Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 335-46-4) and Related Salts

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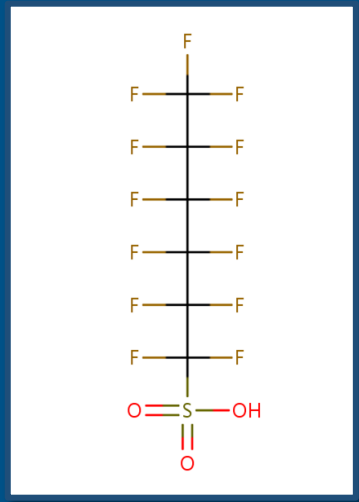
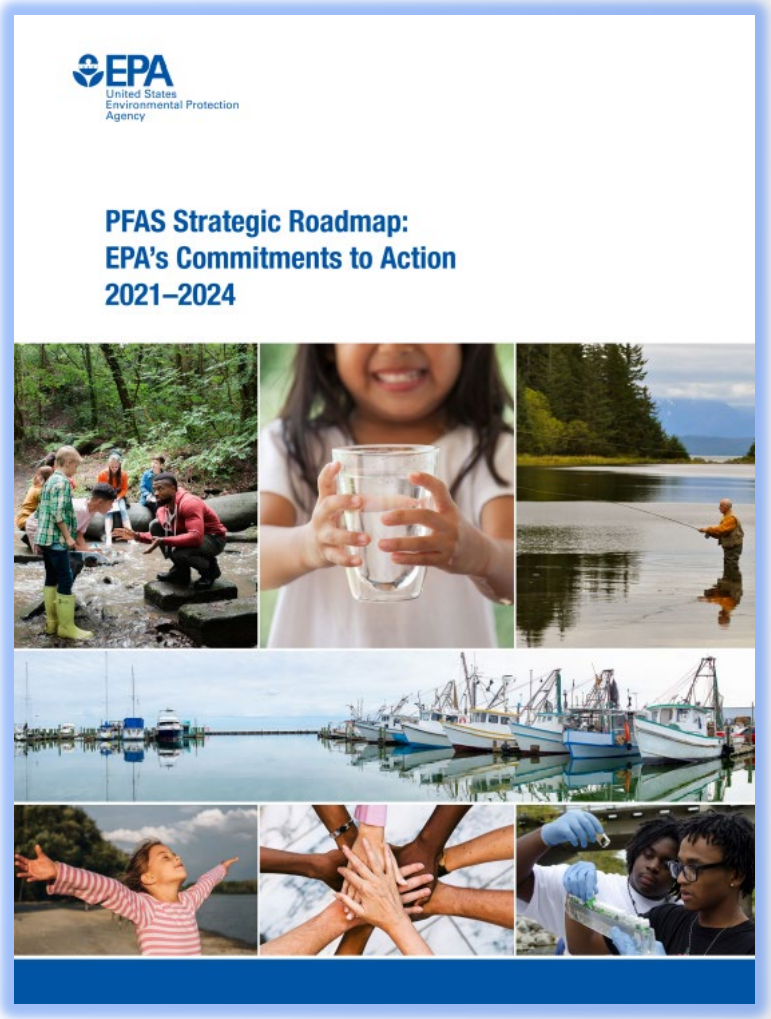


PFHxS and EPA's Broader PFAS Strategic Roadmap

- The IRIS assessment of PFHxS is being produced in parallel with separate IRIS assessments of four other PFAS, specifically PFBA, PFHxA, PFDA, and PFNA.
- The five IRIS assessments represent only one component of EPA's broader actions to address PFAS.
  - PFHxS was added to UCMR5 for public water system monitoring, which applies to 2022–2026 with sample collection occurring between 2023 and 2025.
  - As part of the National Defense Authorization Act for Fiscal Year 2020 (Section 7321), PFHxS was added to the EPA's Toxic Release Inventory list.
- For more information on the EPA's PFAS Strategic Roadmap, visit EPA's PFAS website.



https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

EPA Needs More PFAS Toxicity Information

- Decision-making on PFAS has been limited by narrow number of available human health toxicity assessments
- EPA's Office of Research and Development (ORD), which includes the IRIS Program, is developing federal, peer-reviewed toxicity assessments for priority PFAS
  - ORD assessments are used by EPA Programs and Regions in combination with nationwide- or site-specific exposure information and other considerations to set clean-up and regulatory values
- Developing assessments on individual PFAS cannot address the timing and extent (thousands of PFAS) of the need, but grouping of PFAS is hindered by lack of data
  - Tiered toxicity testing aims to fill data gaps and inform decisions on grouping and prioritization https://www.epa.gov/chemical-research/pfas-chemical-lists-and-tiered-testing-methods-descriptions
  - Systematic evidence maps collect and inventory the current data on thousands of PFAS

EPA-ORD Efforts on PFAS and Human Health

Individual Toxicity Assessments (e.g., IRIS)

- For PFAS with more robust datasets
- Toxicity values support regulatory decisions and can serve as index values in read-across for data-poor PFAS in their “group”

- New approach methods (NAMs) to fill data gaps
- Testing structurally diverse PFAS using in vitro toxicity and toxicokinetic assays
- Aids grouping for read-across and informs prioritization decisions

- Inventories available toxicity data across the broader PFAS class
- Parallels PFAS tiered toxicity testing
- Highlights data gaps and fit-for-purpose assessment opportunities for emerging PFAS of concern

[1]CCTE - Center for Computational Toxicology and Exposure

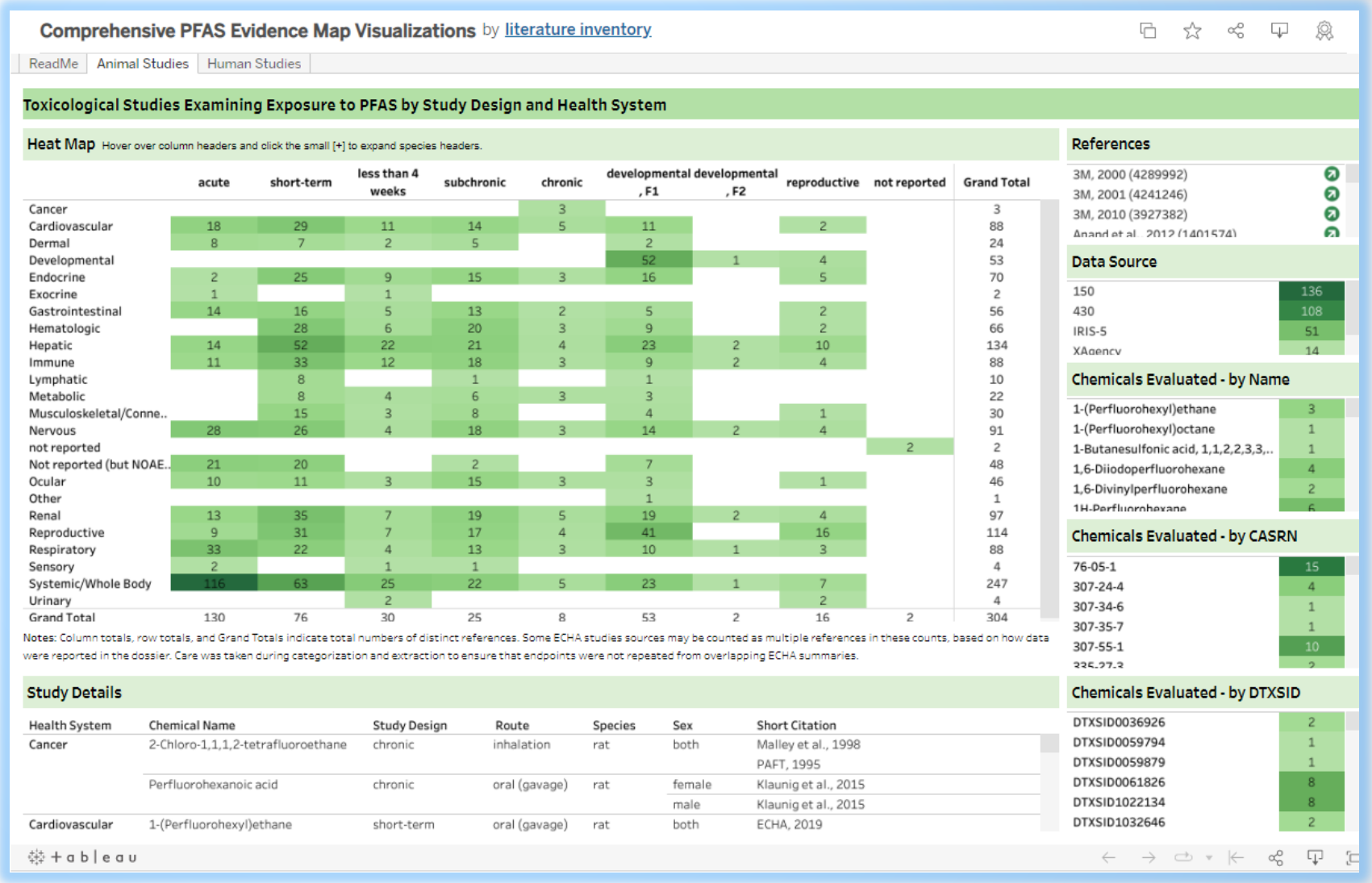
Per- and Polyfluoroalkyl Substances (PFAS) Systematic Evidence Map (SEM) Activities

- SEMs use systematic review methods to identify and summarize animal bioassay and epidemiological evidence. No hazard conclusions or toxicity values.
  - **PFAS 150[1, 2]:** Initial effort from CCTE identified ~150 PFAS chemicals testing a range of PFAS structures, chemistries, and with environmental relevance (first 75 chemicals described in publication by Patlewicz et al. 2019, Patlewicz et al. 2022)
  - **Expanded PFAS[3]:** Expanded effort that includes additional ~345 PFAS
  - **PFAS Universe[4]:** ~15,000 PFAS substances and structures includes most of the chemicals in the EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/chemical_lists/PFASSTRUCTv5)
- Specific goals and uses:
  - Create a repository that is easily updated, web-based, and shareable
  - Identify *in vivo* evidence to inform CCTE efforts to characterize PFAS library
  - Characterize data gaps and key research needs, including tiered toxicity testing
  - Be positioned to quickly address new PFAS assessment needs
- Key findings:
  - Many PFAS are data poor
  - Very few inhalation studies available

[1]Environ Health Perspect. 2022 May;130(5):56001. doi: 10.1289/EHP10343. Epub 2022 May 17. ; [2] Environ Health Perspect. 2022. https://ehp.niehs.nih.gov/doi/10.1289/EHP11185 Sept 2022. [3]Environ Health Perspect. 2024 Feb;132(2):26001. doi: 10.1289/EHP13423.
[4]Manuscript in development.

Combining PFAS Datasets Across EPA

- Comprehensive PFAS Dashboard
- Includes information from PFAS evidence maps, IRIS assessments, and other agency PFAS Assessments (GenX, PFOA, PFOS, PFBS, etc.)



Comprehensive PFAS Evidence Map Visualizations by literature inventory

ReadMe | Animal Studies | Human Studies

Toxicological Studies Examining Exposure to PFAS by Study Design and Health System

Heat Map Hover over column headers and click the small [+] to expand species headers.

	acute	short-term	less than 4 weeks	subchronic	chronic	developmental, F1	developmental, F2	reproductive	not reported	Grand Total
Cancer					3					3
Cardiovascular	18	29	11	14	5	11		2		88
Dermal	8	7	2	5		2				24
Developmental						52	1	4		53
Endocrine	2	25	9	15	3	16		5		70
Exocrine	1		1							2
Gastrointestinal	14	16	5	13	2	5		2		56
Hematologic		28	6	20	3	9		2		66
Hepatic	14	52	22	21	4	23	2	10		134
Immune	11	33	12	18	3	9	2	4		88
Lymphatic		8		1		1				10
Metabolic		8	4	6	3	3				22
Musculoskeletal/Conne..		15	3	8		4		1		30
Nervous	28	26	4	18	3	14	2	4		91
not reported									2	2
Not reported (but NOAE..	21	20		2		7				48
Ocular	10	11	3	15	3	3		1		46
Other						1				1
Renal	13	35	7	19	5	19	2	4		97
Reproductive	9	31	7	17	4	41		16		114
Respiratory	33	22	4	13	3	10	1	3		88
Sensory	2		1	1						4
Systemic/Whole Body	116	63	25	22	5	23	1	7		247
Urinary			2					2		4
Grand Total	130	76	30	25	8	53	2	16	2	304

Notes: Column totals, row totals, and Grand Totals indicate total numbers of distinct references. Some ECHA studies sources may be counted as multiple references in these counts, based on how data were reported in the dossier. Care was taken during categorization and extraction to ensure that endpoints were not repeated from overlapping ECHA summaries.

Study Details

Health System	Chemical Name	Study Design	Route	Species	Sex	Short Citation
Cancer	2-Chloro-1,1,1,2-tetrafluoroethane	chronic	inhalation	rat	both	Malley et al., 1998
						PAFT, 1995
	Perfluorohexanoic acid	chronic	oral (gavage)	rat	female	Klaunig et al., 2015
					male	Klaunig et al., 2015
Cardiovascular	1-(Perfluorohexyl)ethane	short-term	oral (gavage)	rat	both	ECHA, 2019

References

3M, 2000 (4289992)
3M, 2001 (4241246)
3M, 2010 (3927382)
Anand et al., 2012 (1401574)

Data Source

150	136
430	108
IRIS-5	51
XAgency	14

Chemicals Evaluated - by Name

1-(Perfluorohexyl)ethane	3
1-(Perfluorohexyl)octane	1
1-Butanesulfonic acid, 1,1,2,2,3,3,..	1
1,6-Diiodoperfluorohexane	4
1,6-Divinylperfluorohexane	2
1H-Perfluorohexane	6

Chemicals Evaluated - by CASRN

76-05-1	15
307-24-4	4
307-34-6	1
307-35-7	1
307-55-1	10
335-27-3	2

Chemicals Evaluated - by DTXSID

DTXSID0036926	2
DTXSID0059794	1
DTXSID0059879	1
DTXSID0061826	8
DTXSID1022134	8
DTXSID1032646	2

+ableau

https://hawc.epa.gov/summary/visual/assessment/100500256/Comprehensive-PFAS-Dashboard/ Shirke et al. 2024 https://doi.org/10.1289/EHP13423

Interactive Displays: Data Extraction

Chemical	Endpoint	Study	Animal Description	Route	Exposure Duration
6:2 Fluorotelomer alcohol	Liver Weight, Absolute	Mukerji et al. 2015	P0 Mouse, Crl:CD-1(ICR)BR (♀)	oral gavage	14d pre-mating, 14d mating, gestation, lactation
			P0 Mouse, Crl:CD-1(ICR)BR (♂)	oral gavage	109 d (premating-sacrifice)
		Serex T et al. 2014	Rat, Crl:CD(SD) (♀)	oral gavage	90 d
			Rat, Crl:CD(SD) (♂)	oral gavage	90 d
		Unnamed report (2005a) (ECHA summary)	Rat, Crl:CD(SD) (♂♀)	oral gavage	28 d
	Liver Weight, Relative	Mukerji et al. 2015	P0 Mouse, Crl:CD-1(ICR)BR (♀)	oral gavage	14d pre-mating, 14d mating, gestation, lactation
			P0 Mouse, Crl:CD-1(ICR)BR (♂)	oral gavage	109 d (premating-sacrifice)
		ECHA, 2007, 5701160	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
		Serex T et al. 2014	Rat, Crl:CD(SD) (♀)	oral gavage	90 d
		ECHA, 2007, 5701160	Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
		Serex T et al. 2014	Rat, Crl:CD(SD) (♂)	oral gavage	90 d
6:2 Fluorotelomer methacrylate	Liver Weight, Absolute	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
	Liver Weight, Absolute, Recovery	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
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			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
	Liver Weight, Relative, Recovery	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
Trifluoroacetic acid	Liver Weight, Absolute	Unnamed Report (2010a) (ECHA Summary)	P0 Rat, Crl:CD(SD)IGS BR (♀)	oral gavage	GD 6-19
		Unnamed Report (2012b) (ECHA Summary)	P0 Rat, Crl:CD(SD)IGS BR (♀)	oral gavage	up to 57 d (premating-lactation)
			P0 Rat, Crl:CD(SD)IGS BR (♂)	oral gavage	38 d (premating-termination)
		Saillenfait et al. 1997	P0 Rat, Sprague-Dawley (♀)	oral gavage	GD 10-20
			F1 Rat, Sprague–Dawley (♂♀)	oral gavage	GD 10-20
		Unnamed Report (2016a) (ECHA Summary)	Rat, Wistar Rj:Wi (Iops Han) (♀)	oral diet	90 d
			Rat, Wistar Rj:Wi (Iops Han) (♂)	oral diet	90 d
	Liver Weight, Relative	Unnamed Report (2012b) (ECHA Summary)	P0 Rat, Crl:CD(SD)IGS BR (♀)	oral gavage	up to 57 d (premating-lactation)
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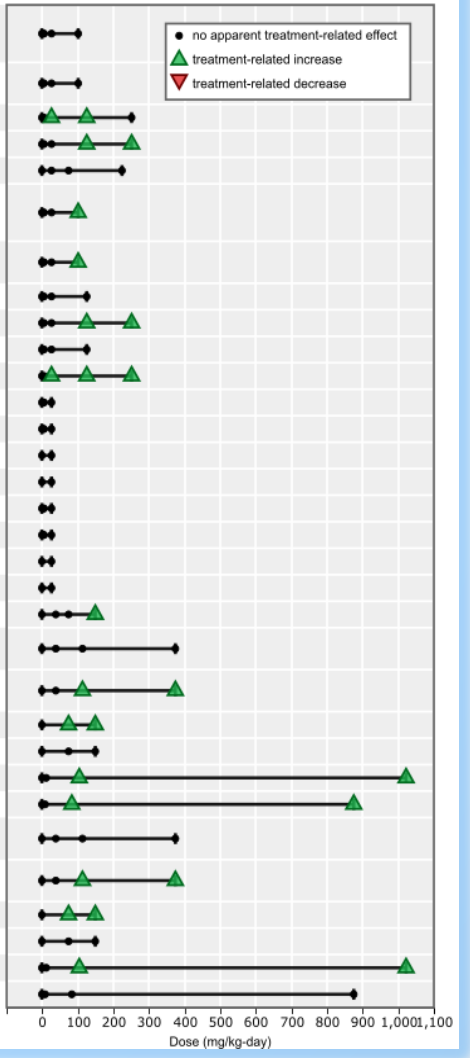

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6:2 Fluorotelomer methacrylate	Liver Weight, Absolute	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
	Liver Weight, Absolute, Recovery	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
	Liver Weight, Relative	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
			Rat, Crl:CD(SD) (♂)	oral gavage	28d (1dose/d)
	Liver Weight, Relative, Recovery	ECHA, 2007, 6299223	Rat, Crl:CD(SD) (♀)	oral gavage	28d (1dose/d)
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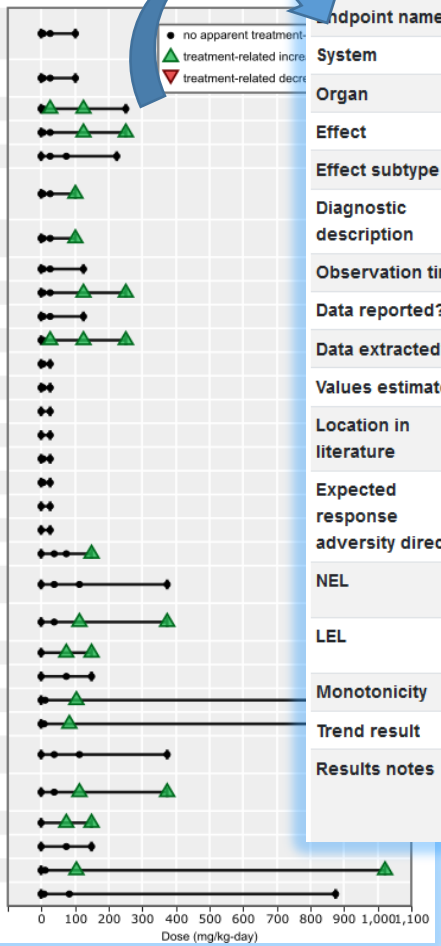
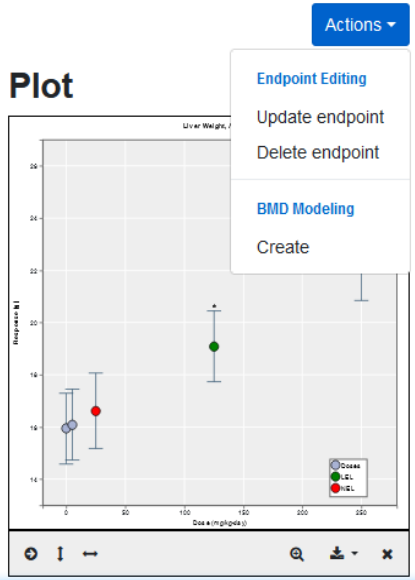


Liver Weight, Absolute

Endpoint Details

Endpoint name	Liver Weight, Absolute
System	Hepatic
Organ	Liver
Effect	Clinical Observation
Effect subtype	Organ Weight
Diagnostic description	Liver, Weight
Observation time	90 d
Data reported?	✔
Data extracted?	✔
Values estimated?	–
Location in literature	Table 5
Expected response adversity direction	---
NEL	25 mg/kg-day
LEL	125 mg/kg-day
Monotonicity	--
Trend result	not reported
Results notes	"Following 90 days of dosing, effects on organ weights were present in the testes, liver and kidney of males (Table 5) and in livers and kidneys

Plot



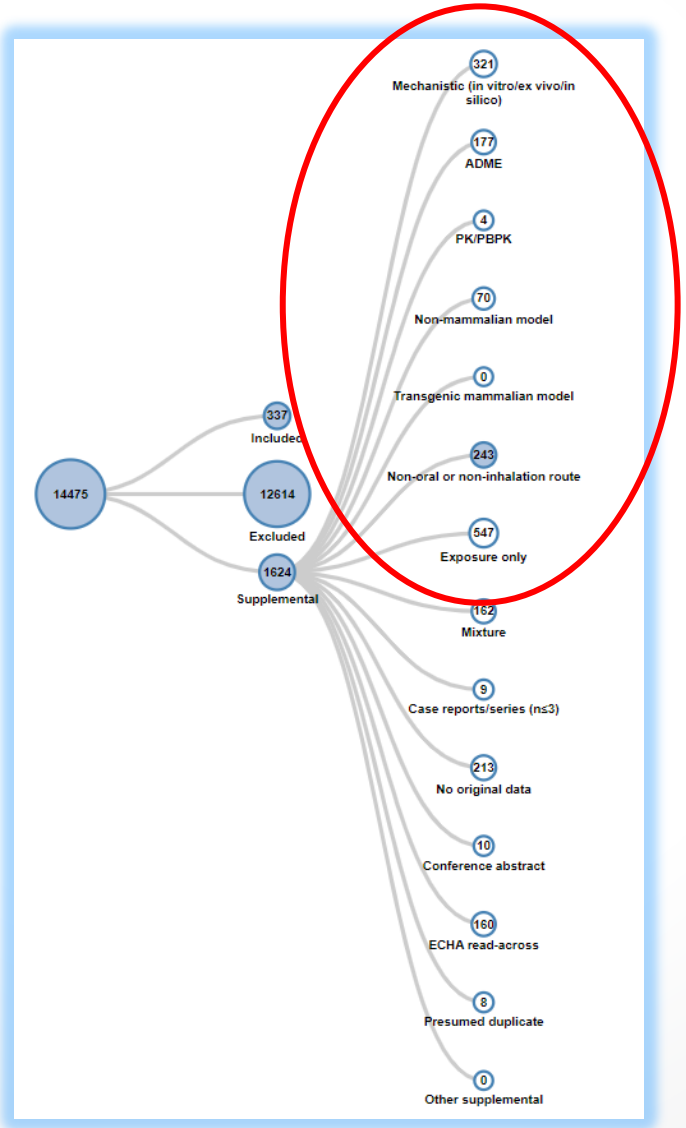
Dataset

Dose (mg/kg-day)	Number of Animals	Response (g)	Standard Deviation
0	10	15.94	1.9
5	10	16.09	1.9
25[a]	10	16.62	2.02
125[b,c]	10	19.09	1.89
250[b]	8	22.84	2.39

[a] NEL (No effect level)
[b] Significantly different from control ($p < 0.01$)
[c] LEL (Lowest effect level)

Carlson et al., 2022 Environ Health Perspect. 2022 May;130(5) May 17. PMID: 35580034; PMCID: PMC9113544.

Interactive Literature Tagtree



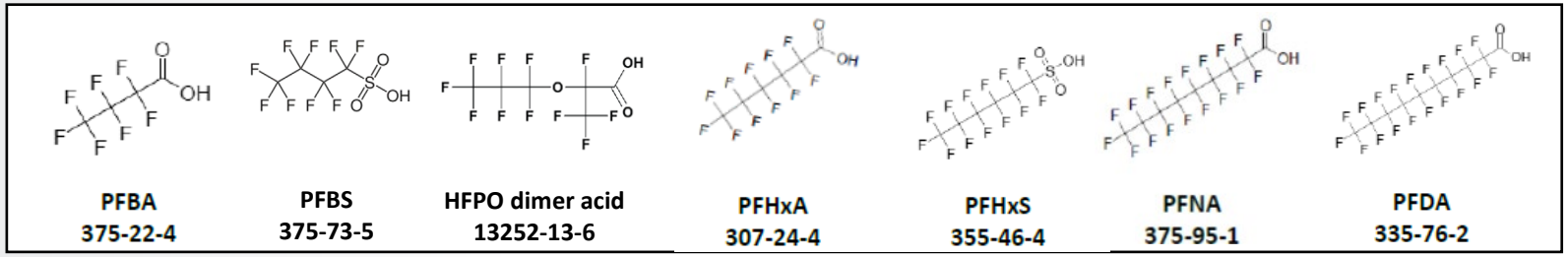
- **Created in 1985 to foster consistency in the evaluation of chemical toxicity across the Agency.**
- **IRIS assessments contribute to decisions across EPA and other health agencies.**
- **Toxicity values**
  - Noncancer: Reference Doses (RfDs) and Reference Concentrations (RfCs).
  - Cancer: Oral Slope Factors (OSFs) and Inhalation Unit Risks (IURs).
- **IRIS assessments have no direct regulatory impact until they are combined with**
  - Extent of exposure to people, cost of cleanup, available technology, etc.
  - Regulatory options.
  - Both of these are the purview of EPA's program offices.

Prioritizing EPA PFAS Toxicity Assessments

Prioritized PFAS (n=7) for EPA toxicity assessments (other than PFOA and PFOS):

- PFBS, GenX chemicals (Office of Water-led), PFBA, PFHxA, PFHxS, PFNA, and PFDA

To better inform read-across, cover a range of carbon chain lengths and functional groups



- These PFAS were selected by an EPA-wide workgroup (not identified by IRIS) based on:
  1. Identified as a priority to inform decision-making for EPA program or regional offices, tribes, or state departments of environmental protection (all 7 PFAS had multiple interested parties)
  2. Include studies of in vivo exposure in animals that could possibly be used to derive toxicity values
  3. Quantifiable in the environment using standardized analytical methods to allow for site-specific application of toxicity values to regulatory decision-making

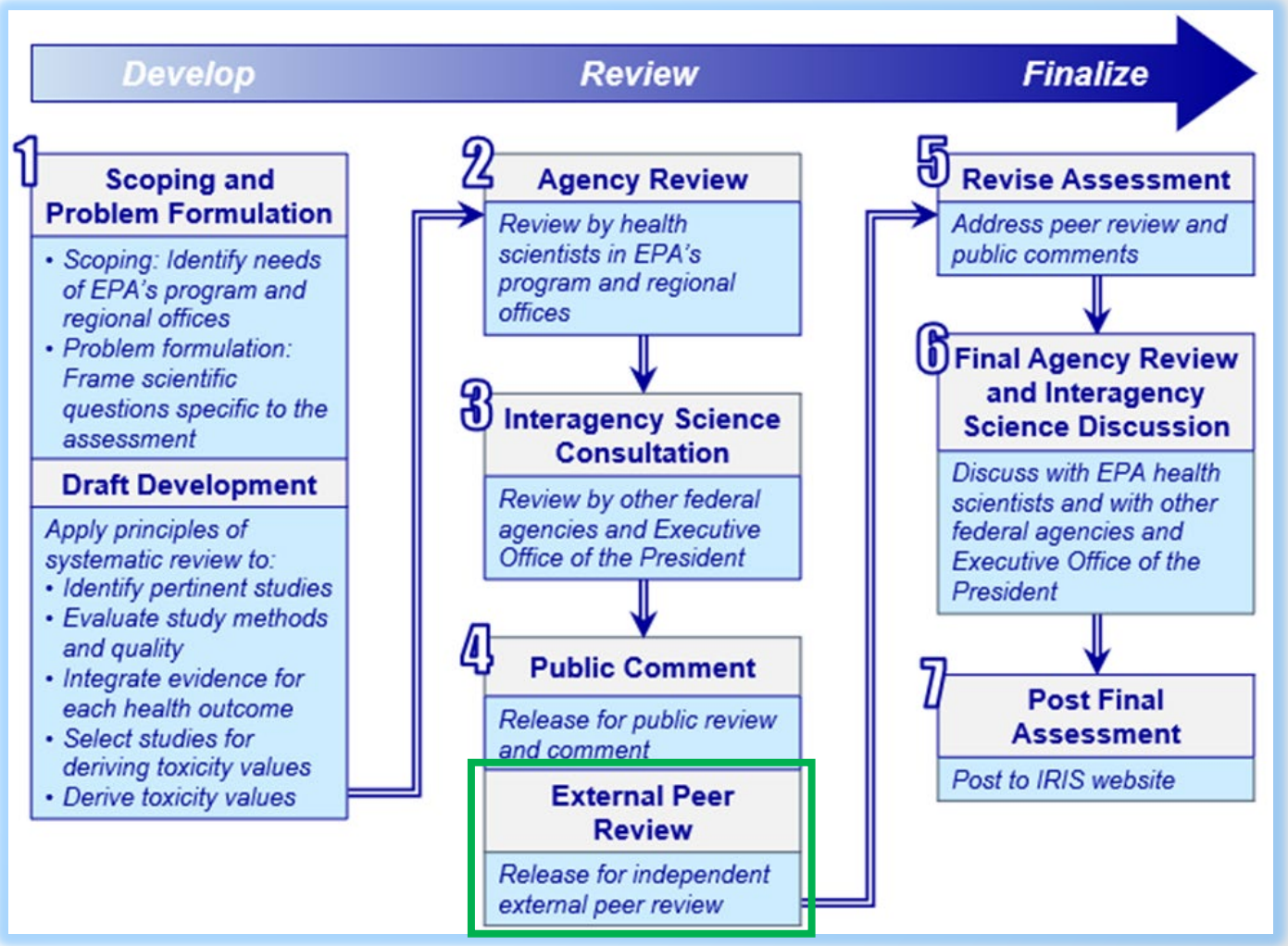
EPA Toxicity Values (ORD and OW)

PFAS	RfD (mg/kg-d)	Critical Effect
PFBA (ORD, ‘22 final)	0.001	Decreased serum total T4 and liver hepatocellular hypertrophy in adult rats
PFBS (ORD; ‘21; final)	0.0003	Decreased serum total T4 in postnatal day (PND)1 (developmental) F_1 mice
PFHxA (ORD; ’23; final)	0.0005	Decreased F1 body weight at PND0 in rats
GenX chemicals (OW; ’21; final)	0.000003	Constellation of liver lesions in F_1 female mice
PFOS (OW; ‘23; draft)	0.0000001	Low birth weight (developmental) and increased cholesterol (cardiovascular) in humans
PFOA (OW; ‘23; draft)	0.00000003	Decreased serum antibodies (immunodevelopmental), low birth weight (developmental), and increased cholesterol (cardiovascular) in humans
PFDA (ORD; ’23; draft)	0.0000000004	Decreased serum antibody concentrations and decreased birth weight in humans
PFPrA (ORD; ‘23 Final)	0.0005	Increased relative liver weight in male rats
HQ-115 (ORD; ‘23 Final)	0.0003	Decreased survival of offspring at PND 4 in rats
PFHxS (ORD; ’23; draft)	0.0000000004	Decreased serum anti-tetanus antibody concentrations in humans

Draft assessment for PFNA is expected in 2024.

IRIS Process

- PFHxS currently in Step 4
- PFHxS released for Public Comment in July 2023 (comment period ended September 2023)
- Received multiple sets of public comments to the public docket
- EPA compiled comments and organized by topic area
- Docket access and compilation of comments provided to Panel to consider during their review; compilation posted to docket

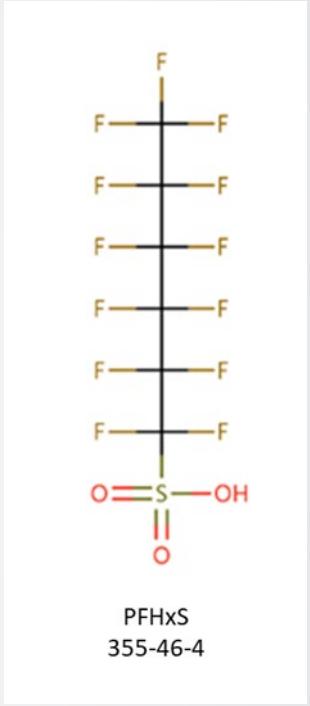


Available PFHxS Assessment Materials

- For peer review
  - Toxicological review: Primary analyses of the evidence for hazard identification and dose-response (IRIS PFHxS Assessment website)
  - Appendices: Supporting analyses and documentation
  - Charge Questions: Detailed questions provided to Panel to ensure feedback on all major conclusions and key areas of scientific complexity
- Other materials
  - EPA compilation of public comments submitted to the public docket
  - Handout of new studies submitted by public commentors
- Materials available in docket
  - Public comments

Introduction to PFHxS*

- PFHxS is man-made chemical belonging to the PFAS chemical family of compounds.
- PFAS have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water.
- PFHxS has been used as a surfactant to make fluoropolymers, in water- and stain-protective coatings for carpets, paper, packaging, and textiles; It has also been used in aqueous film-forming foam (AFFF) for fire suppression.
- PFHxS may also be present in certain industrial and consumer products, such as electronics, industrial fluids, food-contact papers, water-proofing agents, cleaning and polishing products either for intentional uses (as surfactants or surface protection agents) or as unintentional impurities from industrial production processes.



PFHxS
355-46-4

*The IRIS Program does not assess the following, which are the purview of other EPA offices performing risk assessment or risk management activities: Chemical production methods or releases to environmental media, Chemical removal or remediation methods, and exposure assessment

PFHxS Human Exposure*

- The general population may be exposed to PFHxS via inhalation of indoor or outdoor air, ingestion of PFHxS-containing drinking water and food, and dermal contact with PFHxS-containing products.
- Exposure to PFHxS may also occur via hand-to-mouth transfer of materials containing these compounds.
- The oral route of exposure is considered the most important route of exposure among the general population.

*Note: The IRIS Program does not conduct exposure assessments; that is the purview of other EPA offices

Summary of Assessment Conclusions

PFHxS Hazard Judgments – Charge Questions 2 and 7

Organ/System	Evidence integration judgment	Summary
Immune and thyroid effects	Evidence indicates (likely)	• See summaries on following slides • Organ/System-specific toxicity values were derived
Developmental*, hepatic, cardiometabolic, renal, and neurodevelopmental effects	Evidence suggests	• An RfD was not developed; However, a POD derived for developmental • No toxicity values were derived
Hematopoietic, female and male reproductive, and other health effects	Evidence is inadequate	• Some human/animal evidence available • Data are limited and/or largely null
Cancer	Inadequate Information	• Inconsistent evidence from human studies • No animal studies

Immune Effects – Charge Question 2a

Human Evidence: *moderate*

- Based on generally consistent evidence for immunosuppression with PFHxS exposure based on lower antibody response in multiple medium confidence studies, supported by coherent but limited results for infectious diseases
- Inverse association between PFHxS exposure and antibody levels following vaccination in children.
- Studies reported higher odds of infectious disease or symptoms with higher PFHxS exposure.

Animal Evidence: *Indeterminate*

- Based primarily on two high confidence studies and one medium confidence study.

Overall: *Evidence indicates (likely)*

Thyroid Effects – Charge Question 2b

Human Evidence: **Indeterminate**

- Some human studies report an inverse association between thyroid hormones and PFHxS exposure, but most of the available studies reported null findings.

Animal Evidence: **Moderate**

- Based on decreased Free T4 and serum T3 observed in rat studies
- Studies in rats reported significant decreases in TH levels. Decreased free T4 in both adult male and female rats; decreased serum T3 in adult male rats; no changes in TSH after PFHxS exposure. Developmental study showed decreased T4 in dams and their F1 offspring.

Overall: *Evidence indicates (likely)*

Developmental Effects – Charge Question 2c

Human evidence: **Slight**

- Based on consistent evidence for birth weight reductions, the most sensitive endpoint, with coherence across some other developmental endpoints (e.g., birth length, head circumference).
- Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth weight deficits.

Animal evidence: **Indeterminate***

- Mixed, but largely null results across studies using rats and mice.
- *Additional study raised for peer reviewers (see new studies table provided separately) expected to change animal evidence conclusion to *slight.* This would not affect overall conclusion.

Overall: *Evidence suggests*

Hepatic Effects - Charge Question 2D

Human evidence: **Slight**

- Based largely on consistent, but uncertain, increases in ALT in adults.
- Unexplained inconsistency for biomarkers other than ALT.
- Unclear biological significance of small changes in ALT.
- Direction of association with other liver biomarkers varied within and across studies.

Animal evidence: **Slight**

- Based on consistent, coherent, and dose-dependent increases in organ weight and related histopathology.
- Unclear biological significance (adversity) of histopathological changes (e.g., no necrosis observed) as well as the combined hepatic findings in animals across endpoints

Overall: *Evidence suggests*

Neurodevelopmental Effects – Charge Question 2E

Human evidence: **Slight**

- Some evidence of an association between PFHxS exposure or ADHD and related behaviors, but uncertainty remains.
- Other outcomes did not contribute to this judgment
- Unexplained inconsistency for outcomes

Animal evidence: **Indeterminate**

- 2 medium confidence studies reported no effects on functional observation battery parameters, motor activity, or learning and memory.
- Low confidence study observed decreases in spontaneous behaviors.

Overall: *Evidence suggests*

Cardiometabolic Effects – Charge Question 2F

Human evidence: **Slight**

- Generally consistent findings for total cholesterol in adults. Evidence for other related outcomes and age groups is inconsistent.
- Lack of coherence across outcomes in low confidence studies.
- Unexplained inconsistency among studies.

Animal evidence: **Indeterminate**

- No observed PFHxS-induced effects on heart weight or histopathology in short-term, potentially insensitive studies.
- Unclear biological significance of dose-dependent decreases in serum cholesterol and triglycerides.
- Inconsistent findings across studies

Overall: *Evidence suggests*

Hematopoietic Effects, Male and Female Reproductive Effects, and Renal Effects – Charge Question 2g

Noncancer health effects	Evidence integration summary judgement
Hematopoietic Effects	Human evidence: Indeterminate
	Animal evidence: Slight
Female Reproductive Effects	Human evidence: Indeterminate
	Animal evidence: Indeterminate
Male Reproductive Effects	Human evidence: Indeterminate
	Animal evidence: Indeterminate
Renal Effects	Human evidence: Slight
	Animal evidence: Indeterminate

Overall (for each noncancer health effect above): *Evidence is inadequate*

Evaluation of PK approaches for estimating human equivalent doses (HEDs) – Charge Question 5

Two PK modeling approaches were considered in the draft review

1. Published PBPK models were evaluated for estimation of PFHxS dosimetry in experimental animals and humans but did not pass QA / validation
    - The PBPK model of Kim et al. (2018) failed to predict PFHxS dosimetry after IV dosing.
    - EPA believes the key issue is how to best account for plasma protein binding. EPA's judgment: significant additional research is needed to resolve the issue.
2. A classical PK model was also developed and evaluated
    - PK model predictions for adult rats did not match NTP PK (validation) data well.
    - PK model poorly fit the mouse developmental PK data (note: no validation for PFHxS were available; the only such validation data were for PFNA).
    - Hence, the PK model generally did not appear to be sufficiently accurate.

DDEF approach for extrapolation – Charge Question 5

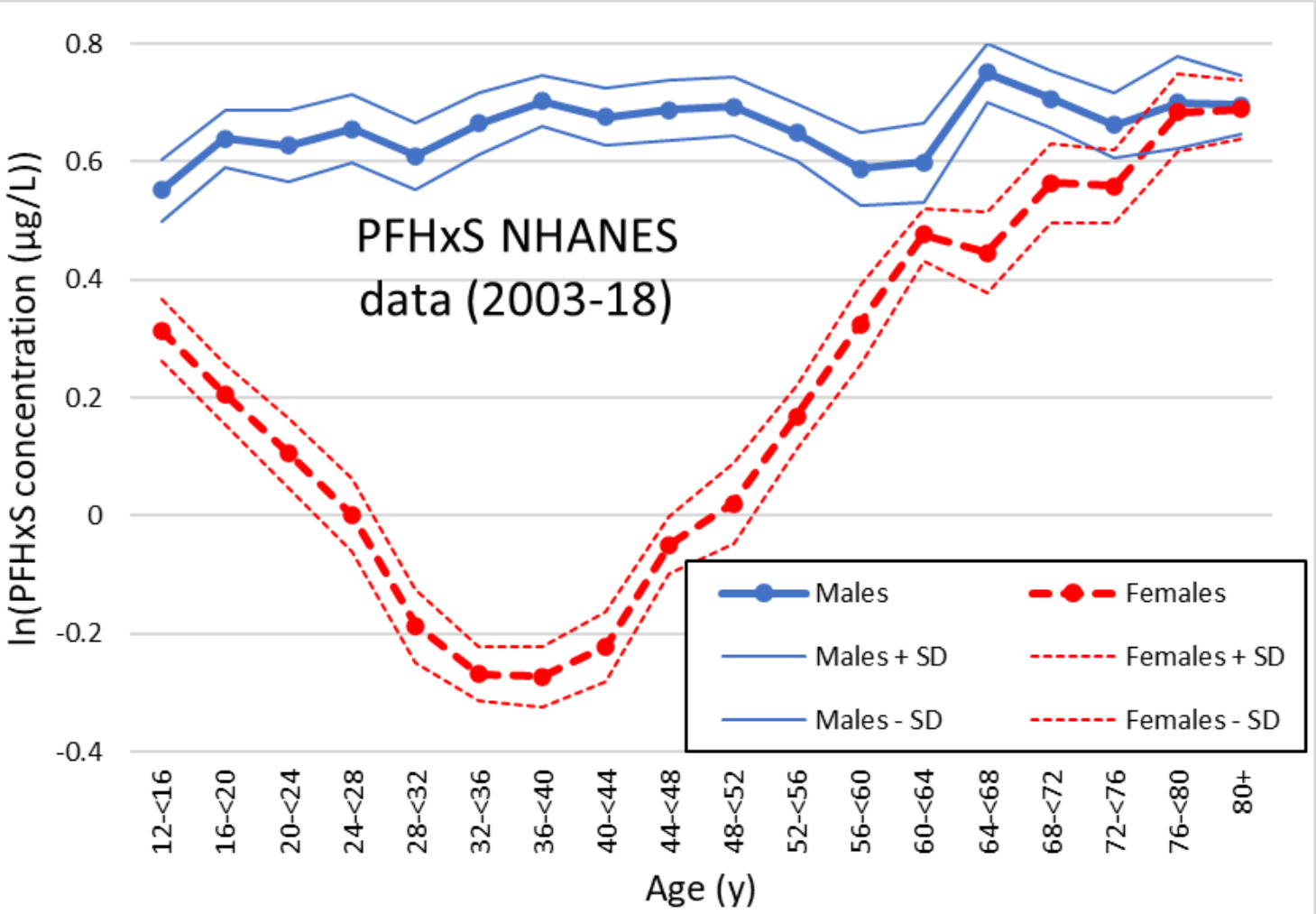
A data-derived extrapolation factor (DDEF) approach was therefore selected as the best alternative for HED calculation from animal toxicity endpoints

- Each DDEF is the ratio of clearance in humans (CL_H) for the appropriate sex and lifestage to clearance in the animal species and sex of endpoint observation ($CL_{species,sex,endpoint}$).
- CL for male & female rats & mice from hierarchical Bayesian model to include all available data.
- For women of reproductive age (12.4-50 y), CL_H was assumed to be increased by 0.033 mL/kg-d based on estimated menstrual fluid loss from Verner et al. (2015).
- A weighted geometric mean total CL_H was estimated for men and women outside of reproductive age while attempting to incorporate all available data.

Further Evidence for Higher CL in Women - Charge Question 5

NHANES serum concentration data for never-pregnant women vs. men indicate significantly higher clearance in women of reproductive age.

Using the CL_H values estimated in the draft, a difference of 0.6 log-units is predicted at steady state for men vs. women of reproductive age, given the same exposure.



Clearance & DDEF Values - Charge Question 5

Clearance & DDEF values in current draft for animal-human extrapolation

Sex	Species	Animal clearance ($CL_{A[s]}$) (mL/kg-d)	Human clearance (CL_H) (mL/kg-d)	DDEF (CL_H:$CL_{A[s]}$)
Male	Rat	7.15	All men, women age < 12.4 or > 50 y: **0.041**	5.73×10^{-3}
Female	Rat	84.1		4.88×10^{-4}
Female	Rat	84.1	Women, aged 12.4-50 y: **0.074**	8.80×10^{-4}
	Mouse	3.18		1.8×10^{-2}

Endpoint dependence:

- Endpoints in adult female animals (e.g., hepatic) are assumed to be relevant to adult women of any age, so the lower CL_H of women > 50 y was used.
- For in-utero effects, CL of the human mother (reproductive age) was used, since it determines exposure of both male and female fetuses.
  - Reflects recent human data for PFHxS collected over the course of pregnancy and post-partum period.
  - Specifically addressed in charge questions.

HEDs for PODs obtained from epidemiological analyses were also calculated using the set of CL_H shown on the previous slide

- Simulations with EPA's PK model, using human parameters, predicted that human serum levels approach steady-state given chronic exposure.
- While specific details of human PK may be uncertain, this general feature suggests that the assumption of steady-state in humans is sufficiently health-protective while avoiding uncertainties that exist for specific simulations, such as PK in children.
- For points of departure based on human serum concentrations, CL_H for the lifestage at which the biomarkers were collected is used to estimate the HED.

Dose-Response Decisions for Immune Effects

- **Consideration of confounding across PFAS for antibody response**
  - See human evidence synthesis for Hazard Identification: Section 3.2.2, page 3-77
  - Impact on BMD modeling approaches: Appendix Section D.1.1, pages D-2 to D-3, D-9, D-12 to D-13, D-15
    - Note that dose-response modeling results were available both with and without control of PFOS & PFOA, and that PFNA did not find immune effects.

Dose-Response Decisions for Immune Effects

- **Benchmark Dose Modeling Approaches**
  - Study and Dataset selection
    - Summary: Section 5.2.1, pages 5-5 to 5-7 (anti-tetanus and diphtheria antibody concentrations at age 7, PFHxS measured at age 5)
    - The key dose-response study by Budtz-Jørgensen et al, (2018) was based on the combination of two birth cohorts Grandjean et al., (2012) and Grandjean et al., (2017)
    - Two windows of exposure were evaluated: 1) PFHxS in 5-year old children and antibodies in 7-year old children, and 2) perinatal PFHxS and antibodies in 5-year old children.
    - Using information from Budtz-Jørgensen et al., (2018) with supplemental details provided by the authors, EPA derived eight total POD values for the two-time windows, two types of antibodies, and with and without statistical control of PFOS & PFOA.
  - Selection of the BMR (aka level of minimal adversity)
    - In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, EPA used BMR of 1/2 SD and BMR of 1 SD.
    - Rationale and discussion of alternative BMRs: See BMD modeling approaches: Section D.1.1 , pages D-3 to D-8 (for tetanus antibody concentrations at 7 years and PFHxS measured at 5 years, similar sections available for other models)

Dose-Response Decisions for Immune Effects

- Summary of results and decisions: Complete results are in Appendix D.1.1. and the four selected POD values are presented in Table 5-5 in the main document.
  - The $BMDL_{½SD(HED)}$ of 1.16×10^{-8} mg/kg-day for decreased anti-tetanus antibodies at age 7 and PFHxS at age 5 is selected for the derivation of osRfDs for immune effects. Confidence in the BMDL estimate was highest (medium confidence)
  - The $BMDL_{½SD(HED)}$ of 1.23×10^{-8} mg/kg-day for decreased anti-diphtheria antibodies at age 7 and PFHxS measured at age 5 is also selected for the derivation of osRfDs for immune effects. Confidence in this BMDL estimate was somewhat lower (medium/low confidence) (see Appendix D, Section 1.1 for more details).

- **Strengths and Uncertainties of the Selected POD**
  - An osRfD of 4×10^{-10} mg/kg-day for developmental immune effects was selected based on decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children.
  - The confidence decisions about the study, evidence base, quantification of the POD, and overall RfD for these organ-/system-specific values are described in detail in Table 5-8, along with the rationales for selection of confidence levels.

Intra-Human Uncertainty Factor (UF_H) – Charge Question 6b

- For developmental immune effects in children, a UF_H of either 3 or 10 was considered.
  - Children's immune systems are not fully formed and thus they are expected to be more sensitive to these effects than most other lifestages, leading to consideration of a $UF_H < 10$.
  - However, there are currently no data to compare the responses in children with other lifestages, nor to quantify differences in sensitivity across individual children.

- **<u>Conclusion</u>**
  - **UF_H = 10 applied to address potential differences in pharmacokinetics and pharmacodynamics relating to PFHxS exposure in humans**

Duration Extrapolation Uncertainty Factor (UF_S) for Immune Effects – Charge Question 6c

- The reduced antibody responses were measured in children 5-7 years of age, but PFHxS has a long half life and is expected to accumulate in the body through adulthood.
- The HED calculations used for immune effects assume chronic exposure and the RfD derived for these effects assures that serum PFHxS levels remain below the POD irrespective of exposure duration
- Early life exposure periods (i.e., prenatal, neonatal, juvenile and adolescent period) are recognized as a susceptible lifestage for developmental immunotoxicity.
- The observed effects on immune response are considered to be the result of a cumulative, prolonged exposure of the children and their mothers

Duration Extrapolation Uncertainty Factor (UF_S) for Immune Effects – Charge Question 6c (*continued*)

Conclusion:

- A UFs =1 is applied to derive osRfDs for immune effects in children
- Uncertainties regarding other susceptible lifestages (e.g., advanced age) are addressed as part of the database uncertainty factor (UF_D)

Organ-Specific and Overall Toxicity Values – Charge Question 3 and 4

Organ/System Integration judgement	Toxicity Value	Value (mg/kg-d)	Confidence	UF_C	Basis
		PFHxS			
Immune Evidence indicates (likely)	Lifetime osRfD	4×10^{-10}	Medium	30	Decreased serum anti-tetanus antibody concentrations in children (Grandjean et al., 2012; Budtz-Jørgensen et al., 2018)
	Subchronic osRfD				
Thyroid Evidence indicates (likely)	Lifetime osRfD	1×10^{-7}	Medium	100	Decreased serum Total T4 in F1 Wistar rats (Ramhøj et al., 2018)
	Subchronic osRfD				
Overall Lifetime RfD and Subchronic RfD		**4×10^{-10}**	**Medium**	**30**	**Immune Effects**

Newly Identified Studies: Literature Search Updates

- The evidence base for PFAS continues to rapidly evolve, complicating literature searches.
- The studies identified in the April 2023 literature search were screened using the PFHxS assessment PECO criteria. Studies that met PECO criteria or which were identified as supplemental material are documented Appendix B.3.
- Only studies impacting the key conclusions in the draft (i.e., changing which hazards are identified or notably affecting the final toxicity values) or informing the identified key science issues were incorporated into assessment, with documentation of these decisions in the Appendix.
- In the charge to external peer reviewers, EPA has asked the panel to comment on EPA's disposition regarding newer studies. This request applies to the studies in Appendix B.3.

Newly Identified Studies: Studies Identified by Public Commenters

- The studies identified by public commenters were screened using the PFHxS assessment PECO criteria, with decisions documented in a handout to the external peer reviewers and posted to the PFHxS docket
  - The handout includes EPA's disposition as to whether and why each study would change assessment conclusions in the public comment draft. Only studies impacting the key conclusions in the external review draft (i.e., changing which hazards are identified or notably affecting the final toxicity values) or informing the identified key science issues will be added to the final assessment.
- In the charge to external peer reviewers, EPA has asked the panel to comment on EPA's disposition regarding these studies.

Assessment-Specific Comments

Organized by Topic Area:

- Systematic Review Methods and Documentation **(4)**
- Background and Assessment Methods **(6)**
- Thyroid **(10)**
- Immune Effects **(6)**
- Developmental Effects **(3)**
- Hepatic Effects **(5)**
- Neurodevelopmental Effects **(2)**
- Cardiometabolic Effects **(2)**
- Other Noncancer Toxicity Effects **(3)**
- Carcinogenicity **(2)**
- Toxicity Values (General) **(2)**
- Noncancer Toxicity Values **(14)**
- Pharmacokinetics **(13)**
- Formatting, Editorial, and Text Clarifications **(3)**
- Risk Communication **(2)**

Note: Some comments may be counted more than once as they pertaining to multiple topic areas.

Thank you!

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