervals. In males dosed at 50 mg/kg bw/day a significant increase in liver enzymes (ALT, ALP, and SDH) in serum could be observed. Further results of the study by (Caverly Rae et al., 2015) found, included a significant decrease in GGT and total protein in females dosed 500 mg/kg bw/day and a significant increase in BUN for males administered 50 mg/kg bw/day and females administered 500 mg/kg bw/day. Additionally a significant increase in phosphorus levels was observed for both sexes in the high dose groups and in females in the high dose group increased chloride and potassium levels were observed.

In females, receiving 500 mg/kg bw/day, a minimal but statistically significant increase in pH and urine volume but decrease in urine specific gravity (hinting at minimal diuresis) were observed at the half year and year mark. Whilst minimal, the authors point out that these changes may be in relation with the increasing observance and severity of incidences of microscopically found chronic progressive nephropathy at the 1-year interim sacrifice.

In females dosed at 500 mg/kg bw/day increased kidney weights and changes in the kidney, such as increased incidence of tubular dilation, oedema of the renal papilla, transitional cell hyperplasia, tubular and pelvic mineralization, renal papillary necrosis, and chronic progressive nephropathy could be observed. Macroscopically an "irregular surface" of the kidneys could be seen during the interim sacrifice in one female administered 500 mg/kg bw/day. By the end of the study, this occurrence could be observed in 16 out of 70 females administered the high dose level.

Increases in relative liver weight were observed at interim sacrifice for high-dosed animals of both sexes. In the group of males administered 50 mg/kg bw/day five specimen showed minimal to mild focal necrosis and in three specimen minimal focal cystic degeneration could be found. At dosage levels of 500 mg/kg bw/day centrilobular hypertrophy could be observed in all females at the interims sacrifice at the one-year mark. In the high dosage groups at the sacrifice at the end of the study, microscopic changes, such as an increase in centrilobular hepatocellular hypertrophy could be observed in 7 out of 70 males and 65 out of 70 females and an increase in centrilobular hepatocellular necrosis could be observed in 5 out of 70 males and 7 out of 70 females. Of the 5 males showing signs of centrilobular hepatocellular necrosis, 3 were graded as severe and in the case of the females the effects were graded as mild to severe. Further at 50 mg/kg bw/day a decrease in focal and periportal vacuolization could be observed in males.

Females administered doses of 500 mg/kg bw/day showed a decrease in centrilobular vacuolization, pan lobular hepatocellular hypertrophy, individual cell hepatocellular necrosis, and angiectasis (i.e., blood- or lymph vessel dilation). Further at the interim sacrifice females of the high dosed group showed a decrease in absolute and relative (to brain) spleen weight, however this effect was observed without any accompanying macroscopic changes.

In males which were administered 50 mg/kg bw/day histopathological results showed a statistically significant induction of adenomas/carcinomas in the pancreas and an increase in occurrence of Leydig cell tumours in the testes. The occurrence of Leydig cell tumours could not be regarded as statistically significant, as these occurrences were also found quite often in the control group. A statistically significant induction of hepatocellular adenomas and carcinomas occurred in females dosed 500 mg/kg bw/day. However, as both the occurrence of interstitial cell hyperplasia and interstitial cell adenomas were increased at dosage levels of 50 mg/kg bw/day and were outside the historical control range, the increase of occurrences indicated a correlation to the treatment. During the interim necropsy one interstitial cell adenoma could be found in one of the male specimens in this dosage group. The increase in uterus stromal polyps was within the range of the historical controls.

Based on increases in the A/G ratio in male rats at a dosage of 1 mg/kg bw/day, the NOAEL for chronic toxicity was set at 0.1 mg/kg bw/day. In females the NOAEL for carcinogenicity is set to 50 mg/kg bw/day based on an increase in liver tumours when administered doses of 500 mg/kg bw/day and is set to 1 mg/kg bw/day for males, based on increased occurrence of combined adenoma and carcinoma of the pancreas at dosage levels of 50 mg/kg bw/day.

20.4.1.1.3 Mutagenicity

As cited in (ECHA, 2019), (Donner, 2008; Myhre, 2008) conducted two Ames tests according to OECD test guideline 471 using plate incorporation with different species of prokaryotes dosed up to 5000 ug/plate. Results showed that GenX was negative with and without metabolic activation. In a mammalian cell gene mutation assay according to OECD TG 476 ((Clarke, 2008) as cited in (ECHA, 2019)) GenX was negative with and without metabolic activation at neutral pH. GenX was negative after 4 and 20 hour exposure without metabolic activation in two in vitro mammalian chromosome aberration tests according to OECD TG 473, but positive after 4 hour exposure with metabolic activation at the highest exposure level of 3471 ug/ml ((Glatt, 2009; Glover, 2008) as cited in (ECHA, 2019)).

In an *in viv*o mouse micronucleus test conducted by ((Gudi & Krsmanovic, 2007) as cited in (ECHA, 2019)) according to OECD test guideline 474 at dose levels up to 1300 mg FRD-902/kg bw by gavage a reduction in PCE/EC in the bone marrow was observed, showing that the substance reached the bone marrow without increase in micronucleated PCE. At the highest dose some mortality was observed. A decrease in the mitotic index of bone marrow cells was

observed in a mouse chromosome aberration test according to OECD TG 475 at the same dose levels, but no increase in structural or numerical chromosome aberrations. In a rat unscheduled DNA synthesis test according to OECD TG 486 by ((Pant & Sly, 2007) as cited in (ECHA, 2019)) at dose levels up to 2000 mg FRD-902/kg bw by gavage, no increase in net grains per nucleus was observed

20.4.1.1.4 Toxicity to reproduction

((Edwards, 2010b) as cited in (ECHA, 2019)) conducted a reproduction/developmental toxicity screening study according to OECD TG 421 in Crl:CD1(ICR) mice (N = 25) exposed to GenX by oral gavage at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day (purity 84%). The F0 males were dosed during study days 0 to 84 (70 days prior to pairing through 1 day prior to euthanasia), for a total of 84 to 85 doses. The females that delivered were dosed from study day 56 through the day prior to euthanasia (14 days prior to pairing through lactation day 20) for a total of 53 to 64 doses. No effect on mortality in the parental animals were shown by results. An increased kidney weight was noted in females dosed at 5 mg/kg bw/day. Increased kidney tubular cell hypertrophy was observed in males dosed at 0.5 and 5 mg/kg bw/day. In both sexes increases in liver weight and liver hypertrophy were observed. In all doses groups, incidences of single cell necrosis were observed in males and females, with 24/24 males and 21/24 females exhibiting single cell necrosis at 5 mg/kg bw/day. This effect was graded as minimal at the middle dose (m) and minimal to moderate at the highest dose (m/f). 3 out of 24 females in the middle dosed group and 5 out of 24 females in the high dosed group showed an increase in occurrences of focal/multifocal necrosis and the latter effect was further defined as minimal focal coagulative necrosis. In the study report. At 0.5 mg/kg bw/day hypertrophy and necrosis were observed in males and females. 6 out of 24 males kidney weights increased with 8% at 5 mg/kg bw/day, this correlated with increased kidney tubular cell hypertrophy at 6 out of 24 males at doses 0.5 mg/kg bw/day and at 18 out of 24 males at 5 mg/kg bw/day. In females, absolute and relative kidney weights were increases with 21% and 10% at 5 mg/kg bw/day respectively.

According to (ECHA, 2019), no changes in reproductive performance were reported. At all dosage levels survival, Sex ratio, and physical condition of the F1 pups was unaffected. At a dosage level of 5 mg/kg per day, F1 pups of both sexes showed significantly lower mean body weights when compared to controls at PNDs 4, 7, 14, 21 and 28, with decreases up to and over 20% at weaning (i.e., PND 21). The decrease in mean body weight was still observable in male F1 pups at PNDs 35 and 40 (~10%), whereas female F1 pups mean body weight returned to control group levels at PNDs 35 and 40. Concentrations in serum in pups and parental animals transfer were indicative of only limited transfer of FDR-902 via lactation.

As effects on reproduction could not be observed at any tested dosage level, the NOAEL for reproductive toxicity was indicated as 5 mg/kg bw/day. The NOAEL for systemic toxicity in parental animals was 0.1 mg/kg bw/day based on the occurrences of hepatic single cell necrosis in males dosed at 0.5 mg/kg bw/day. Based on a decrease in body weight in F1 males and females at dosage levels of 5 mg/kg bw/day during the pre-weaning period, the NOAEL for systemic toxicity in the offspring was 0.5 mg/kg bw/day. The results from this study in mice however, according to (ECHA, 2019), cannot be used to draw final conclusions on the reproductive effects, as the highest dosage levels used only showed minimal effects in the parental animals. Therefore, with respect to potential effects on fertility and development, the information is seen as inconclusive.

In a developmental toxicity study in rats according to OECD test guideline 414 ((Edwards, 2010a) as cited in (ECHA, 2019)) pregnant Crl:CD(SD) rats (N = 22) were exposed to GenX (84% purity) at 0, 10, 100, or 1000 mg/kg bw/day by oral gavage during Gestation days 6-20. Dams

were sacrificed on Gestation Day 21 and organs including the ovaries, uterus, and foetuses were examined. On Gestation Day 20 one female died in the highest dose group due to liver and kidney damage. Early deliveries on gestation day 21 were observed for four females in the 100 and 9 females in the 1000mg/kg bw/day groups. Both, mortality in the 1000 mg/kg bw/day group and early deliveries in the 100 and 1000 mg/kg/day groups were considered test substance related.

At 1000 mg/kg bw/day, test-substance related clinical findings (yellow material on various body surfaces, salivation), reduction in food consumption, a decrease in terminal maternal weight and an increase in mean kidney weight could be found, according to (ECHA, 2019). The 100 and 1000 mg/kg bw/day dosed groups showed a decrease in gravid uterine weight of 10% and 25% respectively. However, none of the groups at any dosage level showed a significant decrease in mean corrected body weight gain or mean corrected body weight, when compared to the control group. Increases in liver weight could be observed in animals administered doses of 100 and 1000 mg/kg bw/day, which in some animals was accompanied by focal necrosis. At dosage levels of 1000 mg/kg bw/day two females showed an edematous pancreas and further liver hypertrophy could be observed at this dosage level. A decrease in mean foetal weight of 8.8% could be observed at dosage levels of 100 mg/kg bw/day and of 28.1% at a dosage level of 1000 mg/kg bw/day. The observed decrease in gravid uterine weight at these dosage groups was attributed to the reduction in mean foetal weight. Aside from a higher occurrence of 14th rudimentary ribs at dosage levels of 1000 mg/kg bw/day, no further effects could be observed on malformations, variations or foetal survival.

For verification of the appearance of dose-related early deliveries in the dams on gestation day 21, ((Edwards. 2010a) as cited by (ECHA, 2019)) conducted a second study. The experimental design was kept unchanged but only a control group and a group dosed at 1000 mg/kg bw/day were studied. The study could confirm the increased occurrence in early deliveries. Additionally a decrease in foetal weight and comparable maternal effects as in the above study could be observed.

Based on a decrease in mean body weight, changes in the pancreas and kidney, mortality and food consumption at dosage levels of 1000 mg/kg bw/day and microscopic findings in the liver (focal necrosis) at 100 and 1000 mg/kg bw/day, the NOAEL for maternal toxicity is considered to be 10 mg/kg bw day. The NOAEL for developmental toxicity is 10 mg/kg bw/day based on a decrease in mean foetal weight, early deliveries and a decrease in gravid uterine weight at dosage levels of 100 and 1000 mg/kg bw/day.

20.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

The immune effects of FRD-903 on 12 (6 male, 6 female) mice (C57BL/6) were studied by (Rushing et al., 2017), by administering oral doses of 0, 1, 10 or 100 mg/kg bw/day through gavage for 28 days. The study was replicated twice with 8 weeks time in between each other.

Concentrations of FRD-903 in serum were measured after 1, 5, 14 and 28 days in one of the replicates. Using SRBC (sheep red blood cells) all mice (male and female in both replicated studies) were immunized. At the end of the test SRBC-specific IgM antibody titers in serum (T-cell antibody response, TDAR) were determined and splenic lymphocyte subpopulations were analysed. The animals were sacrificed one day after the final administration through gavage and the weights of the liver, thymus and spleen were determined. Livers were further investigated for peroxisome proliferation (peroxisomal fatty acid oxidation, hepatic acyl CoA oxidase).

At dosage levels of 100 mg/kg bw/day a decrease of relative spleen weight (11%) and suppression of TDAR (7.3%) were observed only in females. In contrast an increase of T lymphocyte numbers could only be observed in males (74% on average). Both sexes did not show a difference in B-lymphocyte numbers. However both sexes showed an increase in liver weight at dosage levels of 10 and 100 mg/kg bw/day. In males at dosage levels of 10 and 100 mg/kg bw/day and in females at a dosage level of 100 mg/kg bw/day an increase in liver peroxisome proliferation (measurement of hepatic acyl CoA oxidase) could be observed.

Based on an increase in lymphocyte numbers in males and suppression of the TDAR in females at dosage levels of 100 mg/kg bw/day, the NOAEL was set at 10 mg/kg bw/day. The authors further conclude that observations made in this study are in line with parameters affected by PFOA. Even though FDR-902 showed less potency, further studies are required for determination of the full immunomodulatory profile of FDR-902 and possible synergism with other PFAS.

20.4.1.2 New data

20.4.1.2.1 Repeated dose toxicity

No relevant data was found.

20.4.1.2.2 Carcinogenicity

No relevant data was found.

20.4.1.2.3 Mutagenicity

(Coperchini et al., 2020) conducted a study to assess the effects of GenX following *in vitro* exposure to FRTL-5 rat thyroid cells. Cells were exposed to increased concentration of GenX 0.0001, 0.001, 0.01, 0.1, or 1 µg/ml) for 24, 48, or 72 hours. Cell viability was assessed by water-soluble tetrazolium salt (WST-1). DNA-damage was assessed by comet assay and further confirmed by micronucleus assay. The proliferation of survived cells was measured by staining with crystal violet and evaluation of its optical density after incubation with SDS. Changes in gene expression levels of thyroid transcription factor genes (TTF-1 and Pax8), as well as Thyroglobulin (Tg), Thyroid Stimulating Hormone-receptor (TSH-R), Sodium-Iodide Symporter (NIS) and Thyreoperoxidase (TPO) genes expression were evaluated by RT-PCR. Results showed that GenX exposure decreased the viability of FRTL-5 cells in a time and dose-dependent manner. Results of the comet assay and micronucleus assay indicated GenX exerted a genotoxic effect at both cytotoxic and noncytotoxic concentrations. At noncytotoxic concentrations, GenX exposure resulted in a significant lowering of the expression of the regulatory gene TTF-1, a significantly higher expression of Pax-8, and a significant down-regulation of NIS compared to controls. The authors concluded that exposure to GenX produces several toxic effects on thyroid cells *in vitro* and promotes DNA-damage and expression of thyroid transcription-factor genes.

20.4.1.2.4 Toxicity to reproduction

Pregnant CD-1 mice were divided into two experimental blocks to achieve a total of 11 to 13 litters per treatment group (Blake et al., 2020). Dams were administered PFOA (0, 1, or 5 mg/kg/day) or GenX (0, 2, or 10 mg/kg/day) via oral gavage from embryonic day (E) 1.5 to E11.5 or from E1.5 to E17.5, in order to evaluate the potential effects GenX has on gestational weight gain (GWG), embryo growth, liver pathology, and placental development/morphology. Dams were euthanized by decapitation on E11.5 or E17.5 and evaluated for internal dosimetry analyses (Block 1), clinical chemistry analyses (Blocks 2), gross observation of the uterus for total implantation sites, viable embryos, nonviable embryos, and resorptions (Blocks 1 and 2), and histological analyses (Block 2).

Results indicated the percent change (relative) in GWG in dams treated with 10mg/kg/day GenX was statistically significantly increased at E11.5, when compared to controls. After controlling for litter size or adjustment for repeated measures of relative GWG, litter size, and gestational/embryonic day in the mixed-effect models, a statistically significant increase in relative GWG was also reported in dams in the 10 mg/kg/day GenX dose group at both time points, when compared to controls. Absolute and relative liver weights of dams were statistically significantly increased in all treated GenX groups at both time points, when compared to controls. Absolute and relative kidney weights of dams were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls. A statistically significant decrease in triglyceride levels was reported in all GenX treated groups at E11.5 and in the high dose group at E17.5, when compared to controls. Cholesterol and high-density lipoprotein levels were statistically significantly increased in the 2 mg/kg/day GenX group at E11.5 and alkaline phosphatase levels were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls.

There were no statistically significant differences reported in the number of implantation sites, viable embryos, non-viable embryos, or resorptions in any treatment groups at E11.5 or E17.5, when compared to controls. There were also no statistically significant differences reported for viable embryo weight, placental weight, or embryo:placenta weight ratios in any treatment groups at E11.5, when compared to controls. However, placental weights were statistically significantly increased and embryo:placenta weight ratios were statistically significantly decreased in the 10 mg/kg/day GenX group at E17.5, when compared to controls. The percent placentas within normal limits (WNL) were statistically significantly decreased and the percent abnormal placentas were statistically significantly increased in the high dose treatment groups as compared to controls, when placentas were evaluated independently without litter origin consideration. Placenta thyroid hormone evaluation showed that T4 levels were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls.

Study authors concluded that although a NOAEL could not be established in this study, GenX produced significant adverse effects at 2 mg/kg/day. Study limitations noted by the authors included performing the experiment over two blocks, half-life variance, the amount of exposure to GenX, and interspecies differences. Study authors reported that GenX produced adverse effects on the maternal-embryo-placenta unit, as well as increased GWG and maternal liver weights, adverse microscopic pathological changes in the maternal liver, and abnormal histopathological lesions in mature placenta.

Time-mated Sprague-Dawley rats were divided into three blocks of 15 dams per block and dosed once daily via oral gavage from gestational days (GD) 14-18 with GenX at concentrations of 0, 62.5, 125, 250, or 500 mg/kg (Block 1) or 0, 1, 3, 10, or 30 mg/kg (Blocks 2 and 3) (Conley et al., 2019). Sample size per dose in each block was n=3. Dams in all Blocks were euthanized by decapitation on GD18, approximately 2 hours after the final dose. Foetal testis testosterone production, foetal testis gene expression, foetal and maternal liver gene expression, foetal body weight, and maternal serum thyroid hormone and lipid concentrations were evaluated across all dose groups in Blocks 1 and 2. Foetal plasma GenX concentrations were evaluated in the third block. Maternal weight gain during dosing, reproductive output (number of foetuses and resorption), maternal serum GenX concentration, and maternal liver weights at necropsy were evaluated in all blocks for all concentrations of GenX.

Results indicated that proliferator-activated receptor (PPAR) signalling pathways analysed in foetal and maternal livers from HFPO-DA exposed litters had a statistically significant (ANOVA $p<0.0001$) dose-responsive up-regulation for 28 different genes in foetal livers. GenX activated PPAR signalling pathways for varying genes at each dose level in foetal livers, with the majority

associated with fatty acid metabolism (*Acaa2, Acadl, Acadm, Acox1, Acsl1, Acsl3, Acsl4, Cpt1a, Cpt1b, Cpt2, Ehhadh, Etfdh, Fads2, Fabp1, Gk, Hmgcs2, Mlycd, and Scd1*), followed by lipid transport (*angptl4, Dgat1, Lpl*), adipogenesis (*Ech1, Lpl*), water transport (*Aqp7*), insulin signalling (*Cpt1a, Dgat1, Pck1*), PPAR transcription factors (Rxrg), or PPAR ligand transporters (Fabp1, Fabp5, Slc22a5, Slc27a2). The up-regulation of 16 genes were shared between maternal and foetal livers, with the majority associated with fatty acid metabolism (*Acaa2, Acadl, Acadm, Acox1, Acs13, Cpt1b, Cpt2, Ehhadh, Fads2, Fabp1, Hmgcs2, and Scd1*). The most highly up-regulated genes shared by maternal and foetal livers were *Ehhadh* (55-fold in maternal liver) and *Cpt1b* (24-fold maternal liver). Statistically significantly up-regulated expression of genes associated with cell proliferation (*Hspd1, Txnip*) and fatty acid metabolism (*Fabp3*) was reported in the maternal liver but not in the foetal liver. The remaining up-regulated maternal genes were similar to that of foetal livers, with associated adipogenesis (*Ech1*), PPAR transcription factors (*Rxrg*), or PPAR ligand transporters (*Slc22a5, Slc27a2*). Statistically significant expressions of varying up-regulated genes were reported at all dose levels, even the lowest dose group (1 mg/kg) for both foetal livers (*Cpt1b, Acox1, Angptl4*) and maternal livers (*Ech1 and Rxrg*), when compared to controls. There were no statistically significant differences between treated and control groups for the expression of genes for detecting phthalate-like effects in the foetal testis or for foetal testis testosterone production.

Evaluation of maternal endpoints indicated statistically significantly increased liver weight (62.5, 125, 250, and 500 mg/kg/d) and statistically significantly decreased body weight gain (250 and 500 mg/kg/d), serum T4 (125, 250, 500 mg/kg/d), serum T3 (30, 62.5, 125, 250, and 500 mg/kg/d), serum triglycerides (500 mg/kg/d), serum HDL (250 and 500 mg/kg/d), serum cholesterol (250 and 500 mg/kg/d), and serum LDL (125, 250, 500 mg/kg/d), when compared to controls. When evaluated across all dose ranges, GenX concentration in maternal serum was reported to saturate at the higher dose levels with a plateau of 112±15 µg/ml. A linear response was reported in the lower dose ranges (0-30 mg/kg/day) for maternal serum and foetal plasma GenX concentrations, although the maternal slope was statistically significantly greater than the foetal slope. Following dose-response analyses, an estimated effect concentration equivalent to a 5% deviation from control (EC_5) for the most sensitive endpoints (maternal liver weight, maternal liver gene expression, and maternal serum [T3] and [T4]) was estimated using maternal serum HFPO-DA concentrations. The most sensitive endpoints with an EC_5 were ranked as follows: maternal [T3] (EC_5= 3.8 µg/ml), liver *Ehhadh* expression (EC_5=14.1 µg/ml), liver weight (EC_5= 17.6 µg/ml), and [T4] (EC_5= 17.8 µg/ml). The study authors concluded that GenX exposure resulted in up-regulation of genes associated with PPAR signalling pathways, maternal hepatomegaly, decreased maternal serum lipids and thyroid hormones, and reduced body and tissue weights in F1 animals. However, hallmarks of phthalate syndrome were not observed following GenX exposure.

Time-mated Sprague-Dawley rats (Crl:CD(SD)) in one block of 30 dams were treated daily via oral gavage with 0, 10, 30, 62.5, 125, or 250 mg/kg GenX from gestational day (GD) 8 to post-natal day (PND) 2 to evaluate neonatal effects (Conley et al., 2021). At termination, maternal serum was collected, maternal liver weight recorded, maternal liver collected for RNA extraction and GenX concentration, and uterine implantation sites scored. Time of pup delivery was recorded, pups counted, and whole litter weight recorded beginning GD22 (i.e., PND0). Two newborn randomly selected pups were euthanized via decapitation and blood and liver samples were used for RNA extraction and gene expression analysis. Diagnostic histopathology was performed on three deceased newborn pups treated with 30, 125, and 250 mg/kg GenX (one from each group). All other pups were euthanized via decapitation and serum blood collected following being sexed, weighed, and anogenital distance (AGD) measured. Liver weight was

recorded from each litter (1 male and 1 female) and GenX concentration was determined from a liver subsample.

Results indicated statistically significantly decreased average pup birth weight in the 30, 62.5, 125, and 250 mg/kg treatment groups, when compared to controls. Lethargy, moribund, and death were reported shortly after delivery in pups from treatment groups of 10mg/kg/d and greater. The first signs of pup mortality in the 10, 30, 62.5, 125, and 250 mg/kg treatment groups were at 10.4±8.4, 13.1±11.3, 11.4±2.6, 9.3±1.0, and 5.1±1.9 hours respectively, following onset of delivery. Statistically significantly decreased pup survival was reported in all treatment groups exposed to 62.5 mg/kg or greater, when compared to controls. No statistically significant effects on body weight or liver weight were reported at necropsy when compared to controls. A statistically significant decrease in pup body weight was reported in all treatment groups of 30 mg/kg or higher, when compared to controls. There was no statistically significant difference in AGD for male or female pups at any treatment level compared to controls. However, pup relative liver weight was statistically significantly increased at all treatment levels, when compared to controls. Glycogen accumulation (percentage of hepatocytes containing glycogen) reported in liver samples collected from two newborn pups prior to nursing (PND0) was statistically significantly lower in all dose groups than in controls. Serum analyses from PND0 pups resulted in statistically significantly decreased albumin (62.5 and 250 mg/kg) and glucose (62.5, 125, and 250 mg/kg) levels and statistically significantly increased cholesterol (125 and 250 mg/kg) and triglyceride (125 and 250 mg/kg) levels, when compared to controls. Serum analyses from PND2 pups resulted in statistically significantly increased aspartate aminotransferase (AST) (≥30 mg/kg) and cholesterol (≥62.5 mg/kg) levels and statistically significantly decreased glucose (≥125 mg/kg) levels, when compared to controls. A statistically significant decrease in mean maternal body weight and gestational weight gain was reported in the 125 and 250 mg/kg dose groups at GD22 and in absolute dam body weight at PND2 in the 125 and 250 mg/kg dose groups, when compared to controls. Absolute (≥30 mg/kg) and relative (≥10 mg/kg) liver weights of dams were statistically significantly decreased, when compared to controls. Clinical chemistry results of dams at PND2 showed statistically significantly reduced total T4 (≥62.5 mg/kg), total T3 (≥62.5 mg/kg), and albumin (250 mg/kg) levels and statistically significantly increased AST (≥10 mg/kg) and triglyceride (≥125 mg/kg) levels, when compared to controls.

(Conley et al., 2021) also evaluated foetal and maternal effects and serum and liver GenX concentrations in two additional blocks of 15 dams each treated daily with 0 (n=3), 1, 3, 10, 30, 62.5, or 125 mg/kg GenX (n=2 per treatment group) from GD17-21. Maternal weight gain during dosing, clinical chemistry of maternal serum, reproductive output, maternal liver weight, collected maternal and foetal liver mRNA, and measured maternal and foetal serum and liver GenX concentrations were evaluated in all dams from both blocks, except for foetal serum levels which were only evaluated in one block. Results showed no statistically significant differences in male or female absolute or mean foetal body or liver weights across dose groups at GD21, when compared to controls. There were also no statistically significant differences in maternal terminal body weight or body weight gain at any treatment level, when compared to controls. Viable foetuses and resorptions reported were similar between treated and control groups. A statistically significant increase in relative liver weight was reported in dams in the 62.5 and 125 mg/kg treatment groups, when compared to controls. Clinical chemistry results of dams at GD21 showed statistically significantly reduced total T4 (≥62.5 mg/kg), total T3 (≥62.5 mg/kg), albumin (3, 62.5, and 125 mg/kg), cholesterol (≥30 mg/kg), and triglyceride (≥10 mg/kg) levels, when compared to controls.

PPAR signalling pathways were determined using foetal (GD21), neonatal (PND0), and maternal (GD21) livers and glucose metabolism was determined using foetal and neonatal livers by

utilizing 84 target genes associated with each endpoint. Results indicated that four genes (*Pck1, Pdk4, G6pc, and Pdp2*) specific to glucose metabolism were statistically significantly upregulated (also the G6pc at ≥ 3 mg/kg) and one gene (*Ugp2*) was statistically significantly downregulated at ≥ 10 mg/kg in the GD21 foetal liver, when compared to controls. Gene expression was also compared to a previous study (Conley et al., 2019) in which PPAR signalling pathways were assessed in foetal livers exposed to GenX on GD14-18. The comparison indicated that all 28 genes upregulated in the previous study were also upregulated in the current study, with 16 of the genes (*Acaa2, Acadm, Acox1, Acsl1, Acsl3, Angptl4, Cpt1a, Dgat1, Ech1, Ehhadh, Fads2, Gk, Mlycd, Pck1, Rxrg, Scd1*) being statistically significantly upregulated, when compared to controls and at a greater effect on GD21 than at GD18. A statistically significantly upregulated housekeeping gene (*Ldha*) was also reported to be more highly upregulated on GD21, when compared to GD18. Gene expression of glucose metabolism and PPAR target genes in newborn pup (PND0) liver indicated that 11 genes were statistically significantly different from controls across all treatment groups at GD21. Gene expression for PPAR signalling pathways were also statistically significantly different for 21 genes across all treatment groups at GD21, when compared to controls. PPAR signalling pathway genes (19 genes) of maternal livers that were reported upregulated on GD18 in the comparison study (Conley et al., 2019), were shown to be statistically significantly upregulated on GD21 in the current study.

GenX concentrations in maternal serum and liver were increased, but not significantly, at GD17-21 and GD8-PND2, indicating that bioaccumulation was not observed at a longer exposure duration. Comparison of GD21 (current study) and GD18 (Conley et al. 2019) foetal serum GenX concentrations indicated that for a given maternal dose, the maternal serum concentration was 2-3-fold greater than the foetal serum concentration. A low lactational transfer and/or rapid neonatal clearance was indicated by the reduced PND2 liver concentrations, when compared to the GD21 liver concentrations, regardless of sex.

Based on these results the authors concluded that GenX exposure produced developmental effects toxicant in Sprague-Dawley rat offspring. While exposure levels of GenX did not induce overt maternal toxicities, decreased pup birth weights, decreased neonatal survival, and increased pup liver weights were reported following *in utero* exposure to GenX. The study authors noted that results of late gestation maternal serum cholesterol and triglyceride dose-response reductions were indicative of the lipid lowering effects of PPAR α active compounds, and insufficient glucose for glycogen synthesis may be the cause for the foetal liver gene changes observed, due to the late term foetal exposure to GenX. The authors concluded that PPAR alpha (α) activation in maternal, foetal, and neonatal livers is supported by the gene expression results in this study and in (Conley et al., 2019).

In a study conducted by (Cope et al., 2021), pregnant CD-1 mice (target of n=10/group) were treated daily via oral gavage with 0, 0.2, 1.0, or 2.0 mg/kg/day GenX beginning at gestational day (GD) 1.5 to GD17.5 in order to evaluate the effects of gestational *in utero* exposure to GenX. Offspring were weaned at PND22 and litters were standardized to 10 pups (5 male and 5 female when possible) and fed either a high fat diet (HFD, 60% kcal fat diet; 5.21 kcal/g diet) or control low fat diet (LFD, 10% kcal fat diet; 3.8 kcal/g diet) from 3 weeks old (PND 22) to 18 weeks old. Results indicated that pup body weight was statistically significantly reduced in 1.0 (6.3%) and 2.0 (6.7%) mg/kg GenX groups. Body weight, liver weight, and relative liver weight of male and female pups at PND22 and in female pups at 6 weeks of age in the low and high fat diet groups were comparable to controls. However, male pups in the low-fat diet group at 6 weeks of age showed a statistically significant increase in body weight (2.0 mg/kg), liver weight (1.0 and 2.0 mg/kg), and relative liver weight (1.0 mg/kg), when compared to controls. Male pups at 18 weeks of age showed a statistically significant increase in body weight and liver weight in the 2.0

mg/kg treatment group fed low fat diets, when compared to controls. Male pups in the 2.0 mg/kg treatment group and fed the low-fat diet showed a statistically significant increase in postnatal weight gain over time (PND22-Week 18), when compared to controls.

Serum lipid analysis at PND22 showed a statistically significant decrease in triglycerides in all treatment groups in female pups and in the 1.0 and 2.0 mg/kg treatment groups in male pups, when compared to controls. A statistically significant increase in the HDL to LDL ratio was reported in females in the 2.0mg/kg group at PND22, when compared to controls. At 6 weeks of age, a statistically significant increase in cholesterol levels was reported in male pups in all treated groups fed the low-fat diet, when compared to controls. A statistically significant increase in HDL to LDL ratio was reported in the 0.2 mg/kg group fed low fat diets and a statistically significant decrease in triglycerides in the 1.0 mg/kg group fed low fat diets was reported in female pups at 6 weeks of age, when compared to controls. Cholesterol and HDL levels were statistically significantly increased in male pups at 18 weeks old in the 1.0 mg/kg treatment group fed low fat diets, when compared to controls.

Fasting insulin levels in 18-week-old male pups were statistically significantly increased in the 2.0 mg/kg GenX group fed the low-fat diet and in the 1.0 mg/kg GenX group fed the high fat diet, when compared to controls. Males in the 2.0 mg/kg treatment group and fed the low fat diet had statistically significantly increased body weight, fat mass, fat:lean, and percent fat and statistically significantly decreased percent lean at Week 17, when compared to controls. Results of liver pathology at Week 18 indicated a statistically significant increase in microvesicular fatty change in the 2.0 mg/kg group males fed the low-fat diet and a statistically significant increase ($p<0.10$) in hepatocyte single cell necrosis in the 1.0 mg/kg group females fed the high fat diet, when compared to controls. Liver pathology results at Week 18 indicated a statistically significant increase in ALT and ALP levels in the 2.0 mg/kg group females fed the high fat diet, when compared to controls.

Gene expression analysis of adipose tissue at Week 18 resulted in a statistically significant upregulation of *Esrrg* gene in all dose groups and of *Pparg* in the 1.0 mg/kg dose group of females fed the low-fat diet, when compared to controls. Statistically significant downregulation was reported for the *Mapk3* gene in the 2.0 mg/kg group of females fed the high fat diet and for *Acaca, Acacb, Adipoq, Fasn, Insr, Irs1, Pparg, Rxra*, and *Srebf1* genes in the 2.0 mg/kg group of males fed the low-fat diet, when compared to controls. A statistically significant upregulation was reported for *Esr1* gene in the 1.0 mg/kg group of males fed the high fat diet, when compared to controls.

Study authors concluded that prenatal exposure to GenX was associated with metabolic disease based on several adverse outcomes predominantly seen in male mice. Study authors reported increased weight gain, fat mass, retained water weight and fatty liver changes, and decreased insulin sensitivity (1 mg/kg) in male mice following prenatal exposure to GenX. Signs of liver damage (hepatocyte single cell necrosis) and increased ALP and ALT levels were reported in female mice at 1 and 2 mg/kg GenX, which study authors suggest produces metabolic disease at lower doses than PFOA. Study authors conclude that GenX may not be a safe replacement for PFOA.

20.4.1.2.5 Other (endocrine, neurotoxicity, immunotoxicity...)

(Guo, Chen, et al., 2021; Guo, Sheng, et al., 2021) evaluated the effects of GenX on fatty acid metabolism, hepatotoxicity and the relationship between liver injury and gene expression. Adult male BALB/c mice were continuously exposed to GenX at doses of 0, 0.4, 2, or 10 mg/kg/d via oral gavage for 28 days. Results indicated increased hepatomegaly and disturbed fatty acid metabolism with increasing doses of GenX. In addition, treatment with GenX produced

significant increases in relative liver weight and bile acid metabolism at all treatment levels. The ratios of primary bile acids to all bile acids increased in the high-dose groups, while the ratios of secondary bile acids showed a downward trend. Thus, bile acid metabolism disorder may be a prominent adverse effect induced by exposure to GenX.

20.4.2 Relevant Human data

No relevant data was found.

20.4.2.1 New data

No relevant data was found.

20.5 Proposal for a starting point for deriving a drinking water limit value

20.5.1 Selected studies

An overview of the existing recommended points of departure (POD) and oral reference values as reported in (ECHA, 2019) for GenX is provided in Table 77 below.

Table 77: Currently Recommended Points of Departure and Oral Reference Values for GenX

Type of Value	Reference	NOAEL	POD	BMD10	Assessment Factors	Value
tTDI oral general population	(RIVM, 2016)	0.1 mg/kg bw/day	Change in A/G ratio in male rats (Caverly Rae et al., 2015)	Not Applicable	Additional toxicokinetics AF: 66[a] Interspecies toxicokinetics AF: 4[b] Interspecies remaining toxicodynamics AF: 1.8[c] Intraspecies AF: 10 Total AF: 4752	21 ng/kg bw/day
Provisional guideline value drinking water general population	(RIVM, 2016, 2017a)	0.1 mg/kg bw/day	Change in A/G ratio in male rats (Caverly Rae et al., 2015)	Not applicable	Additional toxicokinetics AF: 66[a] Interspecies toxicokinetics AF: 4[b] Interspecies remaining toxicodynamics AF: 1.8[c] Intraspecies AF: 10 Total AF: 4752	150 ng/l[d]
Provisional drinking water	(NC DHHS, 2017)	0.1 mg/kg bw/day	Liver single cell necrosis in male mice	No applicable	Interspecies AF: 10	140 ng/l[e]

Type of Value	Reference	NOAEL	POD	BMD10	Assessment Factors	Value
equivalent level (DWEL) general population			(Edwards, 2010b; Haas, 2008b)		Intraspecies AF: 10 Subchronic-to-chronic AF: 10 Total AF: 1000	
Subchronic reference dose	(US EPA, 2021c)	0.1 mg/kg/day	Constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPOdimer acid ammonium salt by gavage for 53–64 days (Dupont, 2010).	0.01 mg/kg/day	Intraspecies uncertainty factor = 10 Interspecies uncertainty factor = 3 Database uncertainty factor = 10	0.03 µg/kg bw/day
Chronic reference dose	(US EPA, 2021c)	0.1 mg/kg/day	Constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPOdimer acid ammonium salt by gavage for 53–64 days (Dupont, 2010).	0.01 mg/kg/day	Intraspecies uncertainty factor = 10 Interspecies uncertainty factor = 3 Extrapolation from subchronic to a chronic exposure duration uncertainty factor = 10 Database uncertainty factor = 10	0.003 µg/kg bw/day
DNEL oral general population	REACH Registration Dossier	1 mg/kg b/day	Observed liver and blood effects in male rats (Caverly Rae et al., 2015)	Not applicable	Interspecies AF: 4 Interspecies AF other: 2.5 Intraspecies AF: 10 Total AF: 100	0.01 mg/kg bw/day

[a] According to (RIVM, 2016) the toxicokinetic factor of 66 is based on differences in half-lives between cynomolgus monkeys and humans as determined for PFOA (1378 days in humans (manly males)/20.9 days in male monkeys = 66) based on study results from (Olsen Geary W. et al., 2007) and (J. L. Butenhoff, Kennedy, Hinderliter, et al., 2004).

[b] Please note that in the opinion of the project team the general interspecies toxicokinetic factor of 4 is not necessary, if the factor of 66 is applied.

[c] Please note that the project team would suggest a interspecies remaining toxicodynamics factor of 2.5 for rats instead of 1.8.

[d] In agreement with the usual method for deriving drinking-water guidelines, 20% of the (t)TDI is allocated to drinking-water. Using a standard adult body weight of 70 kg and a standard drinking-water consumption of 2 L per day, leads to a provisional drinking-water guideline value of 150 ng/l.

[e] Using body weight (7.8 kg) and drinking water intake of bottle fed infants (1.1 L) and a relative source contribution of 20% to account for exposure to HFPO-DA via other sources.

The most conservative oral toxicity value for GenX is the tentative TDI (t-TDI) of 21 ng/kg bw/day, based on an overall NOAEL of 0.1 mg/kg bw/day from a chronic oral study in rats with increased albumin/globulin ratio in serum as the critical effect (RIVM, 2017a). There is no relevant new information available for this substance that would change the NOAEL of 0.1 mg/kg bw/day based on changes in A/G ratio in 2-year oral rat study (Caverly Rae et al., 2015).

20.5.2 Proposal of assessment factors/modification factors with justification

In (RIVM, 2016, 2017a) an overall assessment factor of 4752 was applied (see table above) including an additional toxicokinetic factor of 66 based on the ratio of PFOA half-lives in humans and monkeys (RIVM, 2016). However, this approach might be considered as uncertain as there are no substance specific toxicokinetic data in humans available for GenX and toxicokinetic parameters as determined in (Gannon et al., 2016) suggest a fast elimination of GenX in both rats and monkeys (see chapter 20.3.1.1). Alternatively, standard assessment factors based on the ECHA guidance may be considered.

In a classical approach an overall assessment factor of 100 can be applied.

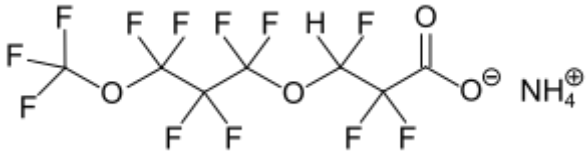
- AF for interspecies differences based on AF of 2.5 for interspecies toxicodynamic differences and AF of 4 for allometric scaling factors for rat to human
- AF of 1 for the time extrapolation (chronic study)
- AF of 10 to account for intraspecies differences

21 Toxicological evaluation of ammonium-4,8-dioxa-3H-4,8-per-fluornonanoat (ADONA)

21.1 Chemical and physical information

ADONA (molecular structure is depicted in Figure 3) is the ammonium salt of 3H-perfluoro-4,8-dioxa-nonanoic acid (DONA). Please note that both substances are chiral. However, no information on the isolated enantiomers is available. For all following physicochemical properties, toxicokinetic and toxicological properties usually racemates were used.

Figure 3: Molecular structure of ADONA



Source: Wikipedia

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 78 and Table 79.

Table 78: Chemical identity of 3H-perfluoro-3-[(3-methoxy-propoxy) propanoic acid], ammonium salt (ADONA, CAS 958445-44-8)

Name	Ammonium-4,8-dioxa-3H-4,8-per-fluornonanoat
English Name	3H-perfluoro-3-[(3-methoxy-propoxy) propanoic acid], ammonium salt
Acronym	ADONA
Mol. Formula	C7H5F12NO4
Mol. Weight (g/mol)	378.1
CAS	958445-44-8; 1280222-90-3 (ammonium salt) 919005-14-4 (acid)
EC	480-310-4 (ammonium salt) 700-835-7 (acid)

Table 79: Physicochemical properties of 3H-perfluoro-3-[(3-methoxy-propoxy) propanoic acid], ammonium salt (ADONA, CAS 958445-44-8)

Properties	Value	Source
Density (g/cm^3)	1.74 (mod.)	(USEPA, 2020)
Melting point (°C)	38	(Gordon, 2011)

Properties	Value	Source
Boiling point (°C)	100 – 105	(Gordon, 2011)
Vapour Pressure (Pa)	9.1 x 10^{-4} – 3.5 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	1.9 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	0.8-1.5 (exp.)	(Baggioli et al., 2018)
Log Koc	No data	
Water Solubility (mg/l)	431 (mod.)[a]	(USEPA, 2020)

(exp = experimental, mod = modelled), [a]OPERA Model: 1.14 x 10^{-3} mol/l = 431 mg/l.

21.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

No quantitative toxicological assessments of ADONA determined by other institutions and drinking water limits were found.

21.3 Toxicokinetics

21.3.1 Animal data

21.3.1.1 New data

ADME profile of ADONA was examined in (Rice et al., 2021). Rice et al evaluated *in vivo* TK study reports on three polyfluorinated ether carboxylate compounds, HFPO-DA, ADONA and EEA-NH4 and examined their TK profiles. Additionally, authors have assessed the quality of these studies to determine whether the TK parameters derived from the data therein could be considered robust enough for use in the human health risk assessment of exposures to these compounds. Based on the available data absorption, distribution, metabolism, and elimination (ADME) profiles were semi-quantitatively determine for the polyfluorinated ether carboxylate compounds including ADONA (Rice et al., 2021).

ADONA is readily absorbed after oral administration in all tested species. The examination of the liver to serum ratios did not indicate marked distribution of the test substances in the liver in male and female rodents (Rice et al., 2021).

ADONA was not metabolized and was eliminated essentially unchanged predominantly in the urine. The elimination of the test substances showed across species, a biphasic decline in concentration over time with a short initial phase and a longer terminal phase after single or repeated oral or iv exposure to ADONA (Rice et al., 2021).

Maximum concentration (C_{max}) and AUC increased with the dose in a 5-day oral toxicity study in male and female rats and were higher in males than females. Mean serum $t_{1/2}$ was between 20 and 40 hours (Rice et al., 2021).

Terminal whole body $t_{1/2}$ of 13.3 days in males and 34.6 days in females at a dose equivalent to 9 mg/kg bw/day was reported in a repeated exposure oral study in SD rats. ADONA was widely distributed in the tissues in the order of plasma >whole blood > liver > GI tract > kidneys > heart. The liver to serum ratios ranged from 0.7 to 4.8 with no significant differences between males and females (Rice et al., 2021).

Based on the data reported in the reviewed TK studies, the overall TK profiles of HFPO-DA and ADONA appear to be qualitatively similar, such that both compounds are rapidly absorbed upon oral exposure followed by no metabolism and a biphasic elimination with a faster initial phase and a slower terminal phase, with most of the test substance eliminated unchanged in the urine. However, due to certain deficiencies in study design and reporting, including absence of adequate raw plasma and/or tissue data, the TK profiles of HFPO-DA and ADONA in plasma and tissues of animals could not be fully characterize under the test conditions of the reviewed studies (Rice et al., 2021).

A basic toxicokinetics in vivo study in cynomolgus monkeys conducted with ADONA was reported in the registration dossier on the ECHA webpage.[6] In the study 3 males and 3 females were dosed with 28.2 mg /kg bw by intravenous injection. The volume of distribution in male cynomolgus monkeys was 331 ml/kg and 160 ml/kg in females. The mean renal clearance in males was 80.3 ml/h kg and 61.8 ml/h kg in females. Mean half-life of the test article: 5.7 h (males), 4.2 h (females).

Another study on serum pharmacokinetics of ADONA in mice was also reported in the registration dossier.[3] In this study ADONA (supplied as a liquid) was investigated in Crl:CD-1 (ICR) mice following a single intravenous dose. ADONA appears to undergo first order elimination (t1/2 Male: 8.1 h, Female: 6.2 h). Mean liver-to-serum ratios at 24 h were 0.66 for males and 0.98 for females. Test article did not preferentially distribute to or accumulate in livers of mice. Based on the results of this study, the test article was rapidly eliminated from the serum of male and female mice following a single intravenous dose of 28 mg/kg bw.

In the review paper of (Chambers et al., 2021) was reported that the bioaccumulation of ADONA and GenX is not well researched.

21.3.2 Human data

21.3.2.1 New data

In the registration dossier on ECHA webpage[3] a human exposure epidemiology study conducted from 2008-2009 is available for ADONA. Serum samples were collected from three employees that were occupationally exposed to the test article. Samples were collected over a 5-week period in which the participants did not have any known additional exposure to the test article. Based on the collected serum samples a half-life of elimination was estimated. Terminal serum elimination half-lives in 3 occupationally exposed male workers were 16.8, 17.6, and 35.5 days. Based on the results of the study, the test article's terminal serum elimination half-life in 3 occupationally exposed male workers ranged from approximately 16-36 days.

21.4 Health effects in humans and/or animals

21.4.1 Relevant Animal data

Publication of (Gordon, 2011) summarizes studies which have been performed to evaluate the toxicity of ADONA by various testing facilities. In this publication many references are made to unpublished studies to which no access is available. Therefore, the relevant studies are cited in the following as secondary literature as (Gordon, 2011).

[6] ECHA registration dossier of ammonium 2,2,3 trifluor-3-(1,1,2,2,3,3-hexafluoro-3-trifluormethoxypropoxy), propionate (EC 480-310-4), https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2602/7/2/2/?documentUUID=e705e8cc-2ef3-4111-a271-8ea63bb0207f (last accessed 25.11.2022)

21.4.1.1 New data

21.4.1.1.1 Repeated dose toxicity

In (Gordon, 2011) two repeated dose toxicity study were reported. Additionally, a 5-day oral toxicity study in rats performed according to OECD 401 is reported.

ADONA was evaluated in a 5-day oral toxicity study in rats performed according to OECD Guideline 401. ADONA was administered by oral gavage once daily for five consecutive days to groups of male and female Sprague–Dawley rats (6/sex/group) at doses of 28, 104, or 298 mg/kg/day. Three rats/sex/group were sacrificed at the end of the treatment period and 3/sex/group were sacrificed at the end of a 7-day post-treatment recovery period. In the study serum ADONA concentrations (Cmax and AUC) for surviving males and females following the last dose were dose-related and were higher in males than in females (Gordon, 2011).

All females in the 298 mg/kg/day dose group died between days 3 and 5. These females exhibited decreased activity, dramatically reduced food consumption, and had dark material on their fur at necropsy. Histopathologic examination of these animals revealed minimal to mild renal congestion, tubular dilation, and tubular degeneration/regeneration. All males survived to scheduled termination and none exhibited clinical signs of toxicity. At the mid-dose, 104 mg/kg/day, the only treatment-related, toxicologically significant findings were minimal to mild renal tubular dilation in 1 of 3 males and 2 of 3 females and minimal renal tubular degeneration in 1 of 3 males. No significant treatment-related effects were observed at the low dose, 28 mg/kg/day in males and observed in females (Gordon, 2011).

In a short-term repeated dose oral toxicity study (OECD 407, GLP) ADONA was administered by oral gavage once daily for 28 consecutive days to groups of male and female Wistar rats (5/sex/group) at doses of 0 (vehicle), 10, 30, or 100 mg/kg/day. A vehicle control group received deionized water (Gordon, 2011). Following evaluations were performed in the study: clinical observations, body weights and food consumption, a functional observational battery (hearing ability, pupillary reflex, static righting reflex, grip strength, and motor activity), serum chemistry, haematology, gross necropsy, organ weights and histopathologic examinations. No treatment-related deaths and no abnormal clinical signs were observed in any ADONA treatment group. There were no significant treatment-related effects on body weight, food consumption, or any functional observational parameters (Gordon, 2011).

Absolute liver weights were significantly increased in males at 30 and 100 mg/kg bw/day (34% and 64%, respectively) and relative liver weights were significantly increased in males in all treatment groups (18%, 29%, and 62% at 10, 30, and 100 mg/kg/day, respectively). The only significant histopathologic change was diffuse midzonal/centrilobular hepatocellular hypertrophy at minimal to moderate (dose-related) severity in the livers of all males in all ADONA treatment groups. There was no evidence of hepatocellular degeneration, necrosis, or other pathologic changes in males at any dose. The minimal liver changes observed in male rats at 10 mg/kg/day were not deemed to be toxicologically significant and this dose was considered to be the NOAEL for males in this study. Based on the results of the study the NOAEL for male was established as 10 mg/kg bw/day and for female as 100 mg/kg bw/day.

In a repeated dose subchronic oral toxicity study (OECD 408, GLP) ADONA was administered by oral gavage once daily for 90 consecutive days to groups of Sprague–Dawley rats (10/sex/group) at doses of 1, 3, or 10 mg/kg/day (males) and 10, 30, or 100 mg/kg/day (females). A vehicle control group received water. The following evaluations were performed: clinical observations, body weights and food consumption, ophthalmoscopy (control and high dose), serum chemistry and haematology, urinalysis and histopathologic examinations (Gordon, 2011).

One high dose female was euthanized. No other deaths occurred, and no abnormal clinical signs were observed in any ADONA treatment group. There were no significant effects on body weight or food consumption, ophthalmoscopic examinations. There were no treatment-related gross lesions noted at necropsy. Absolute and relative liver weights were slightly increased (5– 8%) in high-dose males and females, but the increases were not statistically significant. The minor findings in males at 10 mg/kg/day and in females at 100 mg/kg/day were judged not to be toxicologically significant. NOAEL for male was established as 10 mg/kg bw/day and for female as 100 mg/kg bw/day.

21.4.1.1.2 Carcinogenicity

No relevant data was found.

21.4.1.1.3 Mutagenicity

As reported in (Gordon, 2011) the mutagenic activity of ADONA was evaluated in OECD 471 complaint reverse mutation assays in Salmonella typhimurium (strains TA1535, TA1537, TA98, and TA100) and Escherichia coli (WP2uvrA) up to recommended maximum test concentration of 5 mg/plate using the plate incorporation method. ADONA was not mutagenic in the assay either in the presence or the absence of metabolic activation.

ADONA was found to be not mutagenic on the in vitro HPRT gene mutation assay conducted according to OECD 476 in a Chinese hamster V79 cell line (Gordon, 2011).

Based on the results of in vitro chromosome aberration assay conducted according to OECD 473 ADONA was reported to induce chromosome aberrations in cultured human lymphocytes with and without metabolic activation (Gordon, 2011).

However, ADONA was found to be negative in two in vivo genetic toxicity studies. In the in vivo micronucleus study conducted according to the OECD 474 ADONA was not clastogenic or aneugenic in male or female mice at oral doses up to and including the maximum tolerated dose of 800 mg/kg/bw (males) and 750 mg/kg (females). In the bone marrow cytogenetic study in rats (OECD 475) ADONA was found to be not clastogenic in male or female rats at oral doses up to and including the maximum tolerated dose of 1120 mg/kg/bw (males) and 940 mg/kg/bw (females). The negative findings in the micronucleus study in mice and the bone marrow cytogenetic study in rats indicate that ADONA does not pose a clastogenic hazard under in vivo exposure conditions.

Based on the weight of evidence approach ADONA could be concluded as not genotoxic (Gordon, 2011).

21.4.1.1.4 Toxicity to reproduction

ADONA was evaluated in a developmental toxicity screening study in Sprague–Dawley rats (Gordon, 2011). In the study ADONA was administered to 10 presumed-pregnant female rats once daily by oral gavage at doses of 10, 30, 90, 270, or 500 mg/kg bw/day from gestation day (GD) 0 until delivery or GD 24 for rats that did not deliver. Clinical observations (at least once daily during gestation, throughout parturition, and on postnatal days 1, 4, and 6), body weight and feed consumption (daily), duration of gestation, length of parturition, necropsy, pregnancy status, uterine contents, implantation sited were recorded for dams. Litter and pup (F1) evaluations were performed through postnatal day (PND) 6 and included: litter size, pup viability at birth, pup survival and body weight on PND 1, 4, and 6, clinical observations, and necropsy.

The animals of 500 mg/kg bw/day group were sacrifice due to significant body weight loss, reduced food consumption, and clinical signs that included decreased activity, dehydration, cold to touch, pale extremities, rales, ungroomed coat, urine-stained fur, and ptosis.

At 270 mg/kg/day, 4 of 10 females were found dead between GD 3 and 5 and another was euthanized in moribund condition on GD 21. All females in the 90 and 30 mg/kg/day dose groups survived to scheduled termination. No significant treatment-related clinical signs were observed at 10, 30, and 90 mg/kg bw/day. All surviving pregnant dams in the 10, 30, 90, and 270 mg/kg/day dose groups delivered normally. The mean number of pups per litter, percentage of liveborn pups per litter, and percentage of stillborn pups per litter were not significantly different from controls for any dose group. Pup survival on postnatal days 1,4 and 6 (61.8%, 47.3%, and 47.3%, respectively) was significantly reduced in the 270 mg/kg bw/day group. Mean pup weight per litter was significantly reduced on postnatal days 1, 4 and 6 (25–29%) in the 270 mg/kg bw/day group and on postnatal day 1 (13%) in the 90 mg/kg bw/day group. There were no gross malformations noted at necropsy among pups from any group. Maternal and developmental NOAELs for ADONA in this study were both 30 mg/kg bw/day (Gordon, 2011).

The zebrafish is a widely used in vivo model for toxicity testing. (Gaballah et al., 2020) investigated developmental toxicity and developmental neurotoxicity of ADONA in zebrafish. To determine whether exposure to ADONA caused developmental toxicity in larval zebrafish, embryos were exposed to 0.04–80 μM of PFAS from 0–5 days post fertilization (dpf). Exposure to ADONA did not cause concentration-dependent effects on survival or development.

To evaluate the effects on neurobehavioral development, zebrafish were exposed to 4.4–80.0 μM ADONA from 0–5 dpf and locomotor activity was assessed at 6 dpf. Zebrafish developmentally exposed ADONA did not exhibit differences in locomotor activity at 6 dpf. ADONA was not associated with developmental toxicity and developmental neurotoxicity in zebrafish (Gaballah et al., 2020).

21.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

In vivo PPARa activation was evaluated in rats (Gordon, 2011). ADONA was administered to groups of male and female Sprague–Dawley rats (3/sex/group) at a dose of 30 mg/kg bw either by single oral gavage or by single intravenous injection. Approximately 48 h after dosing, all animals were euthanized, and liver specimens (approximately 300 mg) were analysed by quantitative real-time PCR. Analysis was performed for following mRNA transcripts: peroxisomal bifunctional enzyme (*Ehhadh*), cytochrome P450-4a1 (*Cyp4a1*), acyl coenzyme A oxidase (*Acox*) and DNA damage inducible transcript (*Ddit3*). *Ehhadh, Cyp4a1*, and *Acox* are enzyme markers of PPARa-mediated gene transcription and *Ddit3* is a marker of general tissue injury.

Mean *Ddit3* transcript levels for males and females in the ADONA dose groups were not significantly different from controls. Mean transcript levels of *Cyp4a1, Ehhadh, and Acox* were not statistically significantly increased in males. Mean transcript levels of *Cyp4a1, Ehhadh*, and *Acox* in females administered ADONA were similar to controls. Although not statistically significant, the large fold-increases of *Cyp4a1* and *Ehhadh* in males were found as consistent with ADONA being a *PPARα* agonist in male Sprague–Dawley rats (Gordon, 2011).

(S. Zhang et al., 2021) conducted in vitro tests with rat thyroid cell line FRTL5 and primary normal human thyroid (NHT) cells to evaluate a thyroid disrupting effect of ADONA. To determine the effects of ADONA on thyroid cell viability FRTL5 and primary NTH cells were exposed to 0.1–1000 ng/ ml of ADONA for 24, 48 and 72 hours. ADONA did not alter the viability of cells at the tested concentration and exposure in both cell models. Additionally, treatment

with ADONA did not affect cells proliferation up to concentration of 1000 ng/ml (2531.01 nM) and exposure time 72 hours.

The gene expression of thyroid hormone regulation-related genes (*ttf-1, pax8, nis*, *tg*, *tshr* and *tpo)* in FRTL5 thyroid cells after exposure to 0.1 ng/ml of ADONA for 24 hours was quantified by RT-PCR and Western blot. ADONA increased critical thyroid transcription factor gene (pax*8*) expression. This result was also confirmed on protein level by Western blot. ADONA showed enhancement on PAX8 expression. In primary NHT cells ADONA increased the expression of *pax8* and *tshr (*tsh receptor). Taking together ADONA can affect thyroid cell function by altering the gene expression (S. Zhang et al., 2021).

21.4.2 Relevant Human data

21.4.2.1 New data

No relevant data was found.

21.5 Proposal for a starting point for deriving a drinking water limit value

21.5.1 Selected study

The repeated dose subchronic oral toxicity study (OECD 408, GLP) in rats reported in (Gordon, 2011) was selected as most suitable study to derive a drinking water limit value. Based on result of this study a NOAEL for male rats was established as 10 mg/kg bw/day and for female rats as 100 mg/kg bw/day. Additionally NOAEL of 10 mg/kg bw/day in male rats was established based on the liver effects in the 28-day repeated dose short term toxicity study conducted in rats (Gordon, 2011). ADONA was also found to be PPARα agonist in male Sprague–Dawley rats (Gordon, 2011).

The lowest NOAEL of 10 mg/kg bw/day in male rats based on the evidence of liver effects (increased liver weights and diffuse midzonal/centrilobular hepatocellular hypertrophy) is proposed as the starting point for a TWLW.

21.5.2 Proposal of assessment factors/modification factors with justification

An assessment factor of overall 200 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF for interspecies differences based on AF of 2.5 for interspecies toxicodynamic differences and AF of 4 for allometric scaling factors for rat to human
- AF of 2 to account for the time extrapolation (adjusting for subchronic 90-day exposure to chronic exposure).
- AF of 10 to account for intraspecies differences

22 Toxicological evaluation of 6:2-fluorotelomer sulfonic acid (6:2 FTSA)

22.1 Chemical and physical information

6:2 FTSA (also known as 6:2 FTS) is a fluorotelomer sulfonic acid. It is a derivative of PFOS with 4 fluor atoms substituted by hydrogen atoms (Figure 4) and is therefore also called H_4PFOS.

Figure 4: Molecular structure of 6:2 FTSA

Source: Wikipedia

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 80 and Table 81.

Table 80: Chemical identity of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulfonic acid; 6:2-fluorotelomer sulfonic acid (6:2 FTSA, CAS 27619-97-2)

Name	6:2-Fluortelomersulfonsäure
English Name	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulfonic acid; 6:2-fluorotelomer sulfonic acid
Acronym	6:2 FTSA, H_4PFOS
Mol. Formula	C8H5F13O3S
Mol. Weight (g/mol)	428.2
CAS	27619-97-2
EC	248-580-6

Table 81: Physicochemical properties of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulfonic acid; 6:2-fluorotelomer sulfonic acid (6:2 FTSA, CAS 27619-97-2)

Properties	Value	Source
Density (g/cm^3)	1.64-1.71 (mod.)	(USEPA, 2020)
Melting point (°C)	18.7-126 (mod.)	(USEPA, 2020)
Boiling point (°C)	219-258 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	1.1 x 10^{-4} (mod.)	(USEPA, 2020)

Properties	Value	Source
Henry's Constant (Pa m^3/mol)	1.9 x 10^{-5} (mod.)	(USEPA, 2020)
pKa	0.36 (mod.)	(Steinle-Darling & Reinhard, 2008)
Log Koc	4.0 (±0.2)	(Munoz et al., 2019)
Water Solubility (mg/l)	1.1-428 (mod.)[a]	(USEPA, 2020)

(mod = modelled); [a]EPISuite: 2.48 x 10^{-6} mol/l = 1.1 mg/l, TEST Model: 1.44 x 10^{-5} mol/l = 6.2 mg/l, OPERA Model: 0.001 mol/l = 428 mg/l.

22.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

No quantitative toxicological assessments of 6:2 FTSA determined by other institutions and drinking water limits were found.

22.3 Toxicokinetics

22.3.1 Animal data

22.3.1.1 New data

The study by (Narizzano et al., 2021), investigated toxicokinetics of several per- and polyfluoroalkyl substances in white-footed mice (Peromyscus leucopus). 6:2-FTSA was administered at dose levels of 0, 2.5, 6 and 12.5 mg/kg bw/day via oral gavage for up to 28 days. Serum levels of 6:2-FTSA were measured on day 21 and day 28 of dosing. It was found that serum concentrations were highest in the high dose group after 21 days, followed by the mid and low dose groups. Serum levels decreased between days 21 and 28. At the end of the exposure period (day 28), serum levels of 6:2-FTSA were 6 (±3), 8 (±1) and 18 (±4) ng/ml in both sexes treated with 2.5, 6, and 12.5 mg/kg bw/day. No metabolites could be identified in serum via nontargeted analysis of serum from high dose animals at day 28.

However, the small number of time-points for the follow-up of serum levels did not allow to reliably conclude on typical toxicokinetic parameters, such as area under curve (AUC), half-live ($t_{1/2}$) and maximum concentration (c_{max}) and these were not reported from the study.

In the REACH registration dossier for 6:2-FTSA[7], data from several poorly reported toxicokinetic studies are available. In an *in vitro* study with male rat liver S9 preparations (study report, unnamed, 2008), 2.5 µM of the test substance was incubated for 2 hours to evaluate the extent of potential metabolic conversion of the test substance. Based on the results reported in the registration dossier, no metabolism of the substance occurred. In an additional *in vivo* study in 3 Crl:CD(SD) rats/sex/dose (study report, unnamed, 2007) single gavage administration of the test substance in water at doses of 10 and 30 mg/kg bw lead to tissue:plasma ratios ≤0.1 at the low dose and 0.1 at the high dose level in fat tissue of males, while ratios in females were all below limit of quantification (LOQ). In the liver of male rats, tissue:plasma ratio was 3.0 in the

[7] ECHA registration dossier of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulphonic acid (CAS 27619-97-2) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/24637/7/2/2/?documentUUID=d5f46617-e299-4007-a6a2-a1041f14408b, last modified 27 September 2019 (accessed 24 November 2022)

low dose group and 3.1 in the high dose group, while for females, plasma values were below LOQ and hence no ratio could be calculated.

In a urinary excretion study in 3 male Crl:CD(SD) rats (unnamed study report, 2008), the recovery of a single oral gavage dose of 73 µM/kg 6:2-FTSA in water was 65-68 % after 96 hours. Half-lives of the substance ranged between 20.90 hours via NMR analysis and 23.75 hours via LC/MS.

However, all of these studies were assigned a Klimisch score of 4 (not assignable) and the amount of information that is reported is very limited. Therefore, the reliability of this information cannot be checked against the original study data and the validity of this data is unclear.

22.3.2 Human data

22.3.2.1 New data

No relevant data was found.

22.4 Health effects in humans and/or animals

22.4.1 Relevant Animal data

22.4.1.1 New data

22.4.1.1.1 Repeated dose toxicity

In a study by (Sheng et al., 2017), 20 adult male CD1 mice (6-8 weeks of age) were exposed to a single dose of 5 mg/kg bw/day 6:2 FTSA (>99 % purity) in Milli-Q water containing 2% tween-20 or vehicle alone, by oral gavage for 28 days, consecutively.

There were no mortalities and no changes in body weight. However, absolute and relative liver weights were significantly increased by 19 and 22%, respectively. These findings correlated with significant increases in aspartate transaminase (AST) and albumin (ALB) and non-significant increases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as indications of hepatocyte hypertrophy, hepatocyte necrosis and increased hepatic inflammatory markers. These results indicated signs of liver injury after 6:2 FTSA exposure at 5 mg/kg bw/day.

However, based on the exposure to a single dose showing adverse effects and the resulting lack of dose-response from this data, it was not possible to derive a reliable POD. Nevertheless, the 5 mg/kg bw level can be seen as an adverse effect level, due to signs of liver damage described in the context of this study.

In addition, the REACH registration dossier2 of 6:2-FTSA (CAS No. 27619-97-2) contains data from several other studies with a dose regime corresponding to repeated oral exposure. However, it has to be noted that the information available in the dossier is secondary information provided by the registrants of the substance. Therefore, the original study data is not directly accessible and cannot be checked for validity and reliability.

Among this information, a 14-day repeated dose toxicity study was reported, in which 6:2-FTSA (purity >99 %) was administered to groups of 5 male Crl:CD-1(ICR)BR mice/dose group in the diet at levels of 3, 30, 300 and 3000 ppm for 14 days. Very limited parameters such as body weight, clinical signs, liver weights and gross pathology of the liver were investigated (study report, unnamed, 1995). Significant decreases in mean body weight and weight gain were noted at the 3000 ppm level. In addition, significant increases in mean and absolute liver weight were

observed at 300 ppm and 3000 ppm, which correlated with liver discoloration in 1/5 animals within the 300 ppm group and in 4/5 animals within 3000 ppm group.

Based on these findings, the registrant identified the 30 ppm level as a no observed adverse effect level (NOAEL) from this study.

In addition, data from a combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test according to OECD 422 was reported (unnamed study report, 2018). According to the data reported by the registrant, the potassium salt of 6:2-FTSA (Potassium 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulfonate, reported purity 97.1 %; CAS No. 59587-38-1) was administered to 12 Crl:WI(Han) rats/sex/dose via oral gavage at dose levels of 0 (vehicle control: water), 5, 15 and 45 mg/kg bw/day. Male animals were dosed during a 10-week premating period, during mating and up to sacrifice after a total of 90 days of exposure, while females were dosed during a 10-week premating period, during mating, gestation and lactation until postnatal day 14.

There were no mortalities. No treatment-related clinical signs, haematological findings or effects on food consumption were observed. Reduced body weight gain was observed in high dose males from day 49 to 70 of treatment and in high dose females from day 0-7 and during days 21-28 and 56-70.

Mean total protein and albumin levels were statistically significantly lower in the low and high dose males and urea levels were slightly statistically significantly increased in high dose males. Relative kidney weights were statistically significantly increased in males of the low and high dose groups. In the kidneys from high dose group animals, mild to moderate (multi)focal tubular dilatation in 5/12 males and in 1/12 females were observed, while in lower dose levels no such effects were noted.

In females, relative mean heart weight was slightly statistically significantly decreased in the mid and high dose groups. Although for the changes in relative kidney weight follow-up histopathological results were reported, this was not specifically the case for the histopathological results of the heart. This part of the results section would have been of interest due to the observed reductions in heart weight in high dose group animals.

Without a histopathological follow-up or at least an indication of the extent of weight reduction of the heart it is therefore difficult to assess the adversity of findings in this organ. Based on a conservative worst-case approach, one could consider the mid dose level of 15 mg/kg bw/day as the lowest observed adverse effect level (LOAEL) based on the reduction in heart weight as the most sensitive parameter identified from this study. Although reductions in relative kidney weight were reported even at the low dose level, there did not seem to be a histopathological correlate to this finding. Therefore, it cannot be conclusively evaluated that this effect is non-adverse, based on the available information in the registration dossier, but it is rather unlikely that this isolated finding represents an adverse effect. Consequently, the low dose level of 5 mg/kg bw/day would be considered as a conservative no observed adverse effect level (NOAEL).

22.4.1.1.2 Carcinogenicity

No relevant data was found.

22.4.1.1.3 Mutagenicity

No relevant data was found.

22.4.1.1.4 Toxicity to reproduction

No specific data for the reproductive toxicity endpoint was found. However, in the study by (Narizzano et al., 2021) total sperm counts in white-footed mice (Peromyscus leucopus) were reported to be unaffected by 6:2-FTSA exposure at dose levels of 2.5, 6 and 12.5 mg/kg bw/day via oral gavage for up to 28 days. However, there was also no evidence of adverse effects elicited within the selected dose range as indicated by a lack of body weight changes, gross observations or differences in organ weights among dose groups. The relevance of these observations is therefore unclear, since the adequacy of the selected dose range cannot be judged based on missing toxicological effects in response to administration of the substance.

Additional information on the toxicity to reproduction endpoint was obtained from the data provided by the registrants in the context of the REACH registration dossier[2] for 6:2-FTSA. As described in Repeated dose toxicity section above, a combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECD 422 was conducted with the potassium salt of 6:2-FTSA (Potassium 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanesulfonate, reported purity 97.1 %; CAS No. 59587-38-1) which was administered to 12 Crl:WI(Han) rats/sex/dose via oral gavage at dose levels of 0 (vehicle control: water), 5, 15 and 45 mg/kg bw/day at the same dosing regimen as described above (study report, unnamed, 2018).

With regard to reproductive and developmental parameters, no treatment-related effects on oestrous cyclicity or reproductive performance were observed. One female in the control group was not mated, while one female in the low dose group was pregnant, although initially judged to be not mated. These observations resulted in mating indices of 91.7 % for the control group and 100 % for the high dose group. Male fertility indices ranged between 91.7 % for the control and mid dose groups and 100 % for the low and high dose group. There was no difference between treatment groups in terms of mating days until successful copulation and mean gestational length.

Among F1 offspring, no treatment-related effects on the mean number of liveborn pups were observed. Postnatal survival was marginally reduced in the low dose and mid dose group, in which 1 pup was found missing or dead, respectively, and the high dose group (2 pups found missing/dead). No treatment-related clinical signs were observed. Pup body weight and weight gain were comparable in treatment groups and no dose-related changes were found on mean thyroid weight (absolute or relative), sexual maturation and macroscopic examinations. It has to be noted that a detailed examination of skeletal and visceral malformations is not part of the protocol of the OECD TG 422 and has therefore most probably not been included in this study, according to the information provided. A statistically significant increase in T4 levels was reported for 13 day old male pups only. According to the registrant, the relevance of this finding was questioned due to a large variation between values within dose groups and a lack of statistical significance in the high dose group. Based on the absence of a clear dose response, the registrant rated these findings as not treatment related.

Apart from these findings, no other effects were observed, and the registrant considered a NOAEL for reproductive and developmental toxicity of >45 mg/kg bw/day, i.e., the highest dose tested in this study. However, based on the findings reported for T4 levels, it is unclear, whether this NOAEL can be regarded as robust. Without access to the original report for this study, including the raw data tables for the measured T4 levels, it is not possible to adequately judge the validity of the conclusions made by the registrant.

22.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

No relevant data was found.

22.4.2 Relevant Human data

22.4.2.1 New data

No relevant data was found.

22.5 Proposal for a starting point for deriving a drinking water limit value

22.5.1 Selected studies

Based on the availability of information for the repeated dose toxicity and toxicity to reproduction endpoints, there are two studies which yielded results that could potentially be used for the identification of a point of departure for the derivation of a drinking water limit value.

The first study, which was conducted with the potassium salt of 6:2-FTSA (CAS No. 59587-38-1) was identified from the REACH registration dossier of 6:2-FTSA itself (CAS No. 27619-97-2). According to the information provided, the design of the study was conducted in accordance with a GLP-compliant Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test and the relevant OECD TG 422 in rats. Dose levels of 0, 5, 15 and 45 mg/kg bw/day were administered daily for a period of up to 90 days (study report, unnamed, 2018).

Most critical effects were identified from reduced body weight gains and increased kidney weights in the high dose group, which correlated with histopathological findings of tubular dilatation. In addition, decreased heart weights were described in the mid and high dose groups, but not supported with further data from histopathological investigations.

Based on the nature of the secondary data without provision of additional data tables or access to the original study report, it is difficult to assess the validity and relevance of the findings described. Based on the decreased heart weights ranging down to the mid dose level of 15 mg/kg bw/day, a NOAEL corresponding to the low dose level of 5 mg/kg bw/day can be identified as conservative worst-case approach based on the limited availability of data.

The second study providing information for the identification of a POD for the derivation of a drinking water limit value was a non-GLP non-guideline 28-day study reported in a peer-reviewed publication by (Sheng et al., 2017) with very limited analyses of parameters.

In this study, 6:2 FTSA itself was administered to male mice at a single dose of 5 mg/kg bw/day by oral gavage for 28 days. Most critical effects were described in the liver and included increases in liver weights, concomitant with elevated AST, ALB, ALT and ALP and hepatocyte necrosis and increased hepatic inflammatory markers, indicating signs of liver damage.

However, the reporting of findings within this non-GLP and non-guideline study falls short compared to GLP-compliant guideline studies. For example, incidence and severity of liver necrosis was not described and the reporting of these findings relies on an individual microscopic image obtained after haematoxylin eosin staining, only. In addition, the selection of only one single dose level at 5 mg/kg bw/day is a significant shortcoming in the design of this study, since the dose-response of liver findings could not be evaluated.

It was therefore not possible to identify a reliable NOAEL from this study. However, since liver necrosis can be regarded as an adverse effect, the single dose level of 5 mg/kg bw/day can be considered as an observed adverse effect level for the identification of a POD in a conservative worst-case approach under consideration of additional assessment factors as described in section 22.5.2. A similar approach based on a similar data basis was followed by the Michigan

Department of Environment, Great Lakes, and Energy for the derivation of an initial threshold screening level (ITSL) for 6:2-FTSA[8].

22.5.2 Proposal of assessment factors/modification factors with justification

22.5.2.1 Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECD TG 422 in rats (study report, unnamed, 2018)

An overall assessment factor of 200 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF for interspecies differences based on AF of 2.5 for interspecies toxicodynamic differences and AF of 4 for allometric scaling from rat to human
- AF of 10 to account for intraspecies differences
- AF of 2 to account for the time extrapolation (adjusting for subchronic 90-day exposure to chronic exposure)

22.5.2.2 28-Day repeated dose toxicity study in mice (Sheng et al., 2017)

An overall assessment factor of 3150 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF for interspecies differences based on AF of 2.5 for interspecies toxicodynamic differences and AF of 7 for allometric scaling from mouse to human
- AF of 10 to account for intraspecies differences
- AF of 6 to account for the time extrapolation (adjusting for sub-acute 28-day exposure to chronic exposure)
- AF of 3 to account for the dose-response relationship, from which a LOAEL from a single dose is used as starting point

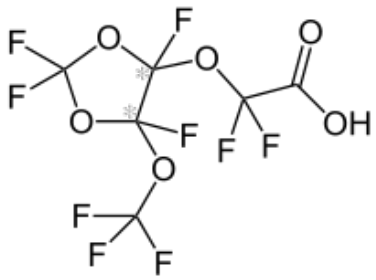
[8] https://www.egle.state.mi.us/aps/downloads/ATSL/27619-97-2/27619-97-2_annual_ITSL.pdf

23 Toxicological evaluation of perfluoro([5-methoxy-1,3-dioxolan-4-yl]oxy) acetic acid (C604)

23.1 Chemical and physical information

The acronym C604 is both used for the perfluoro([5-Methoxy-1,3-dioxolan-4-yl]oxy) acetic acid (depicted in Figure 5) and the corresponding ammonium salt (sometimes also referred to as cC604). Please note that both substances have two stereo centres. However, in study reports usually no information on the stereochemistry is provided. For all following physicochemical properties, toxicokinetic and toxicological properties probably the diastereomeric mixtures were used.

Figure 5: Molecular structure of C604 in its acid form



Source: Wikipedia

Section 1 provides a summary of background information for contextual purposes only. These brief overviews emphasize reviews and other summary information and are not intended to be comprehensive descriptions of the available information. A more comprehensive summary of tabulated values is summarized and consequently updated by (ITRC, 2021) (Interstate Technology and Regulatory Council). The chemical identity and basic physicochemical properties are summarized in Table 82 and Table 83.

Table 82: Chemical identity of Perfluoro-{2-[(5-methoxy-1,3-dioxolan-4-yl)oxy]-acetic acid} (C604, CAS 1190931-41-9)

Name	Perfluor-(2-[(5-methoxy-1,3-dioxo-lan-4-yl)oxy]-essigsäure)
English Name	Perfluoro([5-Methoxy-1,3-dioxolan-4-yl]oxy) acetic acid
Acronym	C604
Mol. Formula	C6HF9O6
Mol. Weight (g/mol)	340.1
CAS	1190931-41-9 (ammonium salt: 1190931-27-1)
EC	682-239-6 (ammonium salt: 682-238-0)

Table 83: Physicochemical properties of Perfluoro-{2-[(5-methoxy-1,3-dioxolan-4-yl)oxy]-acetic acid} (C604, CAS 1190931-41-9)

Properties	Value	Source
Density (g/cm^3)	1.92 (mod.)	(USEPA, 2020)
Melting point (°C)	87.3 (mod.)	(USEPA, 2020)
Boiling point (°C)	184 (mod.)	(USEPA, 2020)
Vapour Pressure (Pa)	0.24-42.0 (mod.)	(USEPA, 2020)
Henry's Constant (Pa m^3/mol)	9.0 x 10^{-6} (mod.)	(USEPA, 2020)
pKa	No data	
Log Koc	No data	
Water Solubility (mg/l)	19.4 (mod.)[a]	(USEPA, 2020)

(mod = modelled); [a]OPERA Model: 0.0569 mol/l = 19.4 mg/l.

23.2 Quantitative toxicological assessments on human data and drinking water limits determined by institutions

No quantitative toxicological assessments of ADONA determined by other institutions and drinking water limits were found.

23.3 Toxicokinetics

23.3.1 Animal data

23.3.1.1 New data

In the ECHA registration dossier of the ammonium salt of C604 (cC604, CAS 1190931-27-1)[9] a study is reported in which groups of Sprague-Dawley rats (4/sex/dose) were administered cC604 by single oral gavage at doses of 0 (purified water; group 3), 20 and 200 mg/kg (groups 4 and 5) in a toxicokinetic study conducted similarly to the OECD Guideline 417 and in compliance with GLP. A separate set of rats were administered 20 mg/kg cC604 ammonium salt by the intravenous route. After oral dosing at 20 and 200 mg/kg, animals of both sexes were exposed to the carboxylated anion. Peak plasma concentrations were achieved within 2 to 6 hours in males and females by the oral route. The AUC (area under the plasma concentration-time curve) was approximately 7-fold and 2-fold higher in males than in females at low and high dose, respectively. The volume of distribution was larger in males than in females. Negligible levels were detected in the male liver (0.02-0.04% of the dose) and kidneys (< 0.01% of the administered dose) at 48 hrs. The substance was not detected in the liver or kidneys of the female rats. The renal route was the main elimination route of the carboxylated anion which was present in the urine. A minor fraction was found in faeces (<1% in males, <2% in females). Majority of the carboxylated anion was eliminated during the first 24-hour collection interval and plasma concentrations dropped rapidly, within 24 hours of administration.

[9] ECHA Registration dossier of Acetic acid, 2,2-difluoro-2-[[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy]-, ammonium salt (1:1) (CAS 1190931-27-1) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5712/7/1 (last accessed 28.11.2022).

Furthermore, in vitro assays were conducted using liver extracts from Sprague-Dawley rats (female/male) treated with cC6O4 ammonium salt at doses of 0, 5, 20, 60 mg/kg in males and 0, 20, 60, 200 (from day 1 to day 19) or 100 (from day 20 to day 28) mg/kg for 28 days conducted according to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents) and in compliance with GLP (as reported in the ECHA registration dossier).[4] A significant dose-dependent increase in activity of peroxisomal β-oxidation of fatty acids consistent with peroxisome proliferation was observed. An increased total cytochrome P450 (unspecified) activity was measured in the liver of rats treated with cC6O4 ammonium salt.

Reliability of the data cannot be assessed as the full study reports are not available.

23.3.2 Human data

23.3.2.1 New data

No relevant data was found.

23.4 Health effects in humans and/or animals

23.4.1 Relevant Animal data

23.4.1.1 New data

23.4.1.1.1 Repeated dose toxicity

In a GLP-compliant sub-chronic repeated dose oral toxicity study conducted according to OECD Test Guideline 408 as reported in the ECHA registration dossier of cC604,[4] cC604 diluted in water was administered daily by oral gavage to groups of Sprague-Dawley rats (10/sex/dose), for 13 weeks at the doses of 0 (vehicle), 0.3, 1, 3 or 10 mg/kg bw/day. Ten additional animals for each sex were included in the medium-high, high dose and control groups for a 6 consecutive weeks recovery assessment. Examinations during the study included: mortality, daily clinical signs, weekly detailed (open field observations) clinical signs, evaluation of sensory reactivity to stimuli and motor activity, body weight, food consumption, ophthalmology, clinical pathology investigations, terminal body weight, haematology, blood chemistry, urinalysis, organ weights, macroscopic observations and histopathological examination which included the evaluation of the staging of spermatogenesis. A No Observed Adverse Effect Level (NOAEL) of 3 mg/kg bw/day in females and 1 mg/kg bw/day in males was identified by the study authors based on changes in clinical pathology parameters and post-mortem findings. The liver was identified as a target organ on the basis of organ weight data and histopathological findings. A second target organ appeared to be the thyroid, where changes were considered to be a secondary effect of the liver toxicity. These effects showed a dose-related trend and appeared to be only partially reversible after the 6-week recovery period. The Lowest Observed Adverse Effect Level (LOAEL) was considered to be 3 and 10 mg/kg bw/day for males and females, respectively.

Reliability of the data cannot be assessed as the full study report is not available.

23.4.1.1.2 Carcinogenicity

No relevant data was found.

23.4.1.1.3 Mutagenicity

The potential for cC6O4 ammonium salt to induce chromosome aberrations in bone marrow cells of the rat was investigated in the ECHA registration dossier of cC604.[4] The animals were treated with a single oral administration of 312.5, 625, or 1250 mg/kg bw in males. Bone marrow cells were collected following 24 hours and 48 hours (only the high dose group) after

dosing. Seven males per test group were evaluated for the occurrence of cytogenetic damage. Per animal 100 well spread metaphases were scored for gaps, breaks, fragments, deletions, multiple aberrations, exchanges, and chromosomal disintegrations. The animals treated with this dose showed clinical signs such as reduction in spontaneous activity, ruffled fur and hunchback posture indicating bioavailability of the substance. No relevant reduction of the mitotic indices could be observed after treatment with the substance, indicating that the substance at the indicated concentrations was not cytotoxic in the bone marrow. No statistically significant increase in the frequency of aberrant cells occurred after treatment with the substance as compared to the vehicle control. The study authors concluded that under the experimental conditions reported, the substance did not induce chromosomal mutations as determined by the chromosome aberration test using rat bone marrow cells. An evaluation of the same study by the European Food Safety Authority (EFSA) concluded that while the substance did not induce chromosome aberrations in bone marrow cells of the rat when administered at the highest tolerated dose (1250 mg/kg bw), the Panel noted that this test was not fully conclusive because there was no evidence that the target tissue was exposed (EFSA, 2014).

The mutagenic potential of cC604 ammonium salt was evaluated by testing for its ability to induce forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells, either in the absence or presence of a metabolic system (S9-mix) for a treatment period of 4 hours or 24 hours in the absence of metabolic activation and 4 hours in the presence of metabolic activation (as reported in the ECHA registration dossier).[4] The highest concentration used in the study was 3640 µg/ml. No substantial and reproducible dose dependent increases in mutant colony numbers were observed in either of the main experiments. No relevant shift of the ratio of small versus large colonies was observed up to the maximal concentration of the cC604 ammonium salt. The study authors concluded that the substance is not mutagenic in the TK mutation test system under the experimental conditions described.

In an in vitro bacterial reverse mutation assay (Ames test) cC604 ammonium salt was tested for mutagenic potential in *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, and in *Escherichia coli* strain WP2 uvrA (pKM101) in the presence and absence of a metabolic activation system (as reported in the ECHA registration dossier).[4] Concentrations of up to 5000 µg/plate were tested. No evidence of mutagenic activity was seen at any concentration of the substance. It was concluded that the substance showed no evidence of mutagenic activity in these bacterial systems under the test conditions employed.

cC6O4 ammonium salt was tested for its ability to cause chromosomal damage in Chinese hamster ovary (CHO) cells, following in vitro treatment in the absence and presence of S9 metabolic activation (as reported in the ECHA registration dossier).[4] Two main experiments for chromosomal damage were performed. In the first experiment, the cells were treated for 3 hours with cC6O4 ammonium salt in the presence and absence of S9 metabolism. Dose levels of up to 5000 µg/ml were used. No statistically significant increase in the incidence of cells bearing aberrations including or excluding gaps was observed in the absence or presence of S9 metabolism. In the presence of S9 metabolism, marked increases in the number of endo-reduplicated cells over the control were observed. An increase in the number of endo-reduplicated cells was also observed in the absence of S9 metabolism at the highest dose selected for scoring. A second main experiment was performed where cells were treated with cC6O4 ammonium salt in the absence of S9 metabolism and harvested after 20 hours. For the second experiment, following treatment with the test item in the absence of S9 metabolism, slight increases but not statistically significant in the incidence of cells bearing aberrations, excluding gaps, over the control values were observed at all dose levels selected for scoring.

More remarkable increases of aberrant cells were observed when including gaps. In the presence of S9 metabolism, statistically significant increases in the incidence of aberrant cells including and excluding gaps were observed at the highest dose selected for scoring (2000 µg/ml). The incidences exceeded the range of the laboratory's historical values for negative controls when excluding gaps. Marked increases in the number of endo-reduplicated cells over the controls were seen at the intermediate and high dose levels selected for scoring. On the basis of these results, it was concluded by the registrant that cC6O4 ammonium salt induces chromosomal aberrations in CHO cells after in vitro treatment under the reported experimental conditions. The registrant also concluded that the substance inhibits cell cycle progression and chromosome segregation under the reported experimental conditions.

Reliability of the data cannot be assessed as the full study reports are not available.

23.4.1.1.4 Toxicity to reproduction

The potential reproductive and developmental effects of cC604 cyclic was investigated in 10 male and 10 female rats and on the development of offspring, when treated for at least 2 weeks before mating and throughout gestation and lactation periods, until Day 3 post-partum (as reported in the ECHA registration dossier). Males received doses of 0, 5, 20 and 60 mg/kg bw/day, and females received doses of 0, 5, 20, and 80 mg/kg bw/day. The potential for liver toxicity was evaluated at a lower dose of 0.5 mg/kg bw/day in a satellite group of 5 males and 5 females. The study design was based on the procedures described in OECD guideline number 421 (Reproduction/ Developmental Toxicity Screening Test) and performed in compliance with GLP. A NOAEL of 20 mg/kg bw/day for reproductive and developmental toxicity was identified by the study authors based on adverse effects seen in the high dose females consisted of reductions in the number of implantation sites and of corpora lutea. At parturition, litters were lost by cannibalism or were smaller in size and weight, before total loss by day 1. Reductions in mean total and individual litter weights (when calculable) were the main treatment-related effects observed in dams dosed at 80 mg/kg/day in this study.

In a GLP-compliant prenatal developmental toxicity study performed according to OECD Guideline 414, cC604 diluted in water was administered by gavage to groups of mated female Sprague-Dawley rats (24 mated females/dose) at doses of 0, 5, 20, 60 and 80 mg/kg bw/day (in terms of dry salt (39.8 %)) from Days 6 to 19 post-coitum (as reported in the ECHA registration dossier).[4] All mated animals were pregnant. No mortality was observed. No relevant clinical signs or signs of reaction to treatment were noted in treated females. No differences of toxicological relevance were noted in body weight, food consumption, gravid uterus weight and litter data of treated females when compared to controls. Swollen and/or enlarged liver was detected at macroscopic examination in some females dosed at 80 mg/kg bw/day, indicating the presence of maternal toxicity at this dose level. Given the absence of a dose-related effect, the findings detected at the external, visceral and skeletal examination of foetuses from all groups were considered by the study author to be incidental. On the basis of the results obtained in this study, the dose of 60 mg/kg bw/day was considered to be the NOAEL (No Observed Adverse Effect Level) for maternal toxicity. The NOAEL for developmental toxicity was considered to be 80 mg/kg bw/day – this being the highest dose tested in this study.

Reliability of the data cannot be assessed as the full study reports are not available.

23.4.1.1.5 Other (endocrine, neurotoxicity, immunotoxicity...)

Rat thyroid cell lines (FRTL5) and normal human thyroid cells (NHT) were incubated with increasing concentrations of C6O4 for 24, 48, 72, and 144 hours to assess cell viability at increasing concentrations of C604 (Coperchini et al., 2021). Long chain PFAS (PFOA and PFOS) were used as positive controls. Various end points were considered including cell viability,

proliferation, induction of apoptosis, and reactive oxygen species (ROS) production. C604 did not exert an adverse effect on the cultured rat and human thyroid cells. In comparison the PFAS reduced cell viability. The study authors concluded that C604 is unlikely to be a safety concern for thyroid cells in vitro.

23.4.2 Relevant Human data

23.4.2.1 New data

No relevant data was found.

23.5 Proposal for a starting point for deriving a drinking water limit value

23.5.1 Selected studies

Based on the available studies for repeated dose toxicity and reproductive/developmental toxicity endpoints, there are three studies which yielded results that could potentially be used for the identification of a point of departure for the derivation of a drinking water limit value. However, for all of these studies all of these studies were assigned a Klimisch score of 4 (not assignable) and the amount of information that is reported is limited. Therefore, the reliability of this information cannot be checked against the original study data and the validity of this data is unclear.

The repeated dose toxicity study was conducted using cC604 (CAS RN: 1190931-27-; EC: 682-238-0) in a GLP-compliant sub-chronic repeated dose oral toxicity study conducted according to OECD Guideline 408. The substance was administered daily by oral gavage to groups of Sprague-Dawley rats (10/sex/dose), for 13 weeks at the doses of 0 (vehicle), 0.3, 1, 3 or 10 mg/kg bw/day. A No Observed Adverse Effect Level (NOAEL) of 3 mg/kg bw/day in females and 1 mg/kg bw/day in males was identified by the study authors based on changes in clinical pathology parameters and post-mortem findings. The liver was identified as a target organ on the basis of organ weight data and histopathological findings. A second target organ appeared to be the thyroid, where changes were considered to be a secondary effect of the liver toxicity. These effects showed a dose-related trend and appeared to be only partially reversible after the 6-week recovery period. The Lowest Observed Adverse Effect Level (LOAEL) was considered to be 3 and 10 mg/kg bw/day for males and females, respectively.

The second study providing information for the identification of a POD for the derivation of a drinking water limit value was a screening study conducted using cC604 (CAS RN: 1190931-27-; EC: 682-238-0) based on the procedures described in OECD guideline number 421 (Reproduction/ Developmental Toxicity Screening Test) and performed in compliance with GLP. Ten male and 10 female rats were included in the study treated for at least 2 weeks before mating and throughout the gestation and lactation periods, until Day 3 post-partum. Males received doses of 0, 5, 20 and 60 mg/kg bw/day, and females received doses of 0, 5, 20, and 80 mg/kg bw/day. The potential for liver toxicity was evaluated at a lower dose of 0.5 mg/kg bw/day in a satellite group of 5 males and 5 females. A NOAEL of 20 mg/kg bw/day for reproductive and developmental toxicity was identified by the study authors based on adverse effects seen in the high dose females consisted of reductions in the number of implantation sites and of corpora lutea. At parturition, litters were lost by cannibalism or were smaller in size and weight, before total loss by day 1. Reductions in mean total and individual litter weights (when calculable) were the main treatment-related effects observed in dams dosed at 80 mg/kg/day in this study.

The third study provides data from a GLP-compliant prenatal developmental toxicity study performed according to OECD Guideline 414 in which cC604 diluted in water was administered by gavage to groups of mated female Sprague-Dawley rats (24 mated females/dose) at doses of 0, 5, 20, 60 and 80 mg/kg bw/day (in terms of dry salt (39.8 %)) from Days 6 to 19 post-coitum. All mated animals were pregnant. No mortality was observed. No relevant clinical signs or signs of reaction to treatment were noted in treated females. No differences of toxicological relevance were noted in body weight, food consumption, gravid uterus weight and litter data of treated females when compared to controls. Swollen and/or enlarged liver was detected at macroscopic examination in some females dosed at 80 mg/kg bw/day, indicating the presence of maternal toxicity at this dose level. Given the absence of a dose-related effect, the findings detected at the external, visceral and skeletal examination of foetuses from all groups were considered by the study author to be incidental. On the basis of the results obtained in this study, the dose of 60 mg/kg bw/day was considered to be the NOAEL (No Observed Adverse Effect Level) for maternal toxicity. The NOAEL for developmental toxicity was considered to be 80 mg/kg bw/day – this being the highest dose tested in this study.

Reliability of the data cannot be confirmed as the full study report are not available.

23.5.2 Proposal of assessment factors/modification factors with justification

23.5.2.1 Repeated Dose Toxicity Study in Rats according to OECD TG 408 (NOAEL 1 mg/kg bw/day)

An overall assessment factor of 200 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF of 1: As the dose descriptor is the NOAEL, the default assessment factor is 1.
- AF of 2: To account for the use of a point of departure taken from a sub chronic (90 day) study to calculate a chronic limit value.
- AF of 4: Allometric scaling to account for differences in allometry in using the rat as a test model.
- AF of 2.5: To account for interspecies toxicodynamic differences.
- AF of 10: To account for intraspecies toxicodynamic differences.
- AF of 1: The database has good quality studies, taking into account completeness, consistency and the standard information requirements, therefore the default factor of 1 applies (however, the project team has no access to the full study report).

23.5.2.2 Reproductive and Developmental Toxicity Screening Study in Rats according to OCED TG 421 (NOAEL 20 mg/kg bw/day)

An overall assessment factor of 100 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF of 1: As the dose descriptor is the NOAEL, the default assessment factor is 1.
- AF of 1: To account for the use of a point of departure taken from a reproductive toxicity study to calculate a chronic limit value. Timescales are not applicable since reproductive and

developmental toxicity does not get worse with longer exposure but is relevant to a critical time window and the experimental exposure adequately covers the pregnancy of the species under investigation.

- AF of 4: Allometric scaling to account for differences in allometry in using the rat as a test model.
- AF of 2.5: To account for interspecies toxicodynamic differences.
- AF of 10: To account for intraspecies toxicodynamic differences.
- AF of 1: The database has good quality studies, taking into account completeness, consistency and the standard information requirements, therefore the default factor of 1 applies (however, the project team has no access to the full study report).

23.5.2.3 Pre-Natal Developmental Toxicity Study in Rats according to OCED TG 414 (NOEAL 60 mg/kg bw/day)

An overall assessment factor of 100 is proposed with the following justifications for its derivation (please refer to information below).

According to the ECHA guidance, an assessment factor can be calculated as follows:

- AF of 1: As the dose descriptor is the NOAEL, the default assessment factor is 1.
- AF of 1: To account for the use of a point of departure taken from a prenatal developmental toxicity study to calculate a chronic limit value. Timescales are not applicable since reproductive and developmental toxicity does not get worse with longer exposure but is relevant to a critical time window and the experimental exposure adequately covers the pregnancy of the species under investigation.
- AF of 4: Allometric scaling to account for differences in allometry in using the rat as a test model.
- AF of 2.5: To account for interspecies toxicodynamic differences.
- AF of 10: To account for intraspecies toxicodynamic differences.
- AF of 1: The database has good quality studies, taking into account completeness, consistency and the standard information requirements, therefore the default factor of 1 applies (however, the project team has no access to the full study report).

24 Relative potency factors developed for PFAS

24.1 Introduction

The relative potency factor (RPF) approach has already been applied to various classes of chemicals (e.g.,(Bosgra et al., 2009)). (Bil et al., 2021) proposed the concept as a screening tool for the assessment of dietary cumulative exposure to PFAS. The RPF is defined as the ratio of the benchmark dose (BMD) of the PFAS index compound (i.e., PFOA) and the BMD of any other PFAS. Subsequently the individual PFAS concentrations per sample are multiplied by their related RPF to obtain the concentration in PFOA equivalents. Against this background, it is possible to compare the sum of all PFOA equivalents to an available drinking water concentration limit or fish consumption limit (Niegowska et al., 2021). One of the biggest challenges facing risk assessors dealing with PFAS is they tend to be found in the environment as diverse mixtures comprising a very large number of compounds for which there is little or no data. The RPF approach is intended to help with that challenge. RPF methodology provides means to assess the risk resulting from mixture exposure. It predicts the combined effect via a common mode of action resulting from exposure to a mixture of chemicals by dose (or concentration) addition, taking potency into account. This methodology is an elaboration of the principle that the cumulative effects of chemicals that contribute to one common toxicological effect can be assessed by dose addition. This methodology has been successfully applied to several other classes of chemicals, such as dioxins and dioxin-like PCBs, organophosphorus, and N-methyl carbamate pesticides (Bosgra et al., 2009). In the RPF approach, the toxic potencies of a set of compounds are expressed relative to the toxic potency of the index compound.

The aim was to provide an extensive literature research on assessments of PFAS using relative potency factors, the description, analysis and critical evaluation of the identified studies calculating RPF for PFAS, analysis of applicability of RPF concept on the group of PFAS in scope of the project, and recommendation for future assessments according to the RPF concept.

24.2 Overview on publications on RPFs

The summary of all studies identified in the literature research and their basic description is presented in Table 84. A recent assessment of RPFs for PFAS has been published by (Zeilmaker et al., 2018) deriving putative RPFs based on data for several liver toxicity endpoints. The results from this report were published later by (Bil et al., 2021). Following to this study RPFs were derived also for internal exposure which could be applied to PFAS concentrations in human blood (Bil et al., 2022). The second recent publication, by Luz et al. (2019) (referenced in (Standards, 2019), investigated potential RPFs based on liver, kidney and body weight effects from the NTP dataset. (Standards, 2019) further explored the utility of the NTP dataset for comparative potency evaluation. For that the authors used more robust dose metrics and benchmark dose analysis with a focus on two sensitive endpoints, namely thyroid hormone and liver effects. One study was found developing the relative potency factor based on *in vitro* toxicity data established for thyroid hormone transport disruption potential (Behnisch et al., 2021).

Table 84: Summary of studies presenting relative potency factors for specific effects and PFAS

Reference	Index compound	PFAS	Health effect/endpoint	Type of study used	RPF calculation
(Bil et al., 2021; Zeilmaker et al., 2018)	PFOA	PFBS, PFHxS, PFOS, PFBA, PFHxA, PFOA, PFNA, PFUnDA, PFDoDA, PFTeDA, PFHxDA, PFODA, HFPO-DA, ADONA, 6:2 FTOH, 8:2 FTOH	liver effects (absolute liver weight, relative liver weight, and liver hypertrophy)	Subchronic *in vivo*	Dose-response modelling (PROAST) - The BMDs correspond to a benchmark response (BMR) of 5% increase in absolute and relative liver weight or to 10% extra risk of liver hypertrophy
(Bil et al., 2021; Zeilmaker et al., 2018)	PFOA	PFPeS, PFHpS, PFDS, PFPeA, PFHpA, PFDA, PFTrDA	liver effects	read-across[10]	read-across
Luz et al. 2019 (Standards, 2019)	PFOA	PFOS, PFNA, PFHxS, PFDA, PFHpA	liver effect (relative liver weight)	Subchronic *in vivo*	Benchmark Dose lower
(Standards, 2019)	PFOA	PFOS, PFNA, PFHxS, PFDA, PFHpA	liver effect (relative liver weight), thyroid effect (free thyroxine)	Subchronic *in vivo*	Bayesian Benchmark Dose - The BMDs correspond to a BMR of 5% in relative liver weight or to 20% for free thyroxine
(Behnisch et al., 2021)	PFOA	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS,	thyroid disruption	*In vitro* study	Potency factor based on PC80: Thyroid disruption - the T4 binding to TTR

[10] No additional information

Reference	Index compound	PFAS	Health effect/endpoint	Type of study used	RPF calculation
		PFOS, PFDS, H4PFOS, PFOSA			
(Bil et al., 2021; Zeilmaker et al., 2018)	PFOA	PFBA, PFHxA, PFNA, PFDoDA, PFOS, HFPO-DA, GenX, PFBS, PFHxS	liver effects	Subchronic *in vivo*, Toxicokinetic model (internal doses)	Dose-response modelling (PROAST)

(Bil et al., 2021) developed on the basis of liver toxicity endpoints potential RPFs for several PFAS. The study identified subchronic studies for 12 PFAS, which show a carbon chain length from C4 to C18. In addition, the RPFs of 7 other perfluoroalkyl acids were estimated based on read across. The authors report as identified common endpoints measures of liver effects, including increases in absolute and relative liver weight and hepatic hypertrophy. For the calculation of BMDs at 5% increases for absolute and relative liver weight or at 10% extra risk for liver hypertrophy using external applied dose (mg/kg-day) as the dose metric fitted dose-response function for each liver effect was used. Division of the BMD for the index chemical PFOA by the BMDs for each of the other PFAS led to reported RPFs. For seven PFAS, insufficient data are reported so that "read across" values were used.

Luz et al. (2019) (Standards, 2019) conducted a relative potency evaluation for seven PFAS tested in the NTP 28-day bioassays (NTP, 2018). Due to a poor model fit for the internal dose metric they calculated RPFs for hepatocellular hypertrophy, kidney weight, liver weight, cholesterol, body weight and reticulocyte count using BMDLs based on applied dose (mg/kg-day). They derived RPFs for five PFAS, which are of relevance for this report. For relative liver weight, the RPF for PFOS was 4, and for both PFNA and PFDA the RPF was 2 compared to PFOA. As a partly explanation for the variability in these results, the authors indicate the use of an external dose metric and the BMDL, rather than the BMD. However, the reported RPF estimates do not show such a difference that allow the conclusion that potencies differ significantly across the compounds.
Based on the assessment of Lutz et al. (2019), (Standards, 2019) Massachusetts Department of Environmental Protection, Office of Research and Standards (MassDEP ORS) further explored the potential utility of the data from the 28-day rat bioassays (NTP, 2018) for deriving RPF for PFOA, PFOS, PFNA, PFHxS and PFDA. For that internal dose and human equivalent dose metrics were used with Bayesian benchmark dose evaluation. The MassDEP ORS identified free thyroxine (fT4) serum concentration and relative liver weight in male rats as the most sensitive endpoints from this data set. This endpoint was selected for subsequent dose-response evaluation and relative potency comparison.

Relative potency factors were also established by (Behnisch et al., 2021) based on results from *in vitro* toxicity test. In the study thyroid hormone transport disruption potential using TTR-TRβ CALUX® bioassay were established for major PFAS. Different technical PFAS mixtures, including aqueous film-forming foam (AFFF) surfactants and chromium mist suppressants (CMS) applications with and without total oxidizable precursor (TOP) were assessed for their thyroid

hormone transport disrupting potential using the TTR-TRβ CALUX® assay. It was confirmed that all PFAS affected the T4 binding to TTR, which is an important plasma thyroid hormone transport protein.

(Bil et al., 2022) derived RPFs at the blood serum level. These RPFs can be applied within mixture risk assessment with primary input data from human biomonitoring studies. The internal exposure in the male rat at the blood serum level has been calculated by using toxicokinetic models for 10 PFAS. By applying dose-response modelling, these internal exposures are used to derive quantitative internal RPFs based on liver effects.

Table 85: Summary of relative potency factors for specific effects and PFAS

PFAS	(Bil et al., 2021; Zeilmaker et al., 2018)	(Behnisch et al., 2021)	Luz et al. 2019 (Standards, 2019)	(Standards, 2019) liver	(Standards, 2019) fT4	(Bil et al., 2022)
PFBS	0.001	0.052	NI	NI	NI	0.2
PFPeS	0.001 ≤ RPF ≤ 0.6	NI	NI	NI	NI	NI
PFHxS	0.6	1.6	0.5	0.2	0.5	0.6
PFHpS	0.6 ≤ RPF ≤ 2	1	NI	NI	NI	NI
PFOS	2	2	4	1	3	3
PFDS	2	NI	NI	NI	NI	NI
PFBA	0.05	0.0018	NI	NI	NI	2
PFPeA	0.01 ≤ RPF ≤ 0.05	0.080	NI	NI	NI	NI
PFHxA	0.01	0.19	NI	NI	NI	10
PFHpA	0.01 ≤ RPF ≤ 1	1.4	NI	NI	NI	NI
PFOA	1	1	1	1	1	1
PFNA	10	0.32	2	1	2	5
PFDA	4 ≤ RPF ≤ 10	0.12	2	2	1	NI
PFUnDA	4	0.052	NI	NI	NI	NI
PFDoDA	3	0.010	NI	NI	NI	10
PFTrDA	0.3 ≤ RPF ≤ 3	0.075	NI	NI	NI	NI
PFTeDA	0.3	0.019	NI	NI	NI	NI
PFHxDA	0.02	NI	NI	NI	NI	NI
PFODA	0.02	NI	NI	NI	NI	NI
HFPO-DA	0.06	NI	NI	NI	NI	9

PFAS	(Bil et al., 2021; Zeilmaker et al., 2018)	(Behnisch et al., 2021)	Luz et al. 2019 (Standards, 2019)	(Standards, 2019) liver	(Standards, 2019) fT4	(Bil et al., 2022)
ADONA	0.03	NI	NI	NI	NI	NI
6:2 FTOH	0.02	NI	NI	NI	NI	NI
8:2 FTOH	0.04	NI	NI	NI	NI	NI
H4PFOS	NI	0.019	NI	NI	NI	NI
PFOSA	NI	0.72	NI	NI	NI	NI

24.3 Evaluation of currently published RPFs

In the open literature the most discussed and applied RPFs are those established by (Bil et al., 2021). The approach has sparked a debate in the letters pages of the journal Environmental Toxicology and Chemistry, with scientists funded by the National Association of Water Companies in the Netherlands saying that the RPF values have "not yet been sufficiently tested for robustness to be used in risk assessment". The Dutch scientists criticise the recommendation to use the RPFs proposed by (Bil et al., 2021) in risk assessment strategies. They argue it is difficult to defend as the values themselves are not robust enough for direct application in risk assessment. The proposed RPF values are based on liver toxicity in rats and that it may be relevant to validate whether they are relevant to other endpoints. This is important given that the current EFSA TWI is based on an immune effect in humans as the critical effect (Rietjens et al., 2022). There are additional items to consider: the toxicity data derived from different studies under different conditions e.g., different laboratories with different strains of rats, dosing via the diet versus oral gavage etc. Further available data on other endpoints needs to be considered as well as other points of departure, the shape of dose-response curves and available human data. At present, it was concluded that the RPF values now proposed by (Bil et al., 2021) have not yet been sufficiently tested for robustness to be used in risk assessment.

For the majority of individual PFAS, little or no data on chemical properties and health effects are available. Hazard and risk assessments for PFAS are usually based on studies with representative lead compounds that have some toxicity and occurrence information such as perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), or perfluorononanoic acid (PFNA). In animals, the most consistent critical toxicity seen after PFAS administration is increased liver weight. A number of PFAS also affected circulating thyroid hormones (both T4 and T3) and induce developmental effects like increased foetal and/or neonatal mortality, reduction in foetal weight and/or postnatal growth, impaired development of mammary glands, and developmental neurotoxic effects (Colnot & Dekant, 2021).

These RPF are estimated based on applied dose. Therefore, the authors suggested differences in pharmacokinetics for the PFAS as a likely reason for some of the differences in the RPF estimates (Zeilmaker et al., 2018). Furthermore, they highlighted that the use of internal or human equivalent doses could yield improved potency comparisons.

Solid information on mode(s) of action (MoA) of PFAS is not available. While interaction of PFAS with the peroxisome proliferation receptorα (PPARα) has been implicated in the liver changes seen and is discussed as MoA for immune system effects, contradictory results question a sole

contribution of PPARα-mediated events to the liver effects. It is also unknown if this MoA accounts for any other of the toxic effects of PFAS. Different PFAS show a wide range of potencies and it is unknown if potencies established for one adverse effect translate to equal potencies for other adverse effects (Goodrum et al., 2021). In addition, large differences in elimination rates between individual PFAS and major differences between species with elimination half-lives in the range of years in humans for PFOS and PFHxS are present (Goodrum et al., 2021; Pizzurro et al., 2019). PFAS toxicokinetic properties in humans and animals may lead to high levels of uncertainty in the quality standards development.

While relative potency factors (RPFs) for some individual PFAS have been derived based on liver weight increases using either external dose or serum concentrations as metric, a number of simplifications had to be integrated and it remains unknown if the RPFs are applicable to other toxicity endpoints (Bil et al., 2021; Goodrum et al., 2021). (Goodrum et al., 2021) considered concentration addition and RPF based methods as inappropriate at this time for PFAS risk assessment due to dissimilarities in the dose-response curves.

Perhaps the greatest challenge of trying to understand PFAS mixtures is that there are thousands of PFAS for which there is little or no information about their individual toxicity or mode of action. In a thorough review and analysis related primarily to mammalian toxicity, (Goodrum et al., 2021) examined PFAS mixtures and concluded that short- and long-chain perfluorinated sulfonates, perfluorinated carboxylates, and fluorotelomer alcohols can interact with nearly two dozen nuclear receptors, indicating multiple MOAs.

In order for the European Commission to prepare policy and proposals related to consumer safety, health and the environment, two independent scientific committees are in place to provide it with scientific advice. One of those, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) gives opinions on questions concerning emerging or newly identified health and environmental risks. The Commission asked SCHEER to evaluate the proposed quality standards for PFAS presented in an Environmental Quality Standards (EQS) dossier drafted by the Joint Research Center (JRC), which is the science and knowledge centre of the European Commission. The SCHEER was also asked to assess the RPF approach presented in the JRC draft dossier. The SCHEER experts agreed on the approach presented by JRC. Stakeholders have however expressed their concern for the uncertainty using the RPF method as basis for Quality Standards since they do not cover relative potency on immune effects which is the basis for the EFSA TWI for PFAS. Additionally, stakeholders argue against the reference supporting RPFs (Bil et al., 2021)as they are only related to a single endpoint and just recently published. In the comments, stakeholders wrote that: “More validation is needed, as stated by the authors themselves. In particular, as the draft EQS proposal notes, extension of the RPFs to other toxicological endpoints, or ecological endpoints, has not been validated”. It is true that immune effects are not available or known for some of the PFAS reported in the dossier. However, it does not impair the consideration that the evaluated PFAS are not equipotent with regard to immune effects either. Similar potency differences were observed for endpoints other than liver toxicity, such as different organ weights, hormone levels, clinical chemistry, white blood cell parameters and pathology endpoints. Therefore, this might also be applicable to immune effects. Re the RPF, the implementation of this methodology, would allow to measure PFAS quantities, resulting in the sum of PFOA equivalents in a mixture, so far, this would include 24 PFAS. It has been already implemented for other substances (e.g. dioxins, PCB) in the legal framework (SCHEER, 2022).

In conclusion, the SCHEER considers the RPF approach as the currently best available means for deriving EQSs. EFSA (2013) was the first to introduce the concept of RPFs without the prerequisite of grouping chemicals based on a common mode of action but rather based on the

same target organ because information on mode of action is often lacking. The committee highlights that different sets of RPFs are available (Bil et al., 2021; Gomis et al., 2018) and that each set will obviously lead to different outcomes. It is therefore recommended to scrutinize any new data sets that may become available to see if they can be used as basis for deriving additional RPF.

24.4 Comparison of toxicological data for PFAS

The RPF method has an important condition, namely a set of comparable toxicity data for individual PFAS. Therefore, the objective of the update was to review the current toxicological and epidemiological research data for 16 PFAS and 4 substitutes. According to RPF methodology, grouping of PFAS should be based on common toxic MOAs and/or target organs. Furthermore, only those PFAS that affect the same target organ/tissue/system should be grouped and assessed for dose additive, or response additive approaches. Thus, the toxicity data of PFAS was screened (out of liver effects) and discussed, if possible, a toxicological framework for applying RPF to the mixture of these compounds.

Table 86: Summary of toxicological data relevant for defining relative potency factors

PFAS	Critical specific effect	Exposure duration	Species	NOAEL (external)	Reference
PFBA	increased incidence of follicular hyperplasia hypertrophy of the thyroid gland hepatocellular hypertrophy	90 days	Sprague Dawley rats	6 mg/kg bw/day	(J. L. Butenhoff, Bjork, et al., 2012)
	increased number of dams with full foetal resorption	GD1-18	CD1 mice		(Das et al., 2008)
PFPeA	no relevant studies identified				
PFHxA	low urine pH values	2 years	Sprague Dawley rats	15 mg/kg/bw/day	(Klaunig et al., 2015)
	lower red blood cell parameters, higher reticulocyte counts and reduced globulin content, increased liver enzyme values		Crl:CD(SD) rats	50 mg/kg bw/day	(Chengelis, Kirkpatrick, Myers, et al., 2009)
	nasal lesions	90 days	Sprague Dawley rats	20 mg/kg bw/day	(Loveless et al., 2009b)
	reduced body weight	70 days before cohabitation to throughout pregnancy and lactation for a total of 4 months	Rats	20 mg/kg bw/day	(Loveless et al., 2009b)

PFAS	Critical specific effect	Exposure duration	Species	NOAEL (external)	Reference
	body weight effects	GD6-GD20	Rats	100 mg/kg bw/day	(Loveless et al., 2009b)
	stillbirths as well as mortality and reduced body weights	6th to 18th of pregnancy		95 mg/kg bw/day	(Iwai & Hoberman, 2014a)
	developmental toxicity end points (incidence of stillborn pups)	GD6-GD18	Crl:CD-1(ICR) mice	175 mg/kg bw/day	(Iwai et al., 2019)
PFHpA	increase of serum testosterone, luteinizing hormone and follicle-stimulating hormone levels sperm production suppressed Leydig cells hyperplasia	21 days	Rats	-	(Z. Li et al., 2021)
	liver-related biochemical markers in the blood liver changes - hepatocellular necrosis	90 (109) days	CD1 mice	0.5 mg/kg/day (LOAEL)	Anonymous (2017)
PFDA	decreases in maternal body weight increased liver weights	GD6-GD21	Mice	1 mg/kg/day (LOAEL)	(M. W. Harris & Birnbaum, 1989)
	hepatic necrosis liver enlargement	28 days	Harlan Sprague-Dawley rats	0.125 mg/kg/day	(Frawley et al., 2018)
	liver enlargement immune cell decrease	28 days	B6C3F1/N mice	0.625 mg/kg/day (LOAEL)	(Frawley et al., 2018)
PFUnDA	centrilobular hypertrophy of hepatocytes	42 days	Crl:CD(SD) rats	0.3 mg/kg bw/day	(Takahashi et al., 2014)
	reduced serum of testosterone, luteinizing hormone (LH) levels, Leydig cell (LC) numbers, body weight and weights of the testes and epididymis	PND35 - PND56	Sprague-Dawley rats	1 mg/kg bw/day (LOAEL)	(Yan et al., 2021)
	reduced body weight, relative epididymis weight, the relative testis weight, serum T	28 days	Sprague-Dawley rats	-	(Xin et al., 2022)

PFAS	Critical specific effect	Exposure duration	Species	NOAEL (external)	Reference
	level, Leydig cell number				
PFDoDA	decreases in male and female body weights and food consumption increases in relative liver weight	42 – 47 days	Crl:CD(SD) rats	0.1 mg/kg/day	(Kato et al., 2015)
	not affect the endocrine status but may impact oestradiol production	28 days	Rats	-	(Z. Shi, Zhang, et al., 2009)
	sperm activity and testicular function	110 days	Rats	-	(Z. Shi et al., 2013)
	inhibition of testicular steroidogenesis	110 days	Rats	-	(Z. Shi et al., 2010)
	hepatoxicity	110 days	Rats	0.02 mg/kg bw/d (LOAEL)	(Ding et al., 2009)
	hepatoxicity	110 days	Rats	-	(H. Liu et al., 2016)
PFTrDA	inhibition of the differentiation of foetal Leydig cells	GD14 – GD21	Rats	1 mg/kg bw (LOEL)	C. Li et al. 2021
PFBS	haematological effects	90 days	Rats	60 mg/kg bw d	(Lieder, Chang, et al., 2009)
	decreases in plasma triglycerides, non-HDL-cholesterol, and an increased excretion of (radioactively labeled) triolein	6 weeks	Mice	-	(Bijland et al., 2011)
	increased liver weights, occurrence of adaptive hepatocellular hypertrophy	10 weeks	Rats	100 mg/kg bw ·d	Lieder, York, et al. 2009
	decreases in total triiodothyronine (T3), total thyroxine (T4), and free T4 increased absolute and relative right kidney, liver weights hepatocellular hypertrophy	28 days	Sprague Dawley rats	-	(NTP, 2022)
	hypothyroxinemic accompanied by deficits	GD 1-20	ICR mice	-	(Feng et al., 2017)

PFAS	Critical specific effect	Exposure duration	Species	NOAEL (external)	Reference
	in prenatal growth, pubertal onset, and reproductive organ development				
PFPeS	no relevant studies identified				
PFHpS	no relevant studies identified				
PFNS	no relevant studies identified				
PFDS	no relevant studies identified				
PFUnDS	no relevant studies identified				
PFDoDS	no relevant studies identified				
PFTrDS	no relevant studies identified				
GenX	Changes in blood parameters, increases in albumin and A/G ratio, reduction in cholesterol and globulin	28 days	Rats	0.3 mg/kg bw/day	Haas, 2008a
	Decrease in globulin and an increase in A/G ratio in both sexes, and reduced haemoglobin, haematocrit	28 days	Rats	0.1 mg/kg bw/day	Haas, 2008b
	Increased relative kidney weight	90 days	Rats	0.1 mg/kg bw/day	Haas, 2009
	Body weight decrements in the F1 males	70 days	Mice	0.5 mg/kg bw/day	Edwards, 2010a
	Mortality, lower mean body weight, food consumption	GD6-GD20	Rats	10 mg/kg bw/day	Edwards, 2010b
	Immune effects	28 days	Mice	10 mg/kg bw/day	Rushing et al., 2017
ADONA	Maternal and developmental	GD0-GD24	Rats	30 mg/kg bw/day	Gordon, 2011
6:2 FTSA	Decreases in mean body weight and weight gain	14 days	Mice	30 ppm	REACH registration dossier
	Reduction in heart weight	90 days	Rats	5 mg/kg bw/day	REACH registration dossier

PFAS	Critical specific effect	Exposure duration	Species	NOAEL (external)	Reference
C604	Changes in clinical pathology parameters	13 weeks	Rats	3 mg/kg bw/day	REACH registration dossier
	Thyroid effects	13 weeks	Rats	3 mg/kg bw/day (LOAEL)	REACH registration dossier
	Reductions in the number of implantation sites and of corpora lutea	2 weeks before mating and throughout gestation and lactation periods, until Day 3 post-partum	Rats	20 mg/kg bw/day	REACH registration dossier

The following table summarises the adverse effects identified within the literature screening and particular PFAS with this kind of effect.

Table 87: Grouping of PFAS per adverse effect

Adverse effect	PFAS
↓ T, LH	PFUnDA, PFOA, PFOS
↑T, LH, FSH	PFHpA
↓T3, T4, fT4	PFBS, PFOA, PFHxS, PFOS
↓ Sperm production, activity	PFHpA, PFDoDA, PFOS
↓ Leydig cells	PFUnDA, PFHpA, PFTrDA, PFOA, PFOS
Testes weight	PFUnDA, PFOS
↓ BW effect[11]	PFHxA, PFDA, PFUnDA, PFBS, PFDoDA, PFOA. PFHxS, PFOS, GenX, 6:2 FTSA
↑ Incidence of follicular hyperplasia	PFBA
Hypertrophy of thyroid gland/Thyroid effects	PFBA, C604
↑ number of dams with full foetal resorption	PFBA
Low pH value	PFHxA
Haematological effect	PFHxA, PFBS, GenX
Immune effect	GenX

[11] BW effect in different target groups such as pups, maternal etc.

Adverse effect	PFAS
Nasal lesions	PFHxA
Reductions in the number of implantation sites and of corpora lutea	C604

As can be seen from the overview presented in the table, the substances affect a number of different parameters and endpoints. Thyroid hormone levels in blood are affected by a number of PFAS but not in a uniform way. Furthermore, effects on Leydig cells and/or testes and sperm were observed. However, it must be noted that there are a number of differences in the biology of the Leydig cells between rats and humans (Steinbach et al., 2015) casting doubt on the relevance of such changes observed in male rats for human risk assessment. Unspecific effects such as a lowered body weight gain were also observed after exposure to various PFAS in different target groups. It is concluded that, based on the overview of endpoints presented in table above, no grouping of substances should be performed.

24.5 Recommendation for future assessment using RPF approach

24.5.1 General recommendation from the open literature

The SCHEER endorses the use of RPFs used in the derivation of quality standards for PFAS for humans. The committee recognises that RPFs will, however, vary depending on the endpoint considered and therefore the SCHEER recommends monitoring the literature in order to signal possible new RPF data sets that may become available (SCHEER, 2022). In the study recently published by (Anderson et al., 2022) expert panellists identified the following data gaps that would need to be filled to conduct a PFAS mixtures risk assessment effectively and efficiently for drinking water exposure:

- Consensus on the relevant critical effects for multiple PFAS
- Mechanisms of toxicity for PFAS such that sub-groups can be constructed with common toxicological endpoints and mechanisms/modes of action
- Potency (dose-response) information for the PFAS of concern
- Data to test the dose additivity assumption
- The role of precursor PFAS and biotransformation pathways
- The contribution of exposure to PFAS from drinking water relative to other routes of exposure

The most critical data gaps identified were (1) exposure, (2) dose-response, and (3) mode of action studies. The panel recommended that future studies focus on these data gaps for individual PFAS. Future steps identified by the panel included the use of exposure information to guide the prioritization of testing PFAS with unknown toxicity profiles. This would also allow prioritization of PFAS sources that are resulting in potentially harmful exposures. Additionally, studies explicitly aimed to define the modes/mechanisms of action of key PFAS are necessary to inform grouping strategies with the assumption of additive risk. Finally, the panel concluded that while whole mixtures for PFAS are likely highly variable, whole mixture studies compared to index compounds could provide valuable information on relative risk. The expert panel

generally supported the approach for development of PFAS drinking water standards for PFAS grouping. Even though launching this concept, the group of experts considered this approach as a pragmatic solution for the current situation where data is lacking. Once more data on toxicological effects and mode of actions is available for more PFAS the concept would need to be further refined.

24.5.2 General recommendation from the contractor

The contractor agrees with the SCHEER committee's conclusion and supports the approach for developing PFAS drinking water standards for PFAS grouping. However, based on the current state of the art of toxicological data for PFAS under this project's scope, new grouping of PFAS does not seem feasible. Thus, the recommendation is to use the RPFs established by Bil et al. 2021 until new RPF data sets may become available.

It should be noted that the RPF approach *"relies on the assumption that the rat-to-human conversion factor for differences in kinetics (e.g., absorption fractions, elimination rates, volumes of distribution) for the other PFAS is equal to that of PFOA."* (Bil et al., 2021). Toxicokinetic data for humans are only available for very few PFAS (e.g., PFHxA). Data from additional kinetics studies on more PFAS allowing for the use of substance-specific data would improve the database for the risk assessment of PFAS.

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A Annex A

A.1 Search strategy for PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFBS

The following search term was used:

(*substance name* OR *synonym*) AND ("health effect" OR "adverse effect" OR "mode of action" OR "cohort" OR "adverse health" OR reprotox* OR toxicokin* OR toxic* OR neurotox* OR expos* OR epidem* OR genotox* OR genetic* OR carc* OR "ADME" OR "PBPK" OR reproduc* OR disease OR neurologic* OR metabol* OR excret* OR "in vivo" OR developmental OR endocrine OR "thyroid disruption" OR "half-life" OR immun* OR "birth weight" OR MOA)

The search was limited to title and abstract.

Time frame: 2017 – 05/2022

A.2 Search strategy for PFUnDA, PFDoDA, PFTrDA, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS

The following search term was used:

(*substance name* OR *synonym*) AND ("health effect" OR "adverse effect" OR "mode of action" OR "cohort" OR "adverse health" OR reprotox* OR toxicokin* OR toxic* OR neurotox* OR expos* OR epidem* OR genotox* OR genetic* OR carc* OR "ADME" OR "PBPK" OR reproduc* OR disease OR neurologic* OR metabol* OR excret* OR "in vivo" OR developmental OR endocrine OR "thyroid disruption" OR "half-life" OR immun* OR "birth weight" OR MOA)

The search was limited to title and abstract.

Time frame: until 05/2022

A.3 Search strategy for PFOA, PFNA, PFHxS, PFOS

The following search term was used:

(*substance name* OR *synonym*) AND ("health effect" OR "adverse effect" OR "mode of action" OR "cohort" OR "adverse health" OR reprotox* OR toxicokin* OR toxic* OR neurotox* OR expos* OR epidem* OR genotox* OR genetic* OR carc* OR "ADME" OR "PBPK" OR reproduc* OR disease OR neurologic* OR metabol* OR excret* OR "in vivo" OR developmental OR endocrine OR "thyroid disruption" OR "half-life" OR immun* OR "birth weight" OR MOA)

The search was limited to title and abstract.

Time frame: 2020 – 05/2022

A.4 Search strategy for HPFO-DA (Gen-X), ADONA, 6:2 FTSA and C604

The following search term was used:

(*substance name* OR *synonym*) AND ("health effect" OR "adverse effect" OR "mode of action" OR "cohort" OR "adverse health" OR reprotox* OR toxicokin* OR toxic* OR neurotox* OR expos* OR epidem* OR genotox* OR genetic* OR carc* OR "ADME" OR "PBPK" OR reproduc* OR disease OR

neurologic* OR metabol* OR excret* OR “in vivo” OR developmental OR endocrine OR “thyroid disruption” OR “half-life” OR immun* OR “birth weight” OR MOA)

The search was limited to title and abstract.

Time frame: until 05/2022

A.5 Search strategy for RPFs

The following search term was used:

(PFAS OR PFC OR PFAA OR polyfluor* OR perfluor* OR organofluor* OR PFBA OR PFPeA OR PFHxA OR PFHpA OR PFDA OR PFUnDA OR PFUDA OR PFDoDA OR PFTrDA OR PFBS OR PFPeS OR PFHpS OR PFNS OR PFDS OR PFUnDS OR PFUDS OR PFDoDS OR PFTrDS OR PFOA OR PFOS OR PFNA OR PFHxS OR GenX OR ADONA OR FTSA OR C604) AND (RPF OR “relative potency”)

The search was limited to title and abstract.

A.6 Inclusion and exclusion criteria

Table 88: Inclusion and exclusion criteria for literature screening based on title/abstract

	Inclusion criteria	Exclusion criteria
PFAS	Relevant PFAS are included	Publication not related to PFAS (or specific PFAS types which are not relevant)
Publication type	Primary literature, Secondary literature (e.g. reviews)	Other types (e.g. corrigendum) without abstracts
Study type	Toxicological, toxicokinetic or epidemiological studies, general risk assessment on health effects	Environmental monitoring, monitoring in articles, analytical methods, ecotoxicity, biomonitoring (animals), biomonitoring (humans without epidemiological data), degradation studies, bioaccumulation, laboratory experiments, studies on removal from environment etc., studies related to dietary exposure
Pre-evaluation of toxicological studies	Species: mammalian species (e.g. rats, mice), zebrafish	Species: nonmammalian model systems, biochemical reactions (e.g. binding assay)
	Exposure: oral	Exposure: dermal, inhalation
	Study period: subacute to chronic	Study period: acute
Pre-evaluation of epidemiological studies	General population, workers	Tested group with already health/adverse effect

Table 89: Inclusion and exclusion criteria for the selection of a POD (full text screening)

	Inclusion criteria	Exclusion criteria
Publication type	Primary data	Review (will be checked for additional studies), secondary data
	English written	Documents not written in or translated to English
Endpoints	Endpoints related to clinical diagnostic criteria, disease outcomes, histopathological examination, genotoxicity, or other apical/phenotypic outcomes, liver (including serum lipids), developmental, reproductive, neurological, developmental neurotoxicity, thyroid disease/disruption, immunological, cardiovascular, and musculoskeletal outcomes.	Endpoints related to diseases outcomes or adverse effects relevant for environment
Toxicological studies	The study period - Sub-chronic (usually refers to a 90-day study), chronic study (usually refers to a 1.5-2 years study), sub-acute (this needs a case-by-case decision)	The study period - acute studies
	The way of exposure - oral studies	The way of exposure - dermal, inhale. studies
	Population - Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages).	Population - Nonmammalian model systems (e.g., fish, amphibians, birds, and Caenorhabditis elegans); studies of human or animal cells, tissues, or biochemical reactions (e.g., ligand binding assays) with in vitro exposure regimens; bioinformatics pathways of disease analysis; and/or high throughput screening data
	Studies reporting on animal-related outcomes relevant for humans	Studies reporting on animals with no statement of findings relevant for humans
	Dose testing - low doses (limit under 10 mg/kg bw); dose groups - at least 1 control and 2 dose (tested) groups,	Dose testing - high doses (higher than 100 mg/kg bw)
	Study design - in vivo, in vitro	Study design - in silico, read across analysis, review, systematic review
	Exposure - Specific PFAS substance tested	Exposure - Mixture(s)
	Outcome - quantitative measures (e.g. NOAEL, LOAEL, etc.)	Outcome - Qualitative characterisation of health outcome
Epidemiological part	Evidence proven for specific PFAS or PFAS group	Evidence proven effect of mixture of contaminants where PFAS is presented

	Inclusion criteria	Exclusion criteria
	Cohort studies, longitudinal studies, case control, prospective, retrospective, medical surveillance studies, case reports etc.	Other study design
	Outcome: exposure-response quantitative results must be presented in sufficient detail such as regression coefficients presented with statistical measure of variation such as RR, HR, OR, or SMR or observed cases vs. expected cases (common in occupational studies); slope or linear regression coefficient (i.e., per unit increase in a continuous outcome); difference in the means; or report means with results of t-test, mean comparison by regression, or other mean-comparing hypothesis test	log-transformed data, data not quantified (e.g. association only qualitative)
Exposure limit	Exposure limits set up only for drinking water	Other exposure limits e.g. air, OEL (Occupational Exposure Limit), etc.

A.7 Risk of Bias (ROB) analysis

The reliability of the publications is assessed according to the OHAT/NTP approach focusing on 9 questions (see next table) from which three have been identified as key questions for in vivo studies. These key questions are highlighted in brackets in the table and were chosen based on the identification of key criteria associated with the most sensitive aspects of the study relative to the overall risk of bias. For each study, an overall ROB appraisal conclusion was determined based on the responses. Therefore, studies determined to have the lowest possibility for risk of bias for the key questions would be the most reliable studies. Based on the risk of bias assessment, each study will be classified as Tier1, 2, or 3. Tier 1 studies will be those rated as "definitely low" or "probably low" risk of bias for the key questions, with most of the other ROB questions answered as "definitely low" or "probably low" risk of bias. A Tier 3 study will be a study appraised to be "definitely high" or "probably high" risk of bias for one or more of the key questions and have most of the other questions answered, "definitely high" or "probably high". Tier 2 studies are those studies that do not meet the requirements for Tier 1 or Tier 3. The algorithm used to combine the answers to the ROB appraisal questions and allocate each study to Tier1, 2, or 3 is presented in the table below.

Table 90: Overview of questions addressed in ROB analysis

Bias Domain	Question
Selection	1.Was the administered dose or exposure level adequately randomized? (key question) 2. Was allocation to study groups adequately concealed?

Bias Domain	Question
Performance	3. Were experimental conditions identical across study groups?
	4. Were the research personnel and human subjects blinded to the study group during the study?
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?
Detection	6. Can we be confident in the exposure characterization? (key question)
	7. Can we be confident in the outcome assessment? (key question)
Selective Reporting	8. Were all measured outcomes reported?
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?

Table 91: Explanation of figures for ROB response

Figure	Meaning
++	Definitely Low risk of bias: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)
+	Probably Low risk of bias: There is indirect evidence of low risk of bias practices OR it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
- or NR	Probably High risk of bias: There is indirect evidence of high risk of bias practices OR there is insufficient information (e.g., not reported or "NR") provided about relevant risk of bias practices
--	Definitely High risk of bias: There is direct evidence of high risk of bias practices (May include specific examples of relevant high risk of bias practices)
	Algorithm to combine ROB answers to ROB questions and allocate studies to Tiers
1 (low ROB)	TIER 1: All key questions are scored +/++ AND maximum 1 non-key question is scored - or --
2	TIER 2: study does not meet criteria for TIER 1 or TIER 3
3 (high ROB)	TIER 3: one (or more) key question is scored -/-- OR -/-- for the majority of the non-key questions

Table 92: ROB analysis of (Crebelli et al., 2019)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Mice
	Strain (source):	C57B1/6
	Sex:	Males

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Doses:	28 mg/l equivalent to 5 mg/kg body weight
	Purity (source)	Not given, Sigma–Aldrich
	Dosing Period:	5 weeks
	Route:	oral in drinking water
	Diet:	commercial laboratory animal feed (Rodent Laboratory Feed, Purina®)
	Controls:	MMS, positive control for genotoxicity assays, was administered by intraperitoneal (i.p.) injection at 40 mg/kg body weight for three times at 0, 24 and 45 h, and mice were sacrificed 3 h after last treatment. CCl4, positive control for lipid peroxidation, was given by i.p. injection 3 h before sacrifice at 1500 mg/kg body weight
	Funding source:	The Istituto Superiore di Sanità (ISS) funded by the Minister of Health
	Author conflict of interest:	No
Health Outcome	Endpoints:	Body weight, organ weight, lipid peroxidation, oxidative stress, biochemical changes in liver, cell death, genotoxicity, DNA damage
	Age at assessment:	11-13 weeks
	Number per group and sex:	Six males in treatment group and 8 males in control group
	Statistical analysis:	Yes, two-tailed Student's t-test. The correlation between results of manual scoring and flow cytometry analysis of micronuclei in reticulocytes was evaluated by Pearson's correlation.
	Control for litter effects:	NR
	Statistical power:	The limit for statistical significance was set at $P < 0.05$
Results	Tabular form based on studies reported results	No mortality or signs of overt toxicity, no changes in body weight gain, In liver: no increase in lipid peroxidation, oxidative stress, ALT and AST enzymes, apoptotic or necrotic cells, no DNA damage in somatic and testis cells, no increase in cytogenesis in reticulocytes and spleen lymphocytes, slight increase in liver weight, increase in plasma levels, bioconcentration factor of 0.7

ROB Questions and Responses - In Vivo Experimental Animal Study

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	NR	
	2. Was allocation to study groups adequately concealed?	NR	
Performance	3. Were experimental conditions identical across study groups?	Yes (+)	
	4. Were the research personnel and human subjects blinded to the study group during the study?	NR	
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	Yes (+)	
Detection	6. Can we be confident in the exposure characterization?	Yes (+)	Trace levels also in serum of untreated mice perhaps due to low level contamination of drinking water and/or animal feed (not investigated). Actual doses deviated less than 10% from calculated.
	7. Can we be confident in the outcome assessment?	Yes (+)	
Selective Reporting	8. Were all measured outcomes reported?	Yes (+)	
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	Yes (+)	
Overall Risk of Bias Appraisal for study:	Probably Low risk of bias		

Table 93: ROB analysis of (Kato et al., 2015)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Crl:CD(SD) (Atsugi Breeding Center, Charles River Laboratories Japan, Inc, Yokohama, Japan)
	Sex:	Male and Female
	Doses:	0, 0.1, 0.5, 2.5 mg/kg/day
	Purity (source)	97% (Exfluor Research Corporation, TX, USA, Lot No. 4103)
	Dosing Period:	Males: 42 days beginning 14 days before mating Females: 14 days prior to mating, throughout gestations and nursing until 5 days after delivery
	Route:	Oral gavage
	Diet:	fed ad libitum with a standard rat diet (CRF-1; Oriental Yeast, Tokyo, Japan) with free access to tap water (Sapporo, Japan).
	Controls:	Vehicle only (0.5% aqueous solution of carboxymethylcellulose sodium (CMC-Na; Maruishi Pharmaceutical, Osaka, Japan)
	Funding source:	Contract grant sponsor: Health and Labour Sciences Research Grant, Ministry of Health, Labour and Welfare, Japan. Contract grant numbers: H22-Kenki-Ippan-006, H25-Kenki-Ippan-007 Contract grant sponsor: Ministry of Health, Labour and Welfare, Japan
	Author conflict of interest:	Not reported
Health Outcome	Endpoints:	Repeated Dose Toxicity: Clinical observation, food consumption, body weight, functional observations (Sensorimotor reactivity to visual, tactile, auditory, pain, proprioceptive stimuli, and air righting reflex; Forelimb and hindlimb grip strength; Spontaneous motor activity), urinalysis, haematology, gross necropsy, histopathology Reproductive/Developmental Toxicity: Oestrous cycle, gestational length, copulation index, fertility index, gestation index, live and dead pups, live birth index, sex ratios, general pup appearance and behaviour, viability index on PND 4, gross external and internal observation, corpora lutea and implantation in uterus, implantation index and delivery index.

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Age at assessment:	10 weeks old at beginning of assessment; 42-day treatment
	Number per group and sex:	12
	Statistical analysis:	Kruskal-Wallis test for trend; Mann-Qwhitney U test to compare between dose and control, chi-square test for oestrus cycles, copulation, fertility, gestation indices, histopathological findings with single frade; chi-square two sample or Fisher's exact to compare between control and each dose group; Mean and standard deviations evaluated by the Varletts test for homogeneity of variances.
	Control for litter effects:	The live birth index, neonatal sex ratio, viability index, and body weight of male and female pups were similarly analysed using the litter as the experimental unit. When homogeneity was recognized ($p>0.05$), a one-way analysis of variance was applied and data without homogeneity ($p\leq0.05$) were subjected to the Kruskal-Wallis test. If a significant difference was identified ($p\leq0.10$), the Dunnett's test or the Mann-Whitney test was used for pairwise comparisons between the control and individual treatment groups.
	Statistical power:	All statistical analyses comparing the control and individual treatment groups were conducted using the 5% level of probability as the criterion for significance.
Results	Tabular form based on studies reported results	Liver hypertrophy, necrosis, and inflammatory cholestasis reported at 0.5 and 2.5 Body weight gain decreased at 2.5 mg/kg/day haematopoiesis in bone marrow decreased, atrophic changes in spleen, thymus and adrenal gland at 2.5 mg/kg/day Decreased spermatid and spermatozoa counts in male reproductive organs, and continuous diestrous in the females at 2.5 mg/kg/day Seven of twelve females receiving 2.5 mg/kg/day died during late pregnancy Authors reports NOAEL for repeated dose toxicity of 0.1 mg/kg/day and 0.5 mg/kg/day for reproductive/developmental toxicity. Four females did not deliver live pups at 2.5 mg/kg/day No reproductive or developmental parameters changed at 0.1 or 0.5 mg/kg/day

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Exposure groups were adequately randomized

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, lot number, sources were all provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported in detail.
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 1 - Low ROB		

Table 94: ROB analysis of (H. Zhang et al., 2008)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	male
	Doses:	0, 5, 10 mg/kg/day
	Purity (source)	> 99% (Sigma-Aldrich, USA)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Dosing Period:	14 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.5 % Tween-20)
	Funding source:	This research was funded by the National Natural Science Foundations of China (20677060 and 20777074) and the Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-128).
	Author conflict of interest:	Not reported
Health Outcome	Endpoints:	Serum and liver triglyceride, absolute and relative liver weights, expression of peroxisome proliferator-activated receptor (PPAR)-α and its target genes
	Age at assessment:	Not reported
	Number per group and sex:	10
	Statistical analysis:	Data were analysed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL) and presented as means with standard errors (mean±SE). Differences between the control and the treatment groups were determined using a one-way analysis of variance (ANOVA) followed by the Ducan multiple range test.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered as statistically significant
Results	Tabular form based on studies reported results	Absolute liver weight significantly decreased at 5 and 10 mg/kg/day Relative liver weight significantly increased at 5 and 10 mg/kg/day Serum and liver triglycerides significantly increased at 10 mg/kg/day Cholesterol significantly increased at 10 mg/kg/day Lipid droplet accumulation was observed in the cytoplasm of the hepatocytes of male rats exposed to PFDoDA, and the lipid droplets were larger in the higher dose PFDoDA groups The rough endoplasmic reticulum (rER) was broken and degranulated in the rats that had received 5 or 10 mg/kg/day. At doses of 10 mg /kg/day, the nuclear membranes became irregular and were surrounded by large lipid droplets

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		The expression of peroxisome proliferator-activated receptor (PPAR)-α and its target genes, and to a lesser extent PPARγ, was induced by PFDoDA.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	-	rats were not randomly divided into four groups but were separated into four groups of ten rats each according to mean body weight.
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 3 - High ROB		

Table 95: ROB analysis of (Greaves et al., 2013)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Li et al., 2013 BRAIN REGION DISTRIBUTION AND PATTERNS OF BIOACCUMULATIVE PERFLUOROALKYL CARBOXYLATES AND SULFONATES IN EAST GREENLAND POLAR BEARS (URSUS MARITIMUS)		
Reference, Animal model, dosing	Species:	Polar Bear
	Strain (source):	NR
	Sex:	Male and female
	Doses:	NA
	Purity (source)	NA
	Dosing Period:	NA
	Route:	NA
	Diet:	NA
	Controls:	NA
	Funding source:	Northern Contaminants Program (Indian and Northern Affairs Canada, to R.J. Letcher)
	Author conflict of interest:	NR
Health Outcome	Endpoints:	Brain distribution
	Age at assessment:	NR
	Number per group and sex:	13 males, six females

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	Statistical analysis:	Statistical analysis was done using Statistica 8.0 (StatSoft, 2008). For statistical analysis, all quantifiable PFAS concentrations were corrected for extractable lipid content in the samples (i.e., transformed from a wet wt concentration to a lipid wt concentration) and subsequently log10-transformed. Normality was tested using a Shapiro-Wilk's W test, with α=0.05. Prior to lipid correction, PFAS concentrations were not normally distributed. Following lipid correction and log10 transformation, the concentrations for the majority of PFSAs and PFCAs had a normal distribution. Statistical tests were not performed for PFASs where >50% of the samples were below the MLOQ. For all other compounds, where <50% of the samples were below the MLOQ, those samples below the MLOQ were assigned a lipid-normalized concentration of the MLOQ/3. The choice of MLOQ/3 was considered adequate after concluding that randomly generated values between 0 and MLOQ showed no statistical difference. Correlations between PFAS wet-weight concentrations and lipid content were assessed using product-moment correlative matrices, with p≤0.05 indicating statistical significance and a Pearson coefficient (r) ≥0.50 indicating a strong correlation. Multiple linear regression analysis was performed to test for confounding factors such as sex and age, with α=0.05.
	Control for litter effects:	NR
	Statistical power:	See above
Results	Tabular form based on studies reported results	The transport of PFTrDA was located in all brain regions, with regions that are close to incoming flow of blood, such as pons/medulla, thalamus, and hypothalamus, consistently containing relatively high levels (ranging from 43 to 49 ng/g wet weight) of PFTrDA. While cerebellum, striatum, and frontal, occipital, and temporal cortices, which are comprised in outer brain regions contained relatively lower levels of PFTrDA. When normalised for lipid content PFTrDA concentrations were not significantly (p>0.05) different among brain regions.

ROB Questions and Responses - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	NA	
	2. Was allocation to study groups adequately concealed?	NR	

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Performance	3. Were experimental conditions identical across study groups?	+	
	4. Were the research personnel and human subjects blinded to the study group during the study?	NR	
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	+	
Detection	6. Can we be confident in the exposure characterization?	NR	
	7. Can we be confident in the outcome assessment?	++	
Selective Reporting	8. Were all measured outcomes reported?	++	
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	
Overall Risk of Bias Appraisal for study:	+		

Table 96: ROB analysis of (Z. Shi et al., 2010)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	male
	Doses:	0, 0.02, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich, St. Louis, MO)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.2 % Tween-20)
	Funding source:	This research was supported by the National Natural Science Foundation of China (20837004 and 20777074). We thank Mr. Xin-Wen Zhou (Fudan University, China) for help with MALDI-TOF/TOF mass spectrometry.
	Author conflict of interest:	The authors declare that there are no conflicts of interest.
Health Outcome	Endpoints:	Concentrations of serum progesterone, testes samples were analysed via a 2-DE approach to investigate the alteration of protein expression in the testes, .
	Age at assessment:	3 weeks old at beginning of study
	Number per group and sex:	6
	Statistical analysis:	For the analysis of protein intensity in 2-DE, fold changes were calculated by comparing the control and treatment groups. For serum progesterone level, lipid peroxidation, enzyme activity, and gene expression, the raw data were analysed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL). All values are expressed as average±S.E.M. Differences between the control and treatment groups were determined by Dunnett's post hoc two-sided t-test described above.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered as statistically significant
Results	Tabular form based on studies reported results	Serum progesterone levels significantly decreased at 0.2 and 0.5 mg/kg/day Matrix-assisted laser desorption/ionization (MALDI) tandem time of flight (TOF/TOF) mass spectrometry analysis identified 40 expressed proteins that were mainly involved in mitochondrial respiration, oxidative stress, sperm activity, cytoskeleton and intracellular signal transduction. PFDoDA led to decreases in activities of superoxide dismutase (SOD), mitochondrial H-ATPase, cytochrome c oxidase, and an increase in lipid peroxidation in testes.

ROB Appraisal of Study - In Vivo Experimental Animal Study

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	rats were randomly divided into four groups
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 1 - Low ROB		

Table 97: ROB analysis of (C. Li et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	rat
	Strain (source):	SD
	Sex:	female
	Doses:	0, 1, 5, and 10 mg/kg
	Purity (source)	No purity mentioned (Sigma–Aldrich (St. Louis, MO))
	Dosing Period:	GD14 to GD21
	Route:	oral gavage
	Diet:	NR
	Controls:	1 control group
	Funding source:	National Natural Science Foundation of China
	Author conflict of interest:	The authors declared that no competing interests exist
Health Outcome	Endpoints:	inhibition of the differentiation of foetal Leydig cells in male pups, anogenital distance, clinical effects, body weight, maternal and pup
	Age at assessment:	GD21
	Number per group and sex:	10 dams/10 pup (male %: 51 ± 2; 48 ± 5; 48 ± 6; 42 ± 4 per dose group respectively)
	Statistical analysis:	All data are expressed as mean ± SEM. One-way ANOVA was used, and then post hoc Dunnett's multiple comparison test were performed to analyse the statistical significance between PFTrDA group and the control by GraphPad software version 6 (GraphPad, San Diego, CA).
	Control for litter effects:	
	Statistical power:	$p < .05, .01, .001$ were considered statistically significant.
Results	Tabular form based on studies reported results	significantly reduced the body weight and anogenital distance of male pups at birth at a dose of 10 mg/kg; significantly decreased serum testosterone levels as low as 1 mg/kg; PFTrDA did not affect foetal Leydig cell number but promoted abnormal aggregation of foetal Leydig cells at doses of 5 and 10 mg/kg; PFTrDA down-regulated the expression of Insl3, Lhcgr, Scarb1, Star, Hsd3b1, Cyp17a1, Nr5a1, and Dhh as well as their proteins. PFTrDA lowered the levels of antioxidants (SOD1, CAT, and GPX1), induced autophagy as shown by increased levels of LC3II and beclin1, and reduced the phosphorylation of mTOR

ROB Appraisal of Study - In Vivo Experimental Animal Study			
ROB Questions and Responses - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	
	2. Was allocation to study groups adequately concealed?	++	
Performance	3. Were experimental conditions identical across study groups?	++	
	4. Were the research personnel and human subjects blinded to the study group during the study?	++	
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	+	
Detection	6. Can we be confident in the exposure characterization?	NR	
	7. Can we be confident in the outcome assessment?	+	
Selective Reporting	8. Were all measured outcomes reported?	+	
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	+	
Overall Risk of Bias Appraisal for study:	+		

Table 98: ROB analysis of (Z. Shi, Zhang, et al., 2009)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimental Animal Central, Beijing, China)
	Sex:	Female
	Doses:	0, 0.5, 1.5, 3 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich)
	Dosing Period:	28 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.5 % Tween-20)
	Funding source:	This research was supported by the National Natural Science Foundation of China (20837004, 20777074 and 20677060).
	Author conflict of interest:	The authors declare that there are no conflicts of interest.
Health Outcome	Endpoints:	Body weight, uterus weight, ovary weight, visible signs of toxicity, age at vaginal opening, body weight at vaginal opening, age at first oestrous, body weight at first oestrous, number of proestrus, oestrous, diestrous, serum cholesterol, oestradiol, luteinizing hormone, follicle stimulating hormone
	Age at assessment:	3 weeks old at beginning of study
	Number per group and sex:	8
	Statistical analysis:	All data were analysed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL). All values are expressed as mean±SEM. The ratio of ovary or uterus to body weight was calculated to yield relative organ weights. The normality of the data was analysed by means of a Shapiro-Wilk test. Significant differences were analysed using a one-way ANOVA followed by a Dunnett's post hoc two-sided t-test between treatment and control groups. Differences in body weight at VO and first oestrous cycle were analysed by a general linear model, adjusted for age, as previously described [29]. In all experiments, the number of follicles in each stage was obtained by

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		individually totalling the number of primordial, primary, preantral and antral follicles in each section.
	Control for litter effects:	Not applicable
	Statistical power:	Values of p < 0.05 were considered as statistically significant
Results	Tabular form based on studies reported results	Significant decrease in body weight at 3 mg/kg/day Significant increase in total serum cholesterol at 3 mg/kg/day Significant decrease in oestradiol at 3 mg/kg/day No significant changes in FSH or LH No abnormal ovarian or uterine structure reported, ovaries of treated rats had normal complement of growing follicles and corpus luteum No significant differences in the number of primordial, primary, preantral or antral follicles in the ovary Altered ovarian expression of genes responsible for cholesterol transport and steroidogenesis, including steroidogenic acute regulatory protein, cholesterol side-chain cleavage enzyme and 17-beta-hydroxysteroid dehydrogenase at 3 mg/kg/day

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	rats were randomly divided into four groups
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 1 - Low ROB		

Table 99: ROB analysis of (H. Zhang et al., 2011)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	male
	Doses:	0, 0.05, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich, St. Louis, MO)
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.2 % Tween-20)
	Funding source:	This research was funded by the National Natural Science Foundations of China (20677060 and 20777074) and the Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-128).
	Author conflict of interest:	Not reported
Health Outcome	Endpoints:	2-D DIGE followed by mass spectrometric analyses of individual protein spots to evaluate potential renal toxicity of PFDoDA. NMR-based metabonomic analysis used for lipid and aqueous kidney extracts to determine the metabolite

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		profiles from normal and PFDoDA treated rats. iTRAQH–LC–MS/MS with 42 internal standards of physiological amino acids and amines was used to detect variations in the profiles of free amino acids in kidneys exposed to PFDoDA
	Age at assessment:	Not reported
	Number per group and sex:	6
	Statistical analysis:	Data were analysed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL) and presented as means with standard errors (mean±SE). Differences between the control and the treatment groups were determined using a one-way analysis of variance (ANOVA) followed by the Ducan multiple range test.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered as statistically significant
Results	Tabular form based on studies reported results	79 differentially expressed proteins between the control and the PFDoDA treated rats (0.2 and 0.5 mg-dosed groups) were identified, that were mainly involved in amino acid metabolism, the tricarboxylic acid cycle, gluconeogenesis, glycolysis, electron transport, and stress response. NMR-based metabonomic analysis showed an increase in pyruvate, lactate, acetate, choline, and a variety of amino acids in the highest dose group. The profiles of free amino acids in the PFDoDA treated groups showed levels of sarcosine, asparagine, histidine, 1-methylhistidine, Ile, Leu, Val, Trp, Tyr, Phe, Cys, and Met increased markedly in the 0.5 mg dosed group, while homocitrulline, a-aminoadipic acid, b-alanine, and cystathionine decreased

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	-	rats were not randomly divided into four groups
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 3 - High ROB		

Table 100: ROB analysis of (Z. Shi et al., 2013)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Vital River Laboratories Beijing, China)
	Sex:	Male
	Doses:	0, 0.02, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich, St. Louis, MO, USA)
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to a standard diet and pure water

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Controls:	vehicle (0.2 % Tween-20),
	Funding source:	This work was supported by the National Key Basic Research Program of China (973 Program: 2013CB945204) and the National Natural Science Foundation of China (Grant 31025006).
	Author conflict of interest:	The authors declare that there are no conflicts of interest
Health Outcome	Endpoints:	Used prefractionation of tryptic peptide mixtures using self-packed reversed phase C18 columns with titanium dioxide (TiO2) and immobilized metal affinity chromatography(IMAC) phosphopeptide enrichment techniques, along with two-dimensional liquid chromatography tandem mass spectrometry (2D-LC MS/MS), the authors analysed the phosphoproteome of normal rat testes and testes after 110 days of PFDoDA exposure
	Age at assessment:	The age of the animals at the beginning of the assessment was not reported.
	Number per group and sex:	6
	Statistical analysis:	The hypergeometric statistical testand the multiple-test Benjamini and Hochberg FDR correction were adopted to derive over-represented functions.
	Control for litter effects:	Not applicable
	Statistical power:	A *p* value of <0.05 was considered statistically significant
Results	Tabular form based on studies reported results	4077 unique phosphopeptides were identified from 1777 proteins with a false discovery rate below 1.0% in the testes of rats exposed to PFDoDA for 110 days. 937 novel phosphorylation sites were discovered in testicular proteins Significant dose related increase in the number of casein kinase 2 kinase-modified peptides. Pathway analysis suggested that the mitogen-activated protein kinase pathway and cell division cycle protein 2 (CDC2) may have contributed to sperm activity and testicular function.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Exposure groups were adequately randomized

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 1 - Low ROB		

Table 101: ROB analysis of (Z. Shi, Ding, et al., 2009)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	Male
	Doses:	0, 0.02, 0.05, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.2 % Tween-20),
	Funding source:	This research was supported by the National Natural Science Foundation of China (20777074 and 20677060).
	Author conflict of interest:	The author declares that there are no conflicts of interest
Health Outcome	Endpoints:	Body weight, testis weight, prostate weight, seminal vesicle weight, vas deferens weights, Hormone levels (LH, FSH, testosterone),serum cholesterol levels, insulin levels, ultrastructure of testis, mRNA expression of key genes and proteins in testosterone biosynthesis
	Age at assessment:	3 weeks old at beginning of study
	Number per group and sex:	6
	Statistical analysis:	All data were analysed using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA). All values are expressed as means±S.E.M. The ratio of testis, prostate, seminal vesicle, and vas deferens organ to body weight was calculated to yield relative weights. Differences in body weight, relative organ weight, serum hormone concentrations, and expression levels of genes and proteins between the control and treatment groups were analysed by one-way ANOVA followed by Dunnett's post hoc two-sided t-test.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered as statistically significant
Results	Tabular form based on studies reported results	Body weights were significantly decreased at 0.5 mg/kg/day No significant changes were reported in any other organ weights. Serum levels of testosterone significantly decreased at 0.2 and 0.5 mg/kg/day Cast-off cells were observed in some seminiferous tubules in testes exposed to 0.5mg/kg/day. Significantly decreased protein levels of steroidogenic acute regulatory protein (StAR) and cholesterol side-chain cleavage enzyme (P450scc), mRNA levels of insulin-like growth factor I (IGF-I), insulin-like growth factor I receptor (IGF-IR), and interleukin 1 (IL-1) in rat testes were reported at 0.2 mg/kg/day and 0.5 mg/kg/day.

ROB Appraisal of Study - In Vivo Experimental Animal Study

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	-	Exposure groups were not reported to be randomized
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 3 - High ROB		

Table 102: ROB analysis of (Z. Shi et al., 2007)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	Male
	Doses:	0, 1, 5, 10 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich)
	Dosing Period:	2 weeks
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.2 % Tween-20),
	Funding source:	This research was supported by the National Natural Science Foundation of China (20677060) and the Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-128).
	Author conflict of interest:	Not reported
Health Outcome	Endpoints:	Body weight, testis weight, Hormone levels (LH, FSH, testosterone),serum cholesterol levels, ultrastructure of testis, mRNA expression of genes involved in cholesterol transport and steroidogenesis
	Age at assessment:	The age of the animals at the beginning of the assessment was not reported.
	Number per group and sex:	10
	Statistical analysis:	All data were analysed using SPSS for Windows 13.0 Software (SPSS, Inc., Chicago, IL). All values are expressed as mean ± SEM. The ratio of testis organ to body weight was calculated to yield relative testis weights. Body weight and relative weight of testis were analysed using one-way ANOVA followed by Dunnett's post hoc two-sided t-test. Differences in testis weight, serum hormone concentrations, and gene expression levels between the treatment and control groups were analysed using a general linear model. Body weight was used as a covariant factor in analysis of these indicators. Dunnett's post hoc two-sided t-test was

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		used to confirm difference between the control and treatment group
	Control for litter effects:	Not applicable
	Statistical power:	A probability (p) of less than 0.05 was chosen as the limit for statistical significance.
Results	Tabular form based on studies reported results	Body weights were significantly decreased at 5 and 10 mg/kg/day Testis weights were significantly decreased at 10 mg/kg/day Relative testis weights were significantly increased at 10 mg/kg/day Serum levels of testosterone significantly decreased at 5 and 10 mg/kg/day Serum luteinizing hormone significantly decreased at 10 mg/kg/day Oestradiol levels significantly decreased at 5 mg/kg/day Leydig cells, Sertoli cells, and spermatogenic cells from rats that received 5 or 10 mg PFDoDA/kg/day, exhibited apoptotic features including dense irregular nuclei, condensed chromatin, ill-defined nuclear membranes, and abnormal mitochondria. Significant declines in mRNA expression of several genes involved in cholesterol transport and steroid biosynthesis at doses of 5 and 10 mg PFDoDA/kg/day Total serum cholesterol significantly increased at 10 mg/kg/day

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	-	Exposure groups were not reported to be randomized
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 3 - High ROB		

Table 103: ROB analysis of (H. Liu et al., 2016)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Vital River Laboratories Beijing, China)
	Sex:	Male
	Doses:	0, 0.05, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma-Aldrich, St. Louis, MO, USA)
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to a standard diet and pure water
	Controls:	vehicle (0.2 % Tween-20),
	Funding source:	This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB14040202) and the National Natural Science

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		Foundation of China (Grants Nos. 31320103915, 21277143 and 21377128).
	Author conflict of interest:	Not reported
Health Outcome	Endpoints:	2-D DIGE followed by mass spectrometric analyses of rat liver both with and without chronic PFDoDA exposure to investigate potential mechanism of hepatotoxicity.
	Age at assessment:	The age of the animals at the beginning of the assessment was not reported.
	Number per group and sex:	10
	Statistical analysis:	For quantitative real-time PCR data and western blot analyses, statistical significance was determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (SPSS for Windows 17.0 Software, Inc., Chicago, IL, USA). Data are presented as means with standard errors (mean ± SE).
	Control for litter effects:	Not applicable
	Statistical power:	A *p* value of <0.05 was considered statistically significant
Results	Tabular form based on studies reported results	73 involved in lipid metabolism, inflammation, stress response and other functions were identified. Six significantly changed proteins (CTE1, MTE1, HADHA, ECH1, ALDH2 and CPS1) were found to be regulated by peroxisome proliferator-activated receptor alpha (PPARα). induction of oxidative stress by PFDoDA exposure was indicated via antioxidant enzyme activity assays of superoxide dismutase and glutathione peroxidase and the content of thiobarbituric acid-reactive substances in the liver. Reactive oxygen species (ROS) content in rat hepatocytes was significantly increased in PPARα knockdown groups, consistent with the PPARα antagonist and agonist treated groups.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Exposure groups were adequately randomized

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	++	test substance purity, and source sources was provided. Exposure was well characterized.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 1 - Low ROB		

Table 104: ROB analysis of (Xin et al., 2022)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Xin et al. Effects of perfluoroundecanoic acid on the function of Leydig cells in adult male rats. Toxicology and Applied Pharmacology 439 (2022) 115903, 2022		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague-Dawley
	Sex:	male
	Doses:	0 (control group, corn oil), 0.1, 0.5, 1 or 5 mg/kg bw/day

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	Purity (source)	not stated; Aladdin (Bay City, MI)	
	Dosing Period:	28 days	
	Route:	oral gavage	
	Diet:	not specified; at libitum.	
	Controls:	vehicle (corn oil)	
	Funding source:	NSFC (81730042 to R.S.G, and 81901467 to Y.W).	
	Author conflict of interest:	The authors declared that no competing interests exist.	
Health Outcome	Endpoints:	serum testosterone levels, Leydig cell (LC) number and LC function	
	Age at assessment:	56 days	
	Number per group and sex:	10 male/dose	
	Statistical analysis:	Data were presented as Mean ± SEM (standard errors). The one-way ANOVA followed by post hoc Dunnett's multiple comparisons was used to compare the difference between the PFUnA group and the control group.	
	Control for litter effects:	not stated	
	Statistical power:	not specified;	
Results	Tabular form based on studies reported results	PFUnA significantly reduced serum testosterone levels as low as 0.5 mg/kg. PFUnA markedly decreased Leydig cell number as low as 0.1 mg/kg	
ROB Qustions and Responses - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	+++ yes	
	2. Was allocation to study groups adequately concealed?	NR	not specified
Performance	3. Were experimental conditions identical across study groups?	+++ yes	
	4. Were the research personnel and human subjects blinded to the	NR	not specified

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	study group during the study?		
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	+	
Detection	6. Can we be confident in the exposure characterization?	+++	gavage
	7. Can we be confident in the outcome assessment?	++ yes	the results are described in sufficient detail
Selective Reporting	8. Were all measured outcomes reported?	+ yes	
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	+	yes
Overall Risk of Bias Appraisal for study:	Definitely Low risk of bias: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)		

Table 105: ROB analysis of (Ding et al., 2009)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague-Dawley (Weitong Lihua Experimental Animal Central, Beijing, China)
	Sex:	Male
	Doses:	0, 0.02, 0.05, 0.2, 0.5 mg/kg/day
	Purity (source)	95% (Sigma Aldrich, St. Louis, MO)
	Dosing Period:	110 days
	Route:	Oral gavage

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Diet:	Type of food and source not reported (access to food and water ad libitum)
	Controls:	Negative controls were used; however, treatment of controls (vehicle or untreated) was not specified.
	Funding source:	This work was funded by the National Natural Science Foundation of China (20837004 for J.D., and 20825520 for H.T., respectively) and we acknowledge partial financial supports from the National Basic Research Program of China (2009CB118804 for Y.W.). We also thank Mr. Hang Zhu of Wuhan Institute of Physics and Mathematics for modifying the MatLab scripts used for color-coding the coefficient plots.
	Author conflict of interest:	No conflict-of-interest statement was included in study.
Health Outcome	Endpoints:	Histopathology of liver slices, food consumption, body weight, liver weight, clinical chemistry for T-Bil, TBA, ALP, ALB, CK, BUN, Cr, TG, LDL-C, and glucose.; NMR Spectroscopy of Serum and Liver Tissue
	Age at assessment:	Not reported
	Number per group and sex:	10
	Statistical analysis:	Values were reported as means (standard error (SE). Statistical differences were determined by one-way ANOVA multiple range test.
	Control for litter effects:	Not applicable
	Statistical power:	Statistical significance was indicated with * to state $p < 0.05$.
Results	Tabular form based on studies reported results	Body weights were significantly decreased at 0.5 mg/kg/day Liver weight were significantly increased at all doses. Relative liver to body weight ratios were significantly increased at all doses. Lipid droplets and widespread disintegrated cell systems were observed in the 0.05-, 0.2-, and 0.5-dosed groups, and the lipid droplets were larger in the high-dosed compared to the low-dosed groups Hydrophic degeneration and steatosis was observed at 0.2 an d0.5 mg/kg/day, as well as swollen and vacuolated hepatocytes with the largest karyons in the 0.5-dosed group Significant increases in the levels of TBA, ALP, BUN, and Cr were detected for the groups receiving a dosage of 0.2 and 0.5, significant increases in the levels of T-Bil were observed in the 0.5-dosed group. The concentrations of CK were significantly increased in the 0.02- and 0.05 dose groups Concentrations of LDL-C were significantly decreased in

ROB Appraisal of Study - In Vivo Experimental Animal Study			
		the 0.02- and 0.05-dosed groups and the levels of TG decreased significantly in the 0.05-, 0.2- and 0.5-dosed groups. No significant changes were observed in the levels of ALT, AST, HDL-C, and T-CHO in the dosed groups NMR-based metabonomics results for both liver tissues and serum demonstrated PFDoDA exposure led to hepatic lipidosis characterized by a severe elevation in hepatic triglycerides and a decline in serum lipoprotein levels. Results of transcriptomic changes induced by PFDoDA corroborated these results with changes in gene transcript levels associated with fatty acid homeostasis.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	+	The rats were randomly assigned to dose groups; however, the randomization method was not reported.
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were identical across all treated groups; however, the authors did not report on however the control animals were dosed (vehicle or diet only).
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	Outcome data was reported completely
Detection	6. Can we be confident in the exposure characterization?	++	Yes, the test substance purity and source were reported
	7. Can we be confident in the outcome assessment?	++	Yes, all methods and assays used were well reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	None identified
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 106: ROB analysis of (Chen et al., 2019)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague-Dawley (Shanghai laboratory Animal Center, Shanghai, China)
	Sex:	Male
	Doses:	0, 5, 10 mg/kg/day
	Purity (source)	Purity not reported (J&K Scientific Ltd. Beijing, China)
	Dosing Period:	daily for 14 days
	Route:	oral gavage
	Diet:	Not reported
	Controls:	The study included an untreated control group; however, the authors did not state if the rats in the control group were gavaged with the vehicle only.
	Funding source:	Supported by NSFC (81730042 to R.S.G., 81701426 to X.L.G., 81601264 to X.H.L.) and Health and Family Planning Commission of Zhejiang Province (2017KY483 to X.H.L., 2018KY523 and 2017KY473 to X.L.G., 11-CX29 to R.S.G.) as well as Zhejiang Provincial NSF (LY15H310008 to R.S.G.).
	Author conflict of interest:	The authors declare no competing financial interest.
Health Outcome	Endpoints:	body weight, testis weight, Serum testosterone; serum LH and FSF, Leydig and Sertoli cell number, gene and protein expression, Leydig cell proliferation
	Age at assessment:	Post-natal day 21 through 35
	Number per group and sex:	8

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Statistical analysis:	The mean ± standard errors (SE) were used for data presentation. Statistical analysis was performed using GraphPad Prism (version 6, GraphPad Software, San Diego, CA) with one-way ANOVA followed by ad-hoc Dunnett's multiple comparison.
	Control for litter effects:	Not applicable
	Statistical power:	The P <0.05 was considered a significant difference.
Results	Tabular form based on studies reported results	Body weight and testis weight were both significantly decreased at 10 mg/kg/day on day 14 of treatment. Levels of testosterone, LH and FSH were significantly decreased at 5 and 10 mg/kg/day on day 14 of treatment. No significant treatment related changes in Leydig or Sertoli cell number. In Leydig cells, PFDoDA down-regulated the expression of Lhcgr and Cyp11a1 at doses of 5 and 10 mg/mg, and Scarb1, Star, Cyp17a1, and Hsd11b1 at 10 mg/kg without affecting Hsd3b1, Hsd17b3, and Nr5a1. PFDoDA did not affect the expression of Sertoli cell genes In Leydig cells, PFDoDA decreased LHCGR, SCARB1, STAR, CYP11A1, CYP17A1, and HSD11B1 levels. PFDoDA decreased CYP11A1 at doses of 5 and 10 mg/kg and HSD11B1 level at 10 mg/kg and had no effect on SOX9 intensity at both doses. No significant effect on the proliferative capacity of Leydig cells was reported in treated rats.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	+	The randomization procedures were not noted; however, the authors state the rats were randomly divided into three groups.
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were identical across all study groups.
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	Outcome data was reported completely
Detection	6. Can we be confident in the exposure characterization?	-	The test substance purity, Lot number and CAS number were not reported. The treatment of the control animals was not reported (e.g., vehicle control, diet only)
	7. Can we be confident in the outcome assessment?	++	Yes, all methods and assays used were well reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	None identified
Overall Risk of Bias Appraisal for study:	Tier 3 - High Risk of Bias		

Table 107: ROB analysis of (H. Zhang et al., 2013)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Weitong Lihua Experimentary Animal Central, Beijing, China)
	Sex:	male
	Doses:	0, 0.2, 0.5 mg/kg/day
	Purity (source)	Not reported (Sigma-Aldrich, St. Louis, MO)
	Dosing Period:	110 days
	Route:	Oral gavage
	Diet:	ad libitum access to food and water
	Controls:	vehicle (0.2 % Tween-20)
	Funding source:	This work was supported by the National Key Basic Research Program of China (973 Program:

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		2013CB945204) and the National Natural Science Foundation of China (Grant No. 31025006).
	Author conflict of interest:	The author declares that there are no conflicts of interest.
Health Outcome	Endpoints:	used TiO2-based phosphopeptide enrichment coupled with LC–MS/MS analysis to identify phosphopeptides in rat livers that were influenced by PFDoDA treatment.
	Age at assessment:	Not reported
	Number per group and sex:	6
	Statistical analysis:	None reported
	Control for litter effects:	Not applicable
	Statistical power:	Not applicable
Results	Tabular form based on studies reported results	total of 1443 unique phosphopeptides from among 769 phosphoproteins identified in normal and PFDoDA-treated rat livers, 849 unique phosphorylation sites were also identified. Of these sites, 143 were considered to be novel phosphorylation sites. Many phosphoproteins were found to be associated with hepatic injuries and diseases, such as hepatotoxicity, regeneration, fatty liver, neoplasms and carcinoma. Furthermore, 25 of the identified phosphoproteins were found to be related to glycogen synthase kinase-3 (GSK3), either directly or indirectly. Western blot and qPCR results suggested that chronic PFDoDA exposure inhibited insulin signal pathways and that inhibition of GSK3 might contribute to the observed increases of lipid levels in the liver.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	-	rats were not randomly divided into four groups
	2. Was allocation to study groups adequately concealed?	+	Not reported but lack of concealment should not produce significant bias
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were reported to be identical across study groups

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	4. Were the research personnel and human subjects blinded to the study group during the study?	-	Blinding of research personal was not reported
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	All outcome data was reported including statistical analysis results and standard deviations.
Detection	6. Can we be confident in the exposure characterization?	-	test substance purity was not reported, only the source was provided.
	7. Can we be confident in the outcome assessment?	++	All methods and results were reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	No other threats to internal validity were noted
Overall Risk of Bias Appraisal for study:	Tier 3 - High ROB		

Table 108: ROB analysis of (Yan et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	rats
	Strain (source):	Sprague-Dawley (Shanghai Laboratory Animal Center (Shanghai, China).
	Sex:	male
	Doses:	0, 1, 5, and 10 mg/kg/day
	Purity (source)	not stated
	Dosing Period:	from postnatal day (PND) 35 to PND 56. In total 21 day
	Route:	orally (gavage)
	Diet:	not reported
	Controls:	vehicle (corn oil)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Funding source:	NSFC (81730042 to R.S.G, and 81901467 to Y·W)
	Author conflict of interest:	The authors declared that no competing interests exist.
Health Outcome	Endpoints:	mortality and morbidity, body weights, serum T and E2 concentrations ,serum LH and FSH concentrations Leydig cell number, Leydig cell steroidogenesis-related gene expression, autophagy in the testis.
	Age at assessment:	PND 35
	Number per group and sex:	9 male rats/group
	Statistical analysis:	All data are expressed as mean ± standard error (SEM). One-way ANOVA was used, and then post hoc multiple comparisons of Dunnett's test were used to analyse the statistical significance by GraphPad (GraphPad, San Diego, CA). P <0.05, 0.01, and 0.001 was considered statistically significant.
	Control for litter effects:	not specified
	Statistical power:	statistical significance :P <0.05, 0.01, and 0.001 was considered statistically significant.
Results	Tabular form based on studies reported results	Serum testosterone and luteinizing hormone levels were remarkably reduced by PFUnDA at ≥1 mg/kg while serum follicle-stimulating hormone levels were lowered at 5 and 10 mg/kg. PFUnDA down-regulated the expression of Lhcgr, Scarb1, Star, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b3, Hsd11b1, Insl3, Nr5a1, Fshr, Dhh, Sod1, and Sod2 and their proteins in the testis and the expression of Lhb and Fshb in the pituitary. PFUnDA reduced Leydig cell number at 5 and 10 mg/kg. PFUnDA induced oxidative stress and increased autophagy.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Rats were randomly divided into 4 groups (9 animals in each group) : 0, 1, 5, and 10 mg/kg/day PFUnDA
	2. Was allocation to study groups adequately concealed?	NR	
Performance	3. Were experimental conditions identical across study groups?	++	

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	4. Were the research personnel and human subjects blinded to the study group during the study?	NR	
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	
Detection	6. Can we be confident in the exposure characterization?	++	Rats were exposed orally by gavage to PFUnDA (dissolved in corn oil) was dosed daily from PND 35 to PND 56 by gavage with vehicle (corn oil) or different doses of PFUnDA. PFUnDA was administered in corn oil (2.5 ml vehicle/kg body weight). The dosing period from PND 35 to PND 56 covers the pubertal LC development.
	7. Can we be confident in the outcome assessment?	++	Appropriate methods were used to measure selected endpoints.
Selective Reporting	8. Were all measured outcomes reported?	++	
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	
Overall Risk of Bias Appraisal for study:	Definitely Low risk of bias: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)		

Table 109: ROB analysis of (Feng et al., 2017)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	mice
	Strain (source):	ICR (Oriental Bio Service Inc. (Nanjing, Jiangsu, China).
	Sex:	female
	Doses:	0, 50, 200, 500 mg/kg/day
	Purity (source)	98% (Sigma-Aldrich, CAS#29420-49-3; St. Louis, Missouri)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Dosing Period:	Gestation day 1 though 20
	Route:	Oral gavage
	Diet:	free access to food and water,
	Controls:	Controls received an equivalent volume of vehicle (0.1% carboxymethyl cellulose).
	Funding source:	National 973 Program (2014CB943303 and 2014CB943301), the National Natural Science Foundation (81471157 and 81671253).
	Author conflict of interest:	The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported herein
Health Outcome	Endpoints:	Perinatal growth and development, puberty onset and oestrous cycle, ovarian and uterine morphology, dam and offspring hormone measurement,
	Age at assessment:	PND 1 through 60
	Number per group and sex:	30
	Statistical analysis:	All statistical analyses were performed using SPSS software, version 16.0 (SPSS Inc., Chicago, Illinois). Differences among means were analysed using 1-/2-factor ANOVA followed by Bonferroni's post hoc analysis.
	Control for litter effects:	The pups were born by natural delivery and housed in the same cages with their dams under the same laboratory conditions. On postnatal day (PND) 21, all offspring were weaned. Female offspring were transferred to other cages (2–4 per cage). Male offspring were used in other experiments.
	Statistical power:	P<.05 and P<.01 were considered statistically significant
Results	Tabular form based on studies reported results	statistically significant reductions of total T3, total T4, and free T4 (reduced 17, 21, and 12%, respectively, relative to control at 200 mg/kg-day and reduced 16, 20, and 11%, respectively, relative to control at 500 mg/kg bw·d) on GD 20 at doses of 200 and 500 mg/kg bw·d Decreased total T3 and total T4 in the female offspring on post-natal days (PNDs) 1, 30, and 60 in offspring gestationally exposed to PFBS at the same doses. Significantly increased thyroid-stimulating hormone (TSH) was reported in dams and pubertal (PND 30) offspring (21 and 14% relative to control at 200 mg/kg bw·d, respectively) exposed gestationally to PFBS. The female offspring exposed to 200 and 500 mg/kg bw·d in utero exhibited significantly decreased perinatal body weight and delayed eye opening

ROB Appraisal of Study - In Vivo Experimental Animal Study			
		compared to control offspring. Significantly delated vaginal opening and first oestrus, as well as significantly prolonged dioestrus in female offspring exposed to 200 and 500 mg/kg bw·d in utero. In pubertal and adult offspring exposed to 200 and 500 mg/kg bw·d in utero significant decreases in serum oestrogen (E2) and progesterone (P4) levels were reported with the elevation of luteinizing hormone levels.	

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	animals were randomly assigned to experimental groups
	2. Was allocation to study groups adequately concealed?	+	Concealment was not reported but is not expected to increase the risk of bias significantly
Performance	3. Were experimental conditions identical across study groups?	++	experimental conditions were identical
	4. Were the research personnel and human subjects blinded to the study group during the study?	++	Research personnel were blinded to the study group
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	outcome data were reported completely with attrition or exclusion
Detection	6. Can we be confident in the exposure characterization?	++	yes, test substance purity and source were reported and methods for exposure were clear.
	7. Can we be confident in the outcome assessment?	++	
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	There were no other threats to internal validity

ROB Appraisal of Study - In Vivo Experimental Animal Study	
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias

Table 110: ROB analysis of (X. Cao et al., 2020)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Mice
	Strain (source):	ICR (Oriental Bio Service, Inc; Nanjing, Jiangsu, China)
	Sex:	Female
	Doses:	0, 50, or 200 mg/kg/day
	Purity (source)	98% (Sigma-Aldrich Company; St. Louis, MO.
	Dosing Period:	14 days
	Route:	Oral gavage
	Diet:	Animals had free access to food and water however, diet supplier was not reported.
	Controls:	vehicle control group received 0.9% saline
	Funding source:	This work was supported by National Natural Science Foundation of China (81671253), Jiangsu provincial Natural Science Foundation of China (BE2016765).
	Author conflict of interest:	The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
Health Outcome	Endpoints:	PFBS in orbital blood Measurement of follicle-stimulating hormone (FSH), testosterone (T), oestrogen (E2), progesterone (P4), luteinizing hormone (LH), triiodothyronine (tT3) and total thyroxine (tT4) using commercial enzyme linked immunosorbent assay (ELISA) kits (Uscn Life Science, Inc., Houston, TX, USA) according to the manufacturer's instructions Number of follicles in ovaries (primordial, primary, secondary, early antral, antral, atretic follicles) were counted in every 6th section for a total number of follicles in each ovary. Immuno-staining of ovaries Western blot total RNA
	Age at assessment:	Not reported
	Number per group and sex:	Not reported

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Statistical analysis:	The group data were expressed as the means ± standard error (SE). All statistical analyses were performed using SPSS software, version 16.0 (SPSS, Inc., Chicago, IL, USA). Differences among means were analysed using one/two-factor analysis of variance (ANOVA) followed by Bonferroni post hoc analysis.
	Control for litter effects:	Not applicable
	Statistical power:	Differences at $P < 0.05$, $P < 0.01$ were considered statistically significant.
Results	Tabular form based on studies reported results	AT 24 h after the last administration, the levels of serum PFBS in control, 50, and 200 mg/kg/day mice were 0.59 ± 0.11 ng/ml, 10.81 ± 1.91 ng/ml and 46.46 ± 6.90 ng/ml, respectively 200 mg/kg/day significantly decreased the levels of serum total triiodothyronine and thyroxine, which depended on the activation of peroxisome proliferator-activated receptor a (PPARa) 200 mg/kg/day significantly decreased the numbers of secondary follicles, early antral follicles, and antral follicles 200 mg/kg/day significantly decreased levels of Akt, mTOR and p70S6K phosphorylation in ovarian granular cells and cumulus cells, which suppressed the proliferation of these cells and enhanced autophagic death of granular cells and cumulus cells. 200 mg/kg/day significantly reduced the levels of serum oestradiol and progesterone with a low expression of the steroidogenic genes Star and P450scc in ovarian tissues.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	NR	Randomization of the dose groups was not reported by the authors
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	++	Experimental conditions were identical across all study groups.
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	Outcome data was reported completely
Detection	6. Can we be confident in the exposure characterization?	++	Test substance purity and source were reported
	7. Can we be confident in the outcome assessment?	++	Yes, all methods and assays used were well reported
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	None identified
Overall Risk of Bias Appraisal for study:	Tier 2		

Table 111: ROB analysis of (NTP, 2022)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rat
	Strain (source):	Sprague Dawley (Hsd:Sprague Dawley SD) (Harlan Laboratories, Inc., Indianapolis, IN, now part of Envigo, Inc.)
	Sex:	Male and female
	Doses:	0, 62.6, 125, 250, 250, 500, 1000 mg/kg/day (one half dose administered twice per day)
	Purity (source)	>97% (Lot 15414TE; Sigma Aldrich; St. Louis, MO)
	Dosing Period:	7 days per weeks for 28 days
	Route:	Oral gavage administered in deionized water with 2% Tween 80 at one half dose twice daily.
	Diet:	NTP-2000 irradiated wafer (Zeigler Brothers, Inc., Gardners, PA), available ad libitum; changed weekly. Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Controls:	vehicle control
	Funding source:	National Toxicology Program
	Author conflict of interest:	The interpretive conclusions presented in the Toxicity Study Reports are based only on the results of these NTP studies.
Health Outcome	Endpoints:	Observed twice daily; animals were weighed, and clinical findings were recorded on day 1, weekly thereafter, and at the end of the study. Necropsies were performed on all rats. Organs weighed were right adrenal gland, heart, right kidney, liver, lung, spleen, right testis, thymus, thyroid gland, and uterus/cervix/vagina. Blood was collected from the abdominal aorta of all rats at the end of the studies for haematology and clinical chemistry. Haematology and clinical chemistry analysis Complete histopathology was performed on rats in the 0, 500, and 1,000 mg/kg per day groups. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, epididymis, oesophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), lung, lymph nodes (mandibular and mesenteric), mammary gland, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, stomach (forestomach and glandular), trachea, urinary bladder, and uterus. The bone marrow, kidney, liver, nose, ovary, pancreas, spleen, testes, thymus, and thyroid gland were examined in all dose groups. Blood was collected from the abdominal aorta of all rats at the end of the study and analysed for testosterone levels. Spermatid and sperm samples were collected from 0, 125, 250, and 500 mg/kg per day males at study termination. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for 16 consecutive days prior to the end of the study from females in the 0, 125, 250, and 500 mg/kg per day groups for vaginal cytology evaluations. The percentage of time spent in the various oestrous cycle stages and oestrous cycle length were evaluated.
	Age at assessment:	10 to 11 weeks. On receipt, rats were approximately 6 to 8 weeks old. Animals were quarantined for 14 (males) or 15 (female) days.
	Number per group and sex:	10

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Statistical analysis:	The Fisher exact test, Organ and body weight data, which historically have approximately normal distributions, were analysed with the parametric multiple comparison procedures of Dunnett and Williams. Haematology, clinical chemistry, hormones, oestrous cycle length, number of oestrous cycles, parent compound, spermatid, epididymal spermatozoal, hepatic enzymes, and hepatic gene expression data, which have typically skewed distributions, were analysed using the nonparametric multiple comparison methods of Shirley (as modified by Williams) and Dunn. Jonckheere's test was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). a procedure based on the overall proportion of affected animals, was used to determine significance.
	Control for litter effects:	Not applicable
	Statistical power:	To maintain the overall significance level at 0.05, the trend as well as the pairwise differences from the vehicle control group were declared statistically significant if $p \leq 0.025$.
Results	Tabular form based on studies reported results	All male rats and 8 of the 10 female rats in the high dose group died. The reported reductions in rat total T3 were up to 57% (male) and 43% (female), in free T4 up to 86% (male) and 77% (female), and in total T4 up to 97% (male) and 71% (female). Thyroid gland weight, thyroid histopathology, and TSH levels were not changed after 28 days of PFBS exposure in male or female rats at doses up to 1,000 mg/kg-day Significantly increased absolute and relative right kidney weights in male rats at 500 mg/kg bw·d, and relative kidney weights in female rats at all tested PFBS doses (≥62.6 mg/kg bw·d). Significantly increased absolute and relative liver weights in males at doses of 125 and 62.6 mg/kg bw·d and greater, respectively, and females at doses of 250 and 125 mg/kg bw·d and greater, respectively. Significantly increased incidence of hepatocellular hypertrophy was reported in male (≥125 mg/kg bw·d) and female (≥500 mg/kg bw·d) rats. Significantly increased cytoplasmic alteration of hepatocytes was observed in these rats (male and female at ≥500 mg/kg bw·d).

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Animals were distributed randomly into groups of approximately equal initial mean body weights and identified by tail tattoo.
	2. Was allocation to study groups adequately concealed?	+	Concealment was not reported but is not expected to increase the risk of bias significantly
Performance	3. Were experimental conditions identical across study groups?	++	experimental conditions were identical
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Blinding of personnel was not reported by the study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations.
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	outcome data were reported completely with attrition or exclusion
Detection	6. Can we be confident in the exposure characterization?	++	yes, test substance purity and source were reported and methods for exposure were clear.
	7. Can we be confident in the outcome assessment?	++	
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 112: ROB analysis of (Conley et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rats
	Strain (source):	Sprague-Dawley rats (Crl:CD(SD))
	Sex:	female

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Doses:	One block of 30 dams: water vehicle, 10, 30, 62.5, 125, or 250 mg/kg HFPO-DA from GD8-PND2; Two blocks of 15 dams each: 0 (n=3), 1, 3, 10, 30, 62.5, or 125 mg/kg HFPO-DA (n=2 per treatment group) from GD17-21
	Purity (source)	HFPO-DA ammonium salt (CAS:62037-80-3; Product No.: 2122-3-09; Lot: 00005383) purchased from SynQuest Laboratories (Alachua, FL, USA). HFPO-DA purity was 100% as determined by the supplier via perchloric acid titration.
	Dosing Period:	One block of 30 dams from Gestational Day 8 – PND2 (PND0 = day of parturition). Two blocks of 15 dams each from GD17-GD21.
	Route:	oral gavage
	Diet:	NIH07 Rat Chow and filtered (5µm) municipal tap water (Durham, NC) ad libitum.
	Controls:	water
	Funding source:	This work was supported by the U.S. Environmental Protection Agency Office of Research and Development Chemical Safety for Sustainability Research Action Plan.
	Author conflict of interest:	The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Health Outcome	Endpoints:	Evaluation of neonatal effects (GD 8 – PND 2): maternal body weight, neonatal serum analysis, maternal liver weight, maternal liver collected for RNA extraction and HFPO-DA concentration, and uterine implantation sites scored. Time of pup delivery was recorded, pups counted, and whole litter weight recorded beginning GD22 (i.e., PND0). Pup Anogenital distance measured, pup liver HFPO-DA concentration, and pup liver weight; Evaluation GD17-GD21: Maternal weight gain during dosing, clinical chemistry of maternal serum, reproductive output, maternal liver weight, collected maternal and foetal liver mRNA, and measured maternal and foetal serum and liver HFPO-DA concentrations were evaluated in all dams from both blocks, except for foetal serum levels which were only evaluated in one block. PPAR signalling pathways were determined using foetal (GD21), neonatal (PND0), and maternal (GD21) livers and glucose metabolism was determined using foetal and

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		neonatal livers by utilizing 84 target genes associated with each endpoint.
	Age at assessment:	90 days old
	Number per group and sex:	Evaluation of neonatal effects: One block of 30 dams (n = 5 for each for each) exposed to 0, 10, 30, 62.5, 125, or 250 mg/kg HFPO-DA at gestational day (GD) 8 - postnatal day (PND) 2. Evaluation of foetal and maternal effects during gestation (GD17-21): Two blocks of 15 dams per block were dosed once daily with water vehicle (n = 3) or 1, 3, 10, 30, 62.5, or 125 mg/kg HFPO-DA (n = 2 per treatment). Total sample sizes were n = 6 for vehicle control and n = 4 for HFPO-DA treated dams.
	Statistical analysis:	All values are reported as mean ± standard error of the mean (SEM) and all statistical comparisons were conducted at α = 0.05 significance level except for RT-qPCR gene expression which identified significantly altered genes at both α = 0.01 and α = 0.001 using analysis of variance (ANOVA), followed by pairwise comparison at α = 0.05 to determine differences of treatment compared to control for significant genes. Data were log10-transformed and treatment effects were identified by ANOVA using the PROC GLM statement in SAS (v.9.4, SAS Institute, Cary, NC, USA) and pairwise comparison versus control was performed using the least squares means (LSMEANS) statement using the PDIFF option. For all foetal and neonatal data, litter means were used as the statistical unit to account for the nested effects of individuals within litters. Pup liver glycogen accumulation grades were analysed by non-parametric Kruskal-Wallis test following by Mann-Whitney U test for pairwise J.M. Conley et al. Environment International 146 (2021) 1062044 comparison to control using GraphPad Prism v8.2.1 (GraphPad, Inc., La Jolla, CA, USA). GraphPad Prism was used to generate all figures and to conduct sigmoidal dose–response curve analyses. Maternal and foetal/neonatal liver gene expression data were analysed using the comparative cycle threshold (CT) method. Melt curve analyses were conducted for all genes at all doses, and any well not displaying a clear peak was assigned a CT value of 35. This is critical for the evaluation of genes that are typically unexpressed in control liver, but are upregulated as a result of exposure. Delta CT values were calculated using the equation 2− ΔΔC T and normalized to the mean CT value of the appropriate housekeeping genes

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		for each tissue and gene array. We selected housekeeping genes that did not display a significant treatment effect of HFPO-DA exposure (GD21 foetal liver = Actb, B2m, Hprt1, Rplp1; GD21 maternal liver = Actb, Hprt1, Rplp; PND0 neonatal liver = Actb, Hprt1). Delta CT values were then converted to foldinduction by dividing the treated replicate delta CT by the mea delta CT of the control replicates for each gene. Fold induction values were log10 transformed prior to ANOVA. We previously examined PPAR target genes in foetal and maternal livers from GD14-18 HFPO-DA exposure (Conley et al. 2019) and hypothesized that GD17-21 exposure would display similar alteration of the same genes. As such, we analysed the foetal and maternal PPAR genes from a single block (n = 3 for control, n = 2 per treatment) of GD17-21 exposure with the GD14-18 data using two-way ANOVA with dose and exposure interval as independent variables. Fatal and pup body weights were analysed using litter size as a covariate within PROC GLM. Liver weight was analysed using body weight as a covariate within PROC GLM and liver weight relative to body weight was also calculated for each individual. Pup survival was scored as the number of live pups on PND2 divided by the total number of live pups born and by the number of uterine implantations sites and presented as percentages. Neonatal serum clinical chemistry was analysed using two-way ANOVA with dose and postnatal day as independent variables. HFPO-DA concentrations in maternal and foetal serum from GD17-21 exposure were compared to previously published data for GD14-18 exposure (Conley et al. 2019). Serum concentrations were analysed using two-way ANOVA with dose and interval as independent variables. There was no effect of interval on foetal serum concentrations so GD18 and GD21 data were combined. Maternal serum concentrations significantly varied from GD18 to GD21 and are reported separately. Foetal (GD21) and neonatal (PND2) liver HFPO-DA concentrations were each analysed using two-way ANOVA with dose and sex as class variables. There was no effect of sex on foetal liver concentrations so male and female concentrations were combined; however, there was a significant effect of sex on PND2 liver concentrations and data are reported for males and females separately. Maternal and foetal serum and liver concentrations were log-

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		transformed and fit with regression models using GraphPad Prism.
	Control for litter effects:	For all foetal and neonatal data, litter means were used as the statistical unit to account for the nested effects of individuals within litters.
	Statistical power:	All values are reported as mean ± standard error of the mean (SEM) and all statistical comparisons were conducted at $\alpha = 0.05$ significance level except for RT-qPCR gene expression which identified significantly altered genes at both $\alpha = 0.01$ and $\alpha = 0.001$ using analysis of variance (ANOVA), followed by pairwise comparison at $\alpha = 0.05$ to determine differences of treatment compared to control for significant genes.
Results	Tabular form based on studies reported results	Results of maternal exposure from GD8-PND2 indicated statistically significantly decreased average pup birth weight in the 30, 62.5, 125, and 250 mg/kg treatment groups, when compared to controls. Lethargy, moribund, and death were reported shortly after delivery in pups from treatment groups of 10mg/kg/d and greater. The first signs of pup mortality in the 10, 30, 62.5, 125, and 250 mg/kg treatment groups were at 10.4±8.4, 13.1±11.3, 11.4±2.6, 9.3±1.0, and 5.1±1.9 hours respectively, following onset of delivery. Statistically significantly decreased pup survival was reported in all treatment groups exposed to 62.5 mg/kg or greater, when compared to controls. No statistically significant effects of pup sex on body weight or liver weight were reported at necropsy, when compared to controls. A statistically significant decrease in pup body weight was reported in all treatment groups of 30 mg/kg or higher, when compared to controls. There was no statistically significant difference in AGD for male or female pups at any treatment level, when compared to controls. However, pup relative liver weight was statistically significantly increased at all treatment levels, when compared to controls. Glycogen accumulation (percentage of hepatocytes containing glycogen) reported in liver samples collected from two newborn pups prior to nursing (PND0) was statistically significantly lower in all dose groups than in controls. Serum analyses from PND0 pups resulted in statistically significantly decreased albumin (62.5 and 250 mg/kg) and glucose (62.5, 125, and 250 mg/kg) levels and statistically significantly increased cholesterol (125 and 250 mg/kg) and triglyceride (125 and 250 mg/kg) levels, when compared to controls. Serum

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		analyses from PND2 pups resulted in statistically significantly increased aspartate aminotransferase (AST) (≥30 mg/kg) and cholesterol (≥62.5 mg/kg) levels and statistically significantly decreased glucose (≥125 mg/kg) levels, when compared to controls. A statistically significant decrease in mean maternal body weight and gestational weight gain was reported in the 125 and 250 mg/kg dose groups at GD22 and in absolute dam body weight at PND2 in the 125 and 250 mg/kg dose groups, when compared to controls. Absolute (≥30 mg/kg) and relative (≥10 mg/kg) liver weights of dams were statistically significantly decreased, when compared to controls. Clinical chemistry results of dams at PND2 showed statistically significantly reduced total T4 (≥62.5 mg/kg), total T3 (≥62.5 mg/kg), and albumin (250 mg/kg) levels and statistically significantly increased AST (≥10 mg/kg) and triglyceride (≥125 mg/kg) levels, when compared to controls. Results of maternal exposure from GD17-21 showed no statistically significant differences in male or female absolute or mean foetal body or liver weights across dose groups at GD21, when compared to controls. There were also no statistically significant differences in maternal terminal body weight or body weight gain at any treatment level, when compared to controls. Viable foetuses and resorptions reported were similar between treated and control groups. A statistically significant increase in relative liver weight was reported in dams in the 62.5 and 125 mg/kg treatment groups, when compared to controls. Clinical chemistry results of dams at GD21 showed statistically significantly reduced total T4 (≥62.5 mg/kg), total T3 (≥62.5 mg/kg), albumin (3, 62.5, and 125 mg/kg), cholesterol (≥30 mg/kg), and triglyceride (≥10 mg/kg) levels, when compared to controls. PPAR signalling pathways were determined using foetal (GD21), neonatal (PND0), and maternal (GD21) livers and glucose metabolism was determined using foetal and neonatal livers by utilizing 84 target genes associated with each endpoint. Results indicated that four genes (Pck1, Pdk4, G6pc, and Pdp2) specific to glucose metabolism were statistically significantly upregulated (also the G6pc at ≥ 3 mg/kg) and one gene (Ugp2) was statistically significantly downregulated at ≥ 10 mg/kg in the GD21 foetal liver, when compared to controls. Gene expression was also compared to a previous study (Conley et al. 2019) in which PPAR

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		signalling pathways were assessed in foetal livers exposed to HFPO-DA on GD14-18. The comparison indicated that all 28 genes upregulated in the previous study were also upregulated in the current study, with 16 of the genes (Acaa2, Acadm, Acox1, Acsl1, Acsl3, Angptl4, Cpt1a, Dgat1, Ech1, Ehhadh, Fads2, Gk, Mlycd, Pck1, Rxrg, Scd1) being statistically significantly upregulated, when compared to controls and at a greater effect on GD21 than at GD18. A statistically significantly upregulated housekeeping gene (Ldha) was also reported to be more highly upregulated on GD21, when compared to GD18. Gene expression of glucose metabolism and PPAR target genes in newborn pup (PND0) liver indicated that 11 genes were statistically significantly different from controls across all treatment groups at GD21. Gene expression for PPAR signalling pathways were also statistically significantly different for 21 genes across all treatment groups at GD21, when compared to controls. PPAR signalling pathway genes (19 genes) of maternal livers that were reported upregulated on GD18 in the comparison study (Conley et al. 2019), were shown to be statistically significantly upregulated on GD21 in the current study. HFPO-DA concentrations in maternal serum and liver were increased, but not significantly, at GD17-21 and GD8-PND2, indicating that bioaccumulation was not observed at a longer exposure duration. Comparison of GD21 (current study) and GD18 (Conley et al. 2019) foetal serum HFPO-DA concentrations indicated that for a given maternal dose, the maternal serum concentration was 2-3 fold greater than the foetal serum concentration. A low lactational transfer and/or rapid neonatal clearance was indicated by the reduced PND2 liver concentrations, when compared to the GD21 liver concentrations, regardless of sex.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Dams were weight ranked and stratified then randomly assigned to treatment groups to produce similar mean weights and variances given the range of dam body weights (typically ~10% coefficient of variation).

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	-	Authors didn't specify diet for vehicle control groups.
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	outcome data were reported completely without attrition or exclusion
Detection	6. Can we be confident in the exposure characterization?	++	yes, test substance purity and source were reported and methods for exposure were clear.
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 113: ROB analysis of (Conley et al., 2019)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Rats
	Strain (source):	Sprague-Dawley [Crl:CD(SD)] from Charles River laboratories
	Sex:	Time-mated females

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Doses:	0, 1, 3, 10, 30, 62.5, 125, 250, and 500 mg/kg/day
	Purity (source)	HFPO-DA ammonium salt (CAS: 62037-80-3; Product No.: 2122-3-09; Lit: 00005383) purchased from SynQuest laboratories. Purity was 100% as determined by the supplier via perchloric acid titration.
	Dosing Period:	Gestation Days 14-18
	Route:	oral gavage
	Diet:	NIH07 rodent diet and filtered (5µm) municipal tap water ad libitum.
	Controls:	water
	Funding source:	This work was supported by the U.S. EPA Chemical Safety for Sustainability Research Action Program under the Adverse Outcome Pathway Discovery and Development task. B.S.M. and G.S.T. were supported by the Intramural Research Program of the National Institutes of Health/National Institute of Environmental Health Sciences grants ZIAES102505-09 and ZIAES103316-01.
	Author conflict of interest:	The authors declare they have no actual or potential competing financial interests.
Health Outcome	Endpoints:	Foetal testis testosterone production, foetal testis gene expression, foetal and maternal liver gene expression, foetal body weight, maternal serum thyroid hormone, and lipid concentrations were evaluated across all dose groups in Blocks 1 and 2. Foetal plasma HFPO-DA concentrations were evaluated in Block 3. Maternal weight gain during dosing, reproductive output, maternal serum HFPO-DA concentration, and maternal liver weight at necropsy were evaluated in all blocks for all concentrations of HFPO-DA.
	Age at assessment:	90 days
	Number per group and sex:	A total of three blocks of 15 dams per block; The first block of dams was dosed with control, 62.5, 125, 250, or 500 mg=kg HFPO-DA (n= 3 dams for each). The second and third blocks of dams were dosed with control, 1, 3, 10, or 30 mg=kg HFPO-DA (n= 3 per dose per block). Total sample sizes were n= 9 for control, n= 6 for

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		1, 3, 10, 30 mg=kg, and n= 3 for 62.5, 125, 250, and 500 mg=kg HFPO-DA.
	Statistical analysis:	All values are reported as mean ± standard error (SE) and all statistical comparisons were conducted at a= 0:05 significance level except for PPAR pathway gene expression, which utilized a= 0:0001 to detect highly significant analysis of variance (ANOVA) results and a= 0:01 to determine pairwise differences of treatment as compared with controls for significant genes. Treatment effects as compared with control were identified using ANOVA in SAS (version 9.4; SAS Institute). Foetal and postnatal data were analysed using PROC MIXED to correct for the nested effects of individuals within litters (foetus/pup data nested within litter, litter as random variable); dam data were analysed using PROC GLM. Pairwise comparison of significant ANOVA results was performed using the least squares means (LSMEANS) procedure in SAS. GraphPad Prism (version 7.02; GraphPad, Inc.) was used to generate all figures and to conduct dose–response curve analyses. Foetal testis and maternal/foetal liver gene expression data were analysed using the comparative cycle threshold (CT) method. Briefly, delta CT values were calculated using the equation 2−DDCT and normalized to the mean CT value of the appropriate housekeeping genes. We selected housekeeping genes for each tissue and gene array that did not display a significant (ANOVA p> 0:01) treatment effect of HFPO-DA exposure (foetal liver =Actb, B2m; maternal liver =Actb, Hprt1, Rplp1; and foetal testis =Actb, Gusb, Ldha). Delta CT values were then converted to fold-induction by dividing the treated replicate delta CT by the mean delta CT of the control replicates for each gene. Fold-induction values were then then log10-transformed prior to ANOVA. Foetal testis testosterone production was normalized to the mean control concentration within a given block and analysed as percentage of control values across blocks. Maternal liver weight was analysed using body weight as a covariate within PROC GLM followed by pairwise comparison using LSMEANS, this analysis produces linear regressions of body weight versus liver weight for each dose group. Mean female AGD was subtracted

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		from individual male AGD measures to calculate percentage reduction as compared with control. Serum HFPO-DA concentrations in the mother and the foetus were analysed as a function of oral dose administered to the mother. We utilized nonlinear regression (exponential one-phase association) to describe the increase and saturation of serum HFPO-DA concentrations across the full oral dose range (1–500 mg=kg) for maternal serum. Foetal plasma HFPO-DA concentrations were only analysed in the low-dose range (1–30 mg=kg), which was better described using a linear uptake model. We compared the slopes of the low-dose linear regressions for maternal serum and foetal plasma HFPO-DA concentrations using GraphPad Prism.
	Control for litter effects:	Foetal and postnatal data were analysed using PROC MIXED to correct for the nested effects of individuals within litters (foetus/pup data nested within litter, litter as random variable.
	Statistical power:	$\alpha = 0.05$ except for PPAR pathway gene expression, which utilized $\alpha = 0.0001$ to detect highly significant analysis of variance (ANOVA) results and $\alpha = 0.01$ to determine pairwise differences of treatment as compared with controls for significant genes.
Results	Tabular form based on studies reported results	Results indicated that proliferator-activated receptor (PPAR) signalling pathways analysed in foetal and maternal livers from HFPO-DA exposed litters had a statistically significant dose-responsive up-regulation for 28 different genes in foetal livers and for 16 different genes shared between maternal and foetal livers. The most highly up-regulated genes shared by maternal and foetal livers were *Ehhadh* (55-fold in maternal liver) and *Cpt1b* (24-fold maternal liver). Statistically significantly up-regulated expression of genes associated with cell proliferation (Hspd1, Txnip) and fatty acid metabolism (Fabp3) was reported in the maternal liver but not in the foetal liver. Statistically significant expressions of varying up-regulated genes were reported at all dose levels, even the lowest dose group (1 mg/kg) for both foetal livers (Cpt1b, Acox1, Angptl4) and maternal livers (Ech1 and Rxrg), when compared to controls. There were no statistically significant differences between treated and control groups for the expression of genes for detecting phthalate-like effects in the foetal testis or for foetal testis testosterone production. Evaluation

ROB Appraisal of Study - In Vivo Experimental Animal Study			
		of maternal endpoints indicated statistically significantly increased liver weight (62.5, 125, 250, and 500 mg/kg/d) and statistically significantly decreased body weight gain (250 and 500 mg/kg/d), serum T4 (125, 250, 500 mg/kg/d), serum T3 (30, 62.5, 125, 250, and 500 mg/kg/d), serum triglycerides (500 mg/kg/d), serum HDL (250 and 500 mg/kg/d), serum cholesterol (250 and 500 mg/kg/d), and serum LDL (125, 250, 500 mg/kg/d), when compared to controls. When evaluated across all dose ranges, HFPO-DA concentration in maternal serum was reported to saturate at the higher dose levels with a plateau of 112±15 µg/ml. A linear response was reported in the lower dose ranges (0-30 mg/kg/day) for maternal serum and foetal plasma HFPO-DA concentrations, although the maternal slope was statistically significantly greater than the foetal slope. Following dose-response analyses, an effect concentration for an EC5 for the most sensitive endpoints (maternal liver weight, maternal liver gene expression, and maternal serum [T3]and [T4]) was estimated using maternal serum HFPO-DA concentrations. The most sensitive endpoints with an EC5 were ranked as follows: maternal [T3] (EC5= 3.8 µg/ml), liver Ehhadh expression (EC5=14.1 µg/ml), liver weight (EC5= 17.6 µg/ml), and [T4] (EC5= 17.8 µg/ml).	
ROB Questions and Responses - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Dams were weight-ranked and stratified then randomly assigned to treatment groups to produce similar mean weights and variances.
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	+	Authors didn't specify diet for vehicle control groups.
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	-	Limitations due to performing the experiment over multiple blocks. Outcome data were reported completely without attrition or exclusion.
Detection	6. Can we be confident in the exposure characterization?	++	yes
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	yes
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 114: ROB analysis of (Cope et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	mice
	Strain (source):	Naïve CD-1 mice
	Sex:	female
	Doses:	0, 0.2, 1.0, or 2.0 mg/kg/day GenX
	Purity (source)	GenX (Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate, CAS# 62,037-80-3) was purchased from SynQuest Laboratories (Florida, USA).
	Dosing Period:	gestational day (GD) 1.5 to GD17.5
	Route:	oral gavage
	Diet:	NIH-31 diet (Zeigler, PA, USA) and reverse osmosis deionized (RODI) water was provided ad libitum to dams during gestation and through weaning (PND 22). At weaning, offspring of all litters (50:50 by sex and number, when possible) were

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		randomly assigned to either a high fat diet (HFD, 60% kcal fat diet; 5.21 kcal/g diet; Product #D12492, Research Diets) or control low fat diet (LFD, 10% kcal fat diet; 3.8 kcal/g diet; Product #D12450B, Research Diets, NJ, USA; Table S1). Diets and RODI water were provided ad libitum postweaning.
	Controls:	control low fat diet (LFD, 10% kcal fat diet; 3.8 kcal/g diet; Product #D12450B, Research Diets, NJ, USA;
	Funding source:	This research was funded by NIEHS/DNTP (Z01-ES102785 [S.E.F.]).
	Author conflict of interest:	The authors report no conflicts of interest.
Health Outcome	Endpoints:	GenX serum and urine concentrations, weight gain, serum lipid parameters, body mass composition, glucose tolerance, white adipose tissue gene expression, and liver histopathology
	Age at assessment:	Not provided
	Number per group and sex:	target of n=10/group (5 male and 5 female when possible)
	Statistical analysis:	Data were analysed in R version 1.2.5019 (R Foundation for Statistical Computing; Vienna, Austria). Sample sizes for each endpoint are reported in the accompanying figure legends or tables. A threshold of $P < 0.05$ was used for determining statistical significance unless otherwise noted. For analyses of data obtained by a single measurement at a single time point (e.g. offspring serum and urine dosimetry, litter outcomes at PND 0.5, offspring liver weights, serum lipids, and body mass composition), data were analysed for main effect of treatment group using analysis of variance and the lme4 (Bates et al., 2014) and lmerTest packages (Kuznetsova et al., 20,117). Simultaneous tests for general linear hypotheses were corrected for multiple comparisons of means between all groups using Tukey contrasts in the package multcomp (Hothorn et al., 2009) or by Dunnett's test when comparing treatment groups to the vehicle control group in the same package. Jonckheere-Terpstra's trend test in the package clinfun was used to determine dose-response trends in QUICKI scores [44]. Mixed effects models were used in analyses of data when multiple pups per litter were sampled or

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		when individual pups were measured repeatedly for the same outcome over time (e.g. PND 5.5 pup weights and postweaning weight gain). Analyses of data collected after diet groups were assigned (PND 22 and older) were stratified a priori by offspring sex and diet group. Average PND 0.5 pup weights were calculated as the litter weight divided by the number of pups in the litter. Offspring weights measured after PND 0.5 were obtained from individual pups. Multiple individual pups per litter were weighed for each litter at PND 5.5, therefore pup weights were analysed using mixed effect models and included a priori fixed effects of treatment group and litter size and a random effects term for the dam (litter) using the lme4 package. Point estimates and 95% confidence intervals were determined from the final model using the Wald method. Offspring weights measured from PND 22 and later did not require a random effects term for the dam (litter), as only one pup per sex per litter was measured at these time points. Postnatal weight gain analyses were stratified by diet type and sex, then analysed using mixed effect models and included a priori fixed effects of treatment group and offspring age and a random effects term to account for repeated measures of the same individual using the lme4 package. Point estimates and 95% confidence intervals were determined from the final model using the Wald method. Liver pathology scoring was analysed using pairwise Fisher's exact tests between control and treatment groups, with post-hoc correction of P values using the Holm method [45]. This analysis was done using the RVaidememoire package [46]. RT-qPCR data was analysed using the delta delta Ct (DD Ct) method [43]. Delta Ct (D Ct) was calculated by normalizing to the Rpl19 housekeeping gene and mean D Ct was calculated for each gene in each animal. Mice were stratified by sex and diet, and within these sex and diet groups a one-way ANOVA with Dunnett's post hoc test was used to compare treatment groups to the vehicle using GraphPad Prism (version 8.4.2).
	Control for litter effects:	Analyses of data collected after diet groups were assigned (PND 22 and older) were stratified a priori by offspring sex and diet group.
	Statistical power:	$P < 0.05$
Results	Tabular form based on studies reported results	Results indicated that GenX concentrations in pup serum at PND 5.5 showed no significant differences between treated and control groups

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		and at PND22 there was no detectable GenX concentration in the serum of any treatment group. However, pup urine analyses showed a statistically significant increased GenX concentration in the 1.0 and 2.0 mg/kg treatment groups, when compared to controls. Pup body weight was statistically significantly reduced in 1.0 (6.3%) and 2.0 (6.7%) mg/kg GenX groups, following adjustment for litter size and including a random effects term (dam), when compared to controls. Body weight, liver weight, and relative liver weight of male and female pups at PND22 and in female pups at 6 weeks of age in the low and high fat diet groups were comparable to controls. However, male pups in the low fat diet group at 6 weeks of age showed a statistically significant increase in body weight (2.0 mg/kg), liver weight (1.0 and 2.0 mg/kg), and relative liver weight (1.0 mg/kg), when compared to controls. At 18 Weeks of age, there were no significant differences in body weight, liver weight, and relative liver weight between treated and control groups reported in female pups in either the high or low fat diet groups. Male pups at 18 weeks of age showed a statistically significant increase in body weight and liver weight in the 2.0 mg/kg treatment group fed low fat diets, when compared to controls. Male pups in the 2.0 mg/kg treatment group and fed the low fat diet showed a statistically significant increase in postnatal weight gain over time (PND22-Week 18), when compared to controls. Serum lipid analysis at PND22 showed a statistically significant decrease in triglycerides in all treatment groups in female pups and in the 1.0 and 2.0 mg/kg treatment groups in male pups, when compared to controls. A statistically significant increase in the HDL to LDL ratio was reported in females in the 2.0mg/kg group at PND22, when compared to controls. There were no other significant differences in cholesterol, HDL, or LDL levels reported in male or female pups in any treatment group at PND22, when compared to controls. At 6 weeks of age, a statistically significant increase in cholesterol levels was reported in male pups in all treated groups fed the low fat diet, when compared to controls. There were no other significant differences in HDL, LDL, HDL:LDL, or triglycerides in male pups 6 weeks of age fed either low or high fat diets, when compared to controls. A statistically significant increase in HDL to LDL ratio was reported in the 0.2 mg/kg group fed low fat diets and a statistically significant decrease in triglycerides in the 1.0 mg/kg group fed low fat

ROB Appraisal of Study - In Vivo Experimental Animal Study		
		diets was reported in female pups at 6 weeks of age, when compared to controls. No other significant differences in cholesterol, HDL, or LDL were reported in female pups 6 weeks of age fed either low or high fat diets. Cholesterol and HDL levels were statistically significantly increased in male pups at 18 weeks old in the 1.0 mg/kg treatment group fed low fat diets, when compared to controls. No other serum lipid levels were significantly different from controls in male or female pups 18 weeks old across all treatment groups and fed either diet. Fasting insulin levels in 18 week old male pups was statistically significantly increased in the 2.0 mg/kg GenX group fed the low fat diet and in the 1.0 mg/kg GenX group fed the high fat diet, when compared to controls. A statistically significant decrease in the quantitative insulin sensitivity check index (QUICKI) was reported for male pups in the 2.0 mg/kg group at week 18 fed the low fat diet, when compared to controls. There were no statistically significant differences in glucose tolerance between treated and control groups when measured as area under the curve at 9 or 14 weeks within sex or diet. There were no statistically significant differences between treated and control groups for body mass composition within sex or diet at Week 12. There were also no statistically significant differences in body mass composition in males fed the high fat diet or females fed the low or high fat diet at Week 17, when compared to controls. However, males in the 2.0 mg/kg treatment group and fed the low fat diet had statistically significantly increased body weight, fat mass, fat:lean, and percent fat and statistically significantly decreased percent lean at Week 17, when compared to controls. Results of liver pathology at Week 18 indicated a statistically significant increase in microvesicular fatty change in the 2.0 mg/kg group males fed the low fat diet and a statistically significant increase ($p<0.10$) in hepatocyte single cell necrosis in the 1.0 mg/kg group females fed the high fat diet, when compared to controls. Liver pathology results at Week 18 indicated a statistically significant increase in ALT and ALP levels in the 2.0 mg/kg group females fed the high fat diet, when compared to controls. No other differences in liver enzymes were reported for male or females across dose groups and fed either diet. Gene expression analysis of adipose tissue at Week 18 resulted in a statistically significant upregulated of Esrrg gene in all dose groups and of Pparg in

ROB Appraisal of Study - In Vivo Experimental Animal Study			
		the 1.0 mg/kg dose group of females fed the low fat diet, when compared to controls. Statistically significant downregulation was reported for the Mapk3 gene in the 2.0 mg/kg group of females fed the high fat diet and for Acaca, Acacb, Adipoq, Fasn, Insr, Irs1, Pparg, Rxra, and Srebf1 genes in the 2.0 mg/kg group of males fed the low fat diet, when compared to controls. A statistically significant upregulation was reported for Esr1 gene in the 1.0 mg/kg group of males fed the high fat diet, when compared to controls. Results of the effect of the diet at Week 18 indicated a statistically significant increase in mean body weights of male and females fed the high fat diet as compared to males and females fed the low fat diet.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Animals were assigned to experimenter-blinded color-coded treatment groups.
	2. Was allocation to study groups adequately concealed?	++	Animals were assigned to experimenter-blinded color-coded treatment groups.
Performance	3. Were experimental conditions identical across study groups?	+	Age at assessment was not provided, but did not affect study outcome.
	4. Were the research personnel and human subjects blinded to the study group during the study?	++	Experimenters and technicians were blinded to the identity of the treatment groups throughout the experiments
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	Outcome data were reported completely without attrition or exclusion.
Detection	6. Can we be confident in the exposure characterization?	-	The source and case were provided but not purity
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	yes

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 115: ROB analysis of (Blake et al., 2020)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Mice
	Strain (source):	CD-1
	Sex:	Female
	Doses:	0, 2, or 10 mg/kg
	Purity (source)	GenX (CAS #62,037-80-3) from SynQuest Laboratories.
	Dosing Period:	Embryonic Day (E) 1.5 to E11.5 or E1.5 to E17.5
	Route:	Oral gavage
	Diet:	National Institutes of health (NIH)-31 diet (Zeigler Bros., Inc.); Reverse osmosis deionized water ad libitum
	Controls:	deionized water only
	Funding source:	This work was funded under NIEHS Z0ES102785 (to S.E.F.) and University of North Carolina at Chapel Hill T32 ES007126 (to B.E.B.).
	Author conflict of interest:	Authors declare they have no actual or potential competing financial interests.
Health Outcome	Endpoints:	Gestational weight gain, maternal clinical chemistry, maternal liver histopathology, placental histopathology, embryo weight, placental weight, internal chemical dosimetry, and placental thyroid hormone levels.
	Age at assessment:	7.5 -15.5 weeks
	Number per group and sex:	Total number of dams dosed was not provided but experiment was conducted over two blocks to achieve n=11-13 litters per treatment group;

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Statistical analysis:	Data were analysed in R. Single-observation dam outcomes (e.g. liver weight, relative liver weight, implantation sites, resorptions, viable embryos, and internal dose metrics) were analysed by analysis of variance using the 1me4 and 1merTest packages. Simultaneous tests for general linear hypotheses were corrected for multiple comparisons of means using Tukey contrasts in the package multcomp.
	Control for litter effects:	The first approach assumed the absence of litter effects and considered each placenta evaluated within a treatment group to be a totally independent observation, regardless of its litter of origin. These data were analysed as counts using a generalized linear model with a Poisson regression using the package lme4 (Bates et al. 2014). The second approach considered the litter as the biological unit and compared the relative incidence of placental lesions [e.g., percent within normal limits (WNL)] to adjust for differences in the total number of observations across litters within and between treatment groups.
	Statistical power:	$p<0.05$ were considered statistically significant unless otherwise noted.
Results	Tabular form based on studies reported results	Results indicated the percent change (relative) in GWG in dams treated with 10mg/kg/day GenX was statistically significantly increased at E11.5, when compared to controls. After controlling for litter size or adjustment for repeated measures of relative GWG, litter size, and gestational/embryonic day in the mixed-effect models, a statistically significant increase in relative GWG was also reported in dams in the 10mg/kg/day GenX group at both time points, when compared to controls. Absolute and relative liver weights of dams were statistically significantly increased in all treated GenX groups at both time points, when compared to controls. Absolute and relative kidney weights of dams were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls. A statistically significant decrease in triglyceride levels was reported in all GenX treated groups at E11.5 and in the high dose group at E17.5, when compared to controls. Cholesterol and high density lipoprotein levels were statistically significantly increased in the 2 mg/kg/day GenX group at E11.5 and alkaline phosphatase levels were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls. There were

ROB Appraisal of Study - In Vivo Experimental Animal Study			
		no statistically significant differences reported in the number of implantation sites, viable embryos, non-viable embryos, or resorptions in any treatment groups at E11.5 or E17.5, when compared to controls. There were also no statistically significant differences reported for viable embryo weight, placental weight, or embryo:placenta weight ratios in any treatment groups at E11.5, when compared to controls. However, placental weights were statistically significantly increased and embryo:placenta weight ratios were statistically significantly decreased in the 10 mg/kg/day GenX group at E17.5, when compared to controls. The percent placentas within normal limits (WNL) were statistically significantly decreased and the percent abnormal placentas were statistically significantly increased in the high dose treatment groups as compared to controls, when placentas were evaluated independently without litter origin consideration. Placenta thyroid hormone evaluation showed that T4 levels were statistically significantly increased in the 10 mg/kg/day GenX group at E17.5, when compared to controls.

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Treatment groups were randomly assigned.
	2. Was allocation to study groups adequately concealed?	++	A colour was randomly assigned to treatment groups using a random sequence generator. Experimenters and dosing technicians were blinded to the treatment group to which the colour groups corresponded through the duration of the study, including necropsy.
Performance	3. Were experimental conditions identical across study groups?	+	Authors didn't specify diet for vehicle control groups.
	4. Were the research personnel and human subjects blinded to the study group during the study?	++	A colour was randomly assigned to treatment groups using a random sequence generator. Experimenters and dosing technicians were blinded to the treatment group to which the colour groups corresponded

ROB Appraisal of Study - In Vivo Experimental Animal Study			
			through the duration of the study, including necropsy.
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	-	Outcome data were reported completely without attrition or exclusion. Study authors noted limitations in the study included performing the experiment over two blocks, half-life variance, the amount of exposure to GenX, and interspecies differences.
Detection	6. Can we be confident in the exposure characterization?	+	The source and case were provided but not purity
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	yes
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 116: ROB analysis of (Guo, Chen, et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Mice
	Strain (source):	BALB/C; Vital River Experimental Animal Center (Beijing, China)
	Sex:	Male
	Doses:	0, 0.4, 2, 10 mg/kg/day
	Purity (source)	Sigma-Aldrich, CAS No. 67118-55-2, >97.0% purity

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Dosing Period:	28 days
	Route:	oral gavage
	Diet:	Not reported
	Controls:	Not reported
	Funding source:	This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences and the National Natural Science Foundation of China (21737004 and 21911530145).
	Author conflict of interest:	The authors declare no competing financial interest.
Health Outcome	Endpoints:	RNA sequencing, gene expression, Biochemical Assays and Bile Salt Metabolomics
	Age at assessment:	6 to 8 weeks
	Number per group and sex:	12
	Statistical analysis:	Statistical significance was determined using one-way analysis of variance (ANOVA), followed by multiple comparisons with Duncan's multiple range test.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered statistically significant.
Results	Tabular form based on studies reported results	The GenX treatment groups showed an increase in relative liver weight, and bile acid metabolism in all treatment groups,

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Groups were randomized
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	-	Authors didn't specify details regarding diet or water source and availability

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	outcome data were reported completely without attrition or exclusion
Detection	6. Can we be confident in the exposure characterization?	++	yes, test substance purity and source were reported and methods for exposure were clear.
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 117: ROB analysis of (Guo, Sheng, et al., 2021)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	Mice
	Strain (source):	BALB/C; Vital River Experimental Animal Center (Beijing, China)
	Sex:	Male
	Doses:	0, 0.4, 2, 10 mg/kg/day
	Purity (source)	Sigma-Aldrich, CAS No. 67118-55-2, >97.0% purity
	Dosing Period:	28 days
	Route:	oral gavage
	Diet:	Not reported

ROB Appraisal of Study - In Vivo Experimental Animal Study		
	Controls:	Not reported
	Funding source:	This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences and the National Natural Science Foundation of China (21737004 and 21911530145).
	Author conflict of interest:	The authors declare no competing financial interest.
Health Outcome	Endpoints:	Biochemical Assays; histopathology of the liver; RNA sequencing
	Age at assessment:	6 to 8 weeks
	Number per group and sex:	12
	Statistical analysis:	Statistical significance was determined using one-way analysis of variance (ANOVA), followed by multiple comparisons with Duncan's multiple range test.
	Control for litter effects:	Not applicable
	Statistical power:	Values of $p < 0.05$ were considered statistically significant.
Results	Tabular form based on studies reported results	The GenX treatment groups showed an increase in relative liver weight, and bile acid metabolism in all treatment groups,

ROB Questions and Responses - In Vivo Experimental Animal Study

Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	++	Groups were randomized
	2. Was allocation to study groups adequately concealed?	+	Not reported; however, the lack of allocation concealment at study start is not expected to appreciably bias results
Performance	3. Were experimental conditions identical across study groups?	-	Authors didn't specify details regarding diet or water source and availability
	4. Were the research personnel and human subjects blinded to the study group during the study?	+	Not reported, but lack of adequate allocation concealment was not expected to appreciably bias results for this study

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	++	outcome data were reported completely without attrition or exclusion
Detection	6. Can we be confident in the exposure characterization?	++	yes, test substance purity and source were reported and methods for exposure were clear.
	7. Can we be confident in the outcome assessment?	++	yes
Selective Reporting	8. Were all measured outcomes reported?	++	All measured outcomes were reported
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	There were no other threats to internal validity
Overall Risk of Bias Appraisal for study:	Tier 1 - Low risk of bias		

Table 118: ROB analysis of (Gordon, 2011)

ROB Appraisal of Study - In Vivo Experimental Animal Study		
Reference, Animal model, dosing	Species:	rats
	Strain (source):	Wistar
	Sex:	m/f
	Doses:	1, 3, and 10 mg/kg/day (males) and 10, 30, and 100 mg/kg/day (females).
	Purity (source)	98.5%, Dyneon LLC, Gendorf, Germany.
	Dosing Period:	daily for 90 consecutive days
	Route:	oral gavage
	Diet:	not stated
	Controls:	vehicle control group received water
	Funding source:	not stated

ROB Appraisal of Study - In Vivo Experimental Animal Study			
	Author conflict of interest:	The author is employed by 3M Company. ADONA is manufactured by Dyneon LLC which is a wholly owned subsidiary of 3M.	
Health Outcome	Endpoints:	sub chronic repeated dose toxicity	
	Age at assessment:	not stated	
	Number per group and sex:	10/sex/group	
	Statistical analysis:	Statistical analyses were performed using Dunnett's test for normally distributed continuous data, Steel's rank test for non-normally distributed continuous data, and Fisher's exact test for frequency data. Armitage's test (Armitage, 1971) was performed on selected histopathology data to evaluate trends. All analyses were two-sided, with $p < 0.05$ considered significant.	
	Control for litter effects:	not relevant	
	Statistical power:		
Results	Tabular form based on studies reported results	NOAEL for male was established as 10 mg/kg bw/day and for female as 100 mg/kg bw/day	
ROB Questions and Responses - In Vivo Experimental Animal Study			
Bias Domain	Question	Response	Judgement
Selection	1.Was the administered dose or exposure level adequately randomized?	yes +	
	2. Was allocation to study groups adequately concealed?	yes+	
Performance	3. Were experimental conditions identical across study groups?	yes ++	OECD, GLP study conducted in the CRO
	4. Were the research personnel and human subjects blinded to the study group during the study?	yes ++	OECD, GLP study conducted in the CRO
Attrition/Exclusion	5. Were outcome data complete without attrition or exclusion from analysis?	yes	OECD, GLP study conducted in the CRO

ROB Appraisal of Study - In Vivo Experimental Animal Study			
Detection	6. Can we be confident in the exposure characterization?	yes ++	OECD, GLP study conducted in the CRO, reported in sufficient details.
	7. Can we be confident in the outcome assessment?	yes ++	OECD, GLP study conducted in the CRO
Selective Reporting	8. Were all measured outcomes reported?	yes, +	OECD, GLP study conducted in the CRO, reported in sufficient details
Other Sources	9. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	yes, ++	
Overall Risk of Bias Appraisal for study:	++		Definitely Low risk of bias