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REVIEW ARTICLE

Estimating safe doses of perfluorooctane sulfonate (PFOS): an international collaboration

Michael L. Dourson[1] · Laura C. Green[2] · Edmund A. C. Crouch[3] · Harvey J. Clewell[4] · Thomas Colnot[5] · Tony Cox[6] · Wolfgang Dekant[7] · Linda D. Dell[25] · James A. Deyo[8] · Bernard K. Gadagbui[1] · Helmut Greim[9] · Mahesh Rachamalla Gupta[10] · Tamara House-Knight[11] · Ashish Jachak[12] · Vijayavel Kannappan[13] · Travis R. Kline[14] · Therese Manning[15] · Ravi Naidu[16] · Paul Nathanail[17] · Chijioke Onyema[1] · Frank Pagone[12] · Andrew Pawlisz[18] · Tiago Severo Peixe[19] · Katie Richardson[20] · Anurag Sharma[21] · James S. Smith[22] · Nitin Verma[23] · Andrea Wojtyniak[24] · Jackie Wright[15]

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Abstract

Many government agencies and expert groups have estimated a safe dose (aka a "reference dose," [RfD]) for perfluorooctane sulfonate (PFOS). Notably, these agencies have derived safe doses that vary over at least 600-fold range. The range is larger still if one includes the U.S. Environmental Protection Agency (USEPA) current science-policy position under the Safe Drinking Water Act, which is that the only safe dose of PFOS is zero. This wide range in safe dose-estimates is surprising, since PFOS is a relatively well-studied, and ubiquitous, chemical. The Steering Committee of the Alliance for Risk Assessment (ARA) called for health-scientists interested in attempting to understand and, if possible, narrow this range of estimates. An advisory committee of eight scientists from four countries was selected from nominations received, and a subsequent invitation to scientists internationally led to the formation of three teams comprised of 24 scientists from nine countries. Each team independently reviewed toxicologic and epidemiologic data, and developed PFOS safe dose-estimates. All three teams concluded that currently available epidemiologic data could not form a reliable basis for PFOS safe dose-assessments. In contrast, results of bioassays of PFOS in laboratory monkeys and rats did provide usable bases from which serum-concentration-based "points of departure" were derived. After applying several, necessarily imprecise, uncertainty factors, the three groups derived PFOS safe dose-estimates that ranged, narrowly, from 20 to 100 nanograms (ng) of PFOS/kg body weight/day. In contrast, USEPA's current (United States Environmental Protection Agency (USEPA) (2024) Human health toxicity assessment for perfluorooctane sulfonic acid (PFOS) and Related Salts. EPA Document No. 815R24007.) estimate of the safe dose is 0.1 ng of PFOS/kg-day.

Keywords PFOS · Safe dose · Risk assessment · Regulatory policy · International differences

Introduction

Perfluorooctane sulfonic acid (PFOS) is a synthetic, sulfonated analog of the naturally occurring medium chain fatty acid, octanoic acid. Unlike the natural fatty acid, perfluorooctane sulfonic acid is fully fluorinated; is an extremely strong acid; and cannot be metabolized or otherwise used for energy production. Instead, ingested (or otherwise absorbed) PFOS (as the sulfonate) bioaccumulates, both in fish and other animals, including people, primarily by binding to albumin and other proteins in vivo (Geisy and Kannan 2001; Manzetti 2018).

High-level exposures to PFOS cause adverse effects in laboratory rodents and laboratory monkeys (ATSDR 2021; USEPA 2024); although whether people's essentially ubiquitous—and typically much lower-level—exposures to PFOS have harmed our health is uncertain.

Extended author information available on the last page of the article

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Many expert groups and agencies have estimated a safe dose[1] for PFOS. The estimates vary widely, as shown in Table 1, and have ranged from 0.1 nanograms/kg-day (USEPA 2024) to 60 ng/kg-day (Health Canada 2018). The range is wider still if one includes the policy-based determination made by USEPA (2024) under the Safe Drinking Water Act, which set a maximum contaminant level goal (MCLG) of zero, based on the USEPA's (2024) current judgment that PFOS is a likely human carcinogen, and that no amount of exposure to PFOS should be considered to be safe.

The various expert groups and regulatory agencies working on estimating safe doses of PFOS have differed with regard to their choices of:

(i) Key studies (whether epidemiologic or laboratory animal-based),
(ii) Critical adverse health-effect(s),[2]
(iii) Points of departure in measured and/or assumed exposure–response relationships, and
(iv) Various chemical-specific-adjustments and uncertainty factors.

As noted in Burgoon et al. (2023) for perfluorooctanoate (PFOA), the wide range of its estimated safe doses (from 0.0015 to 160 ng/kg-day) was a primary reason that the Steering Committee of the Alliance for Risk Assessment (ARA)[3] sought out expert health-scientists who might be able to narrow this range for PFOA and PFOS. Of course, it was recognized that some regulatory agencies adopt precautionary approaches, intentionally and substantially erring on the side of safety. Nonetheless, when safe dose-estimates vary by orders of magnitude, we felt it important to probe the bases for these differences.

Despite decades of study, there is still much to learn about the biological and toxicological effects of PFOS in humans and even in laboratory monkeys and other test-species. Thus, the guideline values currently set by regulatory authorities are likely to evolve and perhaps converge, based on increasingly relevant and reliable information.

The intent of the current work is to estimate a plausible range of PFOS safe doses. This range is intended to protect public health, including potentially vulnerable subpopulations, with an ample margin of safety.

Methods

As described in Burgoon et al. (2023), the Steering Committee of the Alliance for Risk Assessment (ARA) solicited nominations from potentially interested scientists, in the autumn of 2022, to form an advisory committee that would shepherd a project entitled "Range of the PFOA/PFOS Safe Dose."[4] After reviewing nominations, an advisory committee was selected, as listed in Supplement 1.

This committee in turn sought out potentially interested health-scientists to participate in an international collaboration to perform this work, focusing first on PFOA. The scientists worked in three teams, as described in Burgoon et al. (2023). The process was then repeated for PFOS, leading to the analyses presented herein.

Each of the three teams focused on (i) choosing key studies for critical toxicological effect(s) apparently caused by PFOS, (ii) evaluating mechanistic evidence regarding potential modes of action (MOAs) for the biological and pathophysiological effects of PFOS, and then (iii) choosing and implementing methods for extrapolating dose–response relationships from the key study or studies, including specifying the types and sizes of uncertainty/safety factors to be applied. These tasks were interspersed with periodic virtual meetings, during which the teams shared their independently developed ideas and interim results. The teams attempted to form consensuses if and when possible.

Results

The results provided below are summarized according to the charges given to the three teams. Teams worked independently on each charge, and then shared results prior to and during periodic virtual meetings.

[1] The term "safe dose" is used throughout to be a dose-rate (of PFOS, in this case) that is estimated to lie just below the population threshold for at which any adverse health effects are expected. In other words, it is a dose-rate set to protect the (presumed) most sensitive subpopulation against harm to their health from PFOS-exposure. The USEPA currently uses the term "reference dose" to connote this safe dose-estimate. All such estimates are derived using some combination of science-based and policy-based formulas. Because of this, complete uniformity across agencies and jurisdictions is not to be expected.

[2] Critical effect is defined here as the first adverse effect, or its known and immediate precursor, that occurs as dose is increased. It is recognized that multiple effects may be critical (occurring at or around the same dose), and that critical effects in laboratory animals may not reflect these same effects found or expected in humans. Nonetheless, if the critical effect is prevented, then it is assumed that all other adverse effects would be prevented.

[3] Please see: https://tera.org/Alliance%20for%20Risk/ARA_Steering_Committee.htm.

[4] Please see: https://www.tera.org/Alliance%20for%20Risk/Projects/pfoatwo.html.

Table 1 Safe Doses of PFOS, as estimated by various expert groups and agencies. Adapted from Dourson et al. 2024

Group and/or Agency	Estimated safe dose (ug/kg-day)	Point of departure (POD_{HED})	Uncertainty factors
Alliance for Risk Assessment (this paper, Table 3)	0.02–0.1	Various (see this text): 2.76 to 32.6 ug/ml of serum	Animal-human kinetic factor = 1 (a) Animal-human dynamic factor = 3 (b) Human toxicodynamic factor = 3 (c) Human toxicokinetic factor = 2.1 (d) Subchronic to chronic factor = 3 (e) Database uncertainty factor = 1 (f) Human clearance = 0.13 ml/day-kg (g)
Bundesministerium fur Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz 2022	0.02	Insignificance threshold values derived on the basis of human toxicological data	Group made a risk assessment call of 0.1 ug/liter This value can be used to estimate the comparable safe dose of ~ 0.02 ug/kg-day by multiplying by 2 L of water consumption per day, by dividing by 0.2 to adjust for a relative source contribution, and by dividing by a 60 kg body weight
European Food Safety Authority (EFSA 2020)	0.0006 (h)	BMD modeling is based on large epidemiological studies	None applied BMD from the general population included potentially sensitive subgroups and risk factors for disease rather than disease outcomes
Food Standards Australia New Zealand (FSANZ 2017)	0.02	0.60 ug/kg-day	Within human variability = 10 Animal to human extrapolation = 3
Health Canada (2018)	0.06	1.5 ug/kg-day	Within human variability = 10 Animal to human extrapolation = 2.5
NHMRC (2024)	0.001 (i)	0.29 ug/kg-day Extramedullary hematopoiesis and bone marrow hypocellularity based on modeled serum BMD10. (j)	Within human variability = 10 Animal to human extrapolation = 3 Subchronic to chronic = 10
US Environmental Protection Agency (2024)	0.0001	Various (human): 0.0012 ug/kg-day (increased serum cholesterol) 0.00113 µg/kg-day (low birth weight)	Within human variability = 10
World Health Organization (2022) (j)	No relevant and reliable health-effects-basis found for safe-dose-estimation		

[a] Factor is not needed since PODs are based on serum concentrations

[b] The use of 3 is the USEPA default position (USEPA, 2014); the IPCS (2005) default is 2.5

[c] The use of 3 is the USEPA and IPCS default position

[d] This value of 2.1 is derived as shown in Supplement 2

[e] This factor was used for the Seacat et al. (2002) monkey study, but a factor 1 for the longer-term rat studies

[f] Data base factor of 1 was considered appropriate for all PODs

[g] This clearance value of 0.13 ml/day/kg assumes steady state

[h] Sum of four PFAS: PFOA, PFNA, PFHxS, and PFOS

[i] It is recognized by NHMRC that there are large discrepancies between the USEPA (2024) estimated BMD_{10} and the lowest experimental NOAEL in the study, and that the reasons for this are not known. NHMRC (2024) identifies the NOAEL as the highest confidence value and the resulting safe dose would be 0.022 ug/kg-day. However, the more stringent value based on the BMD_{10} was used in the derivation of this draft—the reasons for this decision are unclear

[j] WHO, 2022 is apparently undergoing revision

Choice of studies for critical effect(s)

The teams struggled with whether epidemiologic studies involving PFOS could be relied upon for determining a safe dose for this PFAS. Unfortunately, none of these studies is of PFOS per se; and all are observational and environmental rather than experimental and/or occupational.

For example, some observational studies of birth cohorts in the Faroe Islands have reported increased odds of falling below a surrogate "threshold" level of protection (measured as antibody titers) against tetanus and diphtheria of 0.1 IU/mL, at a two-fold increase in serum concentrations of PFOS (Grandjean et al. 2012). This was a prospective study of a birth cohort in the Faroe Islands in which a total of 587 participated in follow-up through 2008. Geometric mean PFOS concentrations were around 17 ng/ml in serum. However, others have noted that immunity against tetanus and diphtheria is achieved at lower levels (at titers greater than 0.01 IU/mL; WHO 2009, 2018); and it is well known that secondary measures of immune function might be unreliable (Van Loveren et al. 1999). This point has been emphasized by Garvey et al. (2023), who again noted that a "vaccine responsiveness threshold" of 0.1 IU/ml is inappropriate in this context. Andersson et al. (2023) reported no association between people's responsiveness to COVID-19 mRNA vaccination and serum concentrations of PFOS or any of six other PFAS.

Zhang et al. (2023) reported that higher red blood cell folate concentrations "modified" an association between PFOS and decreased rubella and mumps antibodies, in that null associations were reported between individuals with higher red blood cell folate concentrations. Their results may suggest that the small decrements in vaccine responsiveness associated with increased PFOS in blood-serum concentrations might be due to increased folate concentrations, akin to the suggestion by Clewell (2024) of a pharmacokinetic bias with regard to PFOA.

These and other available epidemiological studies involving PFOS are difficult to interpret. For example, one regulatory agency, the European Food Safety Authority (EFSA), had derived a *provisional* tolerable daily intake (TDI) for PFOS based on apparently positive, associations between PFOS in serum and cholesterol in serum (EFSA 2018), but within 2 years then rejected this endpoint as a basis for human health risk assessment (EFSA 2020). Moreover, several cross-sectional occupational studies in PFOS production workers at higher levels than the general population, as summarized by USEPA (2024), have been conducted and generally reported mostly null or inconsistent findings with respect to liver, cardiac, cancer, and other effects.

Because of these inconsistent findings in humans, the three teams then turned to bioassays of PFOS in laboratory monkeys and rats, which were all at much higher doses (0.02–10 mg/kg-day average for the studies selected here) than the human observational studies, and generally higher than the occupational studies. The teams chose to rely on dose–response data from (i) a 6-month bioassay of PFOS in monkeys (Seacat et al. 2002), (ii) a lifetime bioassay of PFOS in rats (Butenhoff et al. 2012), and (iii) two-generation studies in rats (Lau et al. 2003; Luebker et al. 2005; Thibodeaux et al. 2003). These choices are listed in Table 3.

Two papers with half-life estimates for PFOS in humans (Li et al. 2022; Zhang et al. 2013), were also relied upon.

The teams' conclusions were that:

1. In laboratory animals, the critical effects for PFOS appear to be alterations in hepatic lipid metabolism and developmental delay.
2. For humans, the epidemiologic studies have yet to provide a reliable basis for human health risk assessment.
3. Translating PFOS-associated response in laboratory animals to potential health-risks in humans is best done on the basis of blood-serum concentrations of PFOS.
4. Serum-concentration–response relationships can be best obtained from these studies:
    - In monkeys, Seacat et al. (2002); and
    - In rats, Butenhoff et al. (2012), Lau et al. (2003), Thibodeaux et al. (2003) and Luebker et al. (2005).

Modes of action (MOAs)

Each of the three teams attempted to discern plausible MOAs for PFOS-induced adverse effects, focusing on MOAs likely to operate in humans, at environmental levels of exposure.

Team 1 noted that immune system effects in laboratory mice were critical effects relied upon in whole or in part by EFSA (2020) and USEPA (2024). However, no immune system-based MOA could be identified for either humans or mice, and as noted above, current epidemiologic studies are unreliable.

Teams 2 and 3 noted that PFOS disrupts lipid processing in the liver in laboratory rodents and monkeys, with effects similar to those of PFOA, involving activation of various nuclear receptors, including PPARα, PPARγ, CAR, FXR, LXR, and PXR (Andersen et al. 2021; Baratcu et al. 2024).

Due to species differences in PFOS-induced proliferation of peroxisomes, rats and mice (but not guinea pigs) are unsuitable models for humans with regard to metabolism of lipids and cholesterol (Corton et al. 2018). The responses in monkeys are likely to be more relevant for humans, although only relatively few PFOS-exposed monkeys have been studied, and none of these studies involved two generations.

After considerable discussion (and as listed in Table 2), consensus positions regarding MOAs were as follows:

1. In laboratory rats, mice, and monkeys, disruption of hepatic processing of fats and cholesterol is an MOA for PFOS.
2. Due to species-differences in proliferation of peroxisomes, rats and mice are more sensitive to the hepatic effects of PFOS than are guinea pigs, monkeys, and presumably, humans.
3. In humans, MOAs for PFOS exposures at environmental levels could not be reliably identified with confidence.

Choice of extrapolation method

The teams collectively discussed information developed by Team 2 that described the development of benchmark doses (BMDs) based on individual animal data gleaned from the laboratory reports of studies found in Table 3. These values are shown together with the study No Observed Adverse Effect Levels (NOAELs). All teams agreed that a 15–20% increase in liver weight with or without concurrent hepatocellular hypertrophy can be used as a relevant benchmark response (BMR) in the absence of other histopathological findings such as necrosis, inflammation, fibrosis, vacuolation, pigmentation, degeneration, hyperplasia, or other effects that are indicative of specific liver toxicity, and so this value was used in the development of these BMD for monkeys. This BMR is consistent with the interpretation of several experts (Hall et al. 2012). In general, these BMDs fall into the same range as the corresponding NOAELs, and in keeping with various agencies' guidelines the group preferred lower confidence limits (BMDLs) on these BMDs as points of departure.

Extended discussion then was initiated on the choice of uncertainty factors to be applied to the BMDLs. The resolution of this discussion was:

> For toxicokinetic variability between experimental animals and humans (UF_{ak}), serum concentrations from the experimental animal studies were assumed to be relevant for humans, and so no uncertainty factor was needed (i.e., $UF_{ak} = 1$).
> The toxicodynamic variability between experimental animals and humans (i.e., UF_{ad}), however, was needed. A default of 2.5 (IPCS 2005) or 3.0 (USEPA 2014) was suggested (i.e., $UF_{ad} = 3$).
> For human toxicokinetic variability (UF_{hk}), the development of a chemical specific adjustment factor (CSAF) was considered to be reasonable based on the variation in half-life seen in Li et al. (2022). The selected value ($UF_{hk} = 2.1$) was obtained from the ratio of the 97.5th percentile to the median of a lognormal distribution fitted to the individual half-life estimates for L-PFOS, combined

Table 2 International Collaboration Consensus Statements

Consensus on critical effect	In laboratory animals, the critical effects for PFOS appear to be alterations in hepatic lipid metabolism and developmental delay
	For humans, epidemiologic studies have yet to provide a reliable basis for human health risk assessment
	Translating PFOS-associated response in laboratory animals to potential health-risks in humans is best done on the basis of blood-serum concentrations of PFOS
	Serum-concentration–response relationships can be best obtained from these studies: • in monkeys, Seacat et al. (2002); and • in rats, Butenhoff et al. (2012), Lau et al. (2003), Thibodeaux et al. (2003) and Luebker et al. (2005)
Consensus on Modes of Action (MOAs)	In laboratory rats, mice, and monkeys, disruption of hepatic processing of fats and cholesterol is an MOA for PFOS
	Due to species-differences in proliferation of peroxisomes, rats and mice are more sensitive to the hepatic effects of PFOS than are guinea pigs, monkeys, and, presumably, humans
	In humans exposed environmentally, MOAs for PFOS could not be identified with confidence
Consensus on Extrapolation Method	A 15–20% increase in liver weight with or without concurrent hepatocellular hypertrophy, but with no other adverse effects, was used as a suitable BMR
	Benchmark doses and serum concentrations are preferred bases for extrapolation to a safe dose range for PFOS in humans
	Uncertainty factors for laboratory animals to humans and for various aspects of the database were developed by taking into account available data or the use of default positions of the IPCS (2005) and/or USEPA (2014)
	A geometric mean half-life estimate from Li et al. (2022) of 2.88 years was considered to be reliable for the development of the PFOS safe dose range; the corresponding clearance value is 0.13 ml/day-kg assuming a volume of distribution of 200 ml/kg

Table 3 Experimental animal studies as the basis of the provisional safe PFOS dose[a]

Study	Test species	Critical effect	NOAEL. (mg/kg-day)	NOAEL basis	POD (serum) (µg/mL)[a]	Uncertainty factors								Human serum RfD (µg/mL)	Clearance (mL/day/kg)[b]	RfD. (ng/kg-day)
						AK	AD	HK	HD	L	S	D	T			
Seacat et al. (2002)	Monkey	Increased liver weight	0.03 or 0.15	Liver weight at 0.15 mg/kg/day was 10% higher, but not statistically significant; however more severe liver effects at 0.75 mg/kg/day	13.2 (NOAEL) 21.1 BMDL-1SD 32.8 BMDL-20%)	1	3	2.1	3	1	3	1	60	0.58	0.13	70
Butenhoff et al. (2012)	Rat	Hepatotoxicity	0.021	Health Canada 2018 selection	2.63 (NOAEL) 2.76 BMDL-0.1	1	3	2.1	3	1	1	1	20	0.15	0.13	20
	Rat (male)	Hepatotoxicity	0.098	Hepatotoxicity at next highest dose (0.242 mg/kg/day)	13.6 (NOAEL)	1	3	2.1	3	1	1	1	20	0.72	0.13	90
	Rat (female)	Hepatotoxicity	0.12	Hepatotoxicity at next highest dose (0.299 mg/kg/day)	23.6 (NOAEL)	1	3	2.1	3	1	1	1	20	1.2	0.13	160
Lau et al. (2003) and Thibodeaux et al. (2003)	Rat	Embryo and fetal toxicity	1	Reduced pup survival, decreased body weight and eye-opening delay at next highest dose (2 mg/kg/day)	19.7 (NOAEL)	1	3	2.1	3	1	1	1	20	7.8E-01	0.13	140
Luebker et al. (2005)	Rat	Parental toxicity	0.1	Developmental effect (decreased body weight gain/food consumption in dams; decreased pup weight and weight gain during lactation) in next highest dose group (0.4 mg/kg/day)	4.52 (NOAEL)	1	3	2.1	3	1	1	1	20	2.3E-01	0.13	30

[a]Please see Supplement 2 for details of the various calculations for selected (bold) values

[b]$Cl = (0.692 \times V_d)/t_{1/2}$, where $t_{1/2} = 2.88$ yrs = 1,052 days, and where we assume that Vd = 0.2 L/kg = 200 mL/kg for both PFOA and PFOS. Therefore: Cl = ln(2) * 200/1051 = 0.13 mL/day/kg

[c]Rounded

with a factor 1.11 to account for the isomer mix observed in this study (Supplement 2).

For human toxicodynamic variability (UF_{hd}), a default factor of 3 (IPCS 2005; USEPA 2014) was considered reasonable since no data were available to suggest otherwise (i.e., $UF_{hd} = 3$).

For length-of-study-exposure (UF_s), a factor of 3 was considered to be appropriate for the monkey studies since the length of exposure in these experimental animals was sub-chronic. A factor of 1 was considered appropriate for the rodent studies since these were of sufficient length for the critical effects being monitored (i.e., $UF_s = 3$ for monkeys and $UF_s = 1$ for rodents).

For use of a Lowest Observed Adverse Effect Level (LOAEL) (UF_l), since the points of departure were BMDs and/or NOAELs a factor of 1 was considered to be appropriate.

For overall database (UF_d), a factor of 1 was considered to be appropriate, since multiple studies in various experimental animals were available that addressed the likely critical effects. The use of this factor is consistent with the judgment of other authorities.

Finally, for PFOS, a geometric mean half-life estimate from Li et al. (2022) of 2.88 years was considered to be reliable by all teams for the development of the PFOS safe dose range. This value was from 114 people exposed to drinking water contaminated with PFAS that had been distributed for decades to one third of households in Ronneby, Sweden. The overall conclusions on the extrapolation approach were that:

1. A 15–20% increase in liver weight with or without concurrent hepatocellular hypertrophy, but with no other adverse effects, was used as a suitable BMR.
2. Benchmark doses and serum concentrations were preferred bases for extrapolation to a safe dose for humans.
3. The uncertainty factors for laboratory animals to humans and for various aspects of the database were developed by taking into account available data or the use of default positions of the IPCS (2005) and/or USEPA (2014).
4. A geometric mean half-life estimate from Li et al. (2022) of 2.88 years was considered to be reliable for the development of the PFOS safe dose range; the corresponding clearance value is 0.13 ml/day-kg assuming a volume of distribution of 200 ml/kg.[5]

[5] $Cl = (\ln(2) \times V_d)/t_{1/2}$, where $t_{1/2} = 2.88$ yrs $= 1051$ days, and where we assume that $Vd = 0.2$ L/kg $= 200$ mL/kg for both PFOA and PFOS. Therefore: $Cl = \ln(2) * 200/1051 = 0.13$ mL/day/kg.

A safe dose range for PFOS

Per the above considerations, the PFOS safe dose range was estimated to be between 20 and 100 ng/kg body weight-day (or perhaps somewhat higher), as shown in Table 3. This safe dose range could be used to develop a range of safe levels in various environmental media, such as drinking water. For example, using typical assumptions of a conservative ingestion of 2 L of drinking water per day for an average 70 kg adult, and a "relative source contribution" of 20%, the safe concentration of PFOS in drinking water would be on the order of 140 to 700 ng/L (parts per trillion; ppt). Note that PFOS concentrations in typical U.S. diets are quite small; PFOS was detected at levels ranged from 0.134 ng/g in a boiled frankfurter to 0.865 ng/g in baked tilapia (FDA 2018). Thus, drinking water PFOS might be "permitted" to supply more than 20% of a person's daily PFOS-exposure. If so, then at least for most of us, our drinking water could contain more than 700 ng PFOS/L, and still be safe.

At present (2025), and in contrast, USEPA's maximum contaminant level (MCL) for PFOS is 4 ng/L.

Discussion

PFOS is persistent, bio-accumulative, and ubiquitous; but whether (and if so how) PFOS has harmed human health remains unclear. We, like others, assumed that PFOS could disrupt lipid processing in humans, as observed in bioassays using laboratory animals.

We also judged that the epidemiologic studies cannot yet serve as a reliable basis for human health risk assessment.

We consider that serum concentration–response data from PFOS-exposed laboratory animal bioassays can be used for purposes of human health risk assessment. Although mice and rats tend to be good models for humans for most chemicals, this is not true for PFOS and other PFAS. Monkeys are much better models; but, of course, the numbers of monkeys that have been PFOS-exposed are small; and the endpoints that have been examined remain limited.

The five PFOS bioassays listed in Table 3 were chosen for developing points of departure from serum levels (BMDL where possible, otherwise NOEL). Uncertainty factors were developed by taking into account available data or the use of default positions of the IPCS (2005) or USEPA (2014). A geometric mean human half-life of PFOS was developed from Li et al. (2022). Our resulting range in estimated safe doses for PFOS RfD is 20–100 nanograms of PFOS/kilogram body weight/day (0.02–0.1 µg/kg-day).

As shown in Table 1, the lower value of this range matches the value derived by (i) WHO (2022), (ii) Bundesministerium fur Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz (2022), and (iii) FSANZ

(2017). Safe doses derived by Health Canada (2018) of 0.06 µg/kg-day, and by Food Standards of Australia and New Zealand,[6] are also comparable to ours.

Our estimated safe-dose range is much higher than the safe dose estimated by EFSA (2020) and USEPA (2024). Largely, this is because those two agencies relied on selected, epidemiologic "evidence" for PFOS toxicity, whereas our teams were wary of the reliability of such reliance.

We note also that the UK Committee on Toxicology (2022) wrote:

> *"Whilst the COT is unable to suggest an alternative to the [EFSA] TWI [tolerable weekly intake] at this time, there are strong caveats when comparing the exposure estimates with the TWI established by EFSA. There is considerable uncertainty as to the appropriateness of the derivation of the TWI, and of the biological significance of the response on which it is based, which complicates interpretation of the possible toxicological significance of exceedances."*

The international process described herein has various strengths. For example, many of the scientists who volunteered for this task are experts in various aspects of PFAS in general, and PFOS in particular, or in one or more of the relevant critical effects, or in one or more of the extrapolation methods used to determine safe doses. Many of these scientists are also familiar with one or more of the agency positions on PFOS, especially in their particular country. Despite (or because of) these credentials and familiarity, uniformity of thought was often not present during the international meetings. Therefore, the eventual consensus of 29 scientists from nine countries over 6 months may be more informative than positions developed with fewer or less diverse viewpoints.

This process also has its weaknesses, similar to those discussed by Burgoon et al. (2023). For example, it depended on the views of scientists who might not fully appreciate the constraints imposed upon specific regulatory agencies. In other words, we might have made choices that are simply not available to agency scientists. Another potential weakness is that no funding was received for this work, which limited individuals' efforts to devote all of the time that might have been needed to analyze the nuances of potentially relevant information.

[6] Last year, a regulatory authority reviewed their existing reference dose for several PFAS, including PFOS (National Health and Medical Research Council, 2024). The Council reviewed the literature published since 2017, which was when it had last assimilated the data. The Council "updated" its reference dose by relying, oddly, on a 28-day bioassay, and on an endpoint (related to hematopoiesis) that showed minimal changes at all doses. It is not clear why the Council rejected reliance on other, longer, and more robust bioassays, and on more well-established endpoints.

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Author contributions All authors participated in conception, data curation, analysis, investigation methods, visualization, and writing, reviewing or editing. In addition, Michael Dourson participated in project administration and he, Ashish Jachak and Tony Cox summarized and/or chaired Zoom meetings.

Declarations

Conflict of interest All authors indicate no financial relationship or conflict of interest with the Alliance for Risk Assessment (*ARA*), the colllaboration which endorsed this research. Furthermore, none of the authors, nor the *ARA,* receive any funding for this work.

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Authors and Affiliations

Michael L. Dourson[1] · Laura C. Green[2] · Edmund A. C. Crouch[3] · Harvey J. Clewell[4] · Thomas Colnot[5] · Tony Cox[6] · Wolfgang Dekant[7] · Linda D. Dell[25] · James A. Deyo[8] · Bernard K. Gadagbui[1] · Helmut Greim[9] · Mahesh Rachamalla Gupta[10] · Tamara House-Knight[11] · Ashish Jachak[12] · Vijayavel Kannappan[13] · Travis R. Kline[14] · Therese Manning[15] · Ravi Naidu[16] · Paul Nathanail[17] · Chijioke Onyema[1] · Frank Pagone[12] · Andrew Pawlisz[18] · Tiago Severo Peixe[19] · Katie Richardson[20] · Anurag Sharma[21] · James S. Smith[22] · Nitin Verma[23] · Andrea Wojtyniak[24] · Jackie Wright[15]

✉ Michael L. Dourson
dourson@tera.org

1 Toxicology Excellence for Risk Assessment, Cincinnati, OH, USA

2 Green Toxicology LLC, Hull, MA, USA

3 Green Toxicology, Arlington, MA, USA

4 Ramboll, Durham, NC, USA

5 CiS Toxicology, Osorno, Chile

6 Cox Associates, Denver, CO, USA

7 University of Würzburg (retired), Würzburg, Germany

8 Environmental Protection Authority, Wellington, New Zealand

9 Technical University of Munich University (retired), Munich, Germany

10 University of Saskatchewan, Saskatoon, Canada

11 GHD, North Little Rock, AR, USA

12 RHP Risk Management, Chicago, IL, USA

13 Washington, DC, USA

14 Geosyntec Consultants, Farmington, MO, USA

15 Environmental Risk Sciences Pty Ltd, Carlingford, Australia

16 CRC CARE, Callaghan, Australia

17 LQM, Nottinghamshire, UK

18 Trihydro, Laramie, WY, USA

19 State University of Londrina, Londrina, Brazil

20 Senversa, Melbourne, Australia

21 Nitte (Deemed to be University), Mangaluru, India

22 US Department of Defense, Portsmouth, VA, USA

23 Chitkara University, Himachal Pradesh, India

24 Geosyntec Consultants International, Toronto, Canada

25 Ramboll, Amherst, MA, USA

Supplement 1

Advisory Committee

- Harvey J. Clewell, Ramboll, Global
- Tony Cox, Cox Associates, USA
- Michael L. Dourson, TERA, USA
- Tamara House-Knight, GHD, Global
- Ravi Naidu, CRC CARE, Australia
- Paul Nathanail, LQM, United Kingdom
- James S. Smith, US DoD, USA
- Nitin Verma, Chitkara University School of Pharmacy, Chitkara University Himachal Pradesh, India

Science Teams

Group 1

- Linda Dell, Ramboll, USA
- Bernard Gadagbui, Toxicology Excellence for Risk Assessment, USA
- Helmut Greim, University of Munich University(retired), Germany
- Vijay Kannappan, USA
- Therese Manning, Environmental Risk Sciences Pty Ltd, Australia
- Paul Nathanail, LQM, UK
- Tiago Severo Peixe, State University of Londrina, Brazil
- Andrea Wojtyniak, Geosyntec Consultants International, Canada

Group 2

- Harvey Clewell, Rambol, USA
- Thomas Colnot, CiS Toxicology, Chile
- Edmund A. C. Crouch, Green Toxicology LLC, USA
- James Deyo, DVM,Environmental Protection Authority, New Zealand
- Mahesh Gupta, University of Saskatchewan, Canada
- Tamara House-Knight, GHD, USA
- Travis Kline, Geosyntec Consultants, USA
- Anurag Sharma, Nitte University, India
- Katie Richardson, Senversa, Australia

Group 3

- Wolfgang Dekant, University of Würzburg (retired), Germany
- Laura C. Green, Green Toxicology LLC, USA
- Andrew Pawlisz. Trihydro, USA
- Frank Pagone, RHP Risk Management, USA
- James Smith, NMCPHC, USA
- Nitin Verma, Chitkara University, India
- Jackie Wright, Environmental Risk Sciences Pty Ltd, Australia

"Secretariat"

- Tony Cox with Cox Associates, USA
- Michael Dourson with TERA, USA
- Ashish Jachak from RHP Risk Management Inc., USA
- Ravi Naidu with CRC CARE, Australia

Supplement 2

Please note that the multiple references associated with the monkey study and rat studies mostly referred to different aspects and/or different reports of the same study.

Human Study

Reference

Li Y, Andersson A, Xu Y, Pineda D, Nilsson CA, Lindh CH, Jakobsson K, Fletcher T. Determinants of serum half-lives for linear and branched perfluoroalkyl substances after long-term high exposure—a study in Ronneby, Sweden. Environment International. 2022 May 1;163:107198. https://doi.org/10.1016/j.envint.2022.107198.

Results

Table S1 shows 113 of the 114 individual half-life estimates of Li et al. (2022) for L-PFOS, as estimated from Li et al. (2022, Figure S3). The half-life of a 95-yr-old male is missing from Figure S3, but can be determined to be not among the highest values. Omission of this value does not significantly affect the results.

The mean and standard deviation of the natural logarithm of these half-lives are 1.056 and 0.3190, giving a 97.5th percentile of 1.869 times the median of 2.875 years for the corresponding lognormal distribution.

Table S1 Individual half-lives estimated from Li et al. (2022, Figure S3)

Males		Females	
Age (yr)	Half-life (yr)	Age (yr)	Half-life (yr)
4	2.485	4	1.639
4	3.171	4	2.096
7	2.084	6	2.808
7	2.456	9	1.828
8	1.438	11	2.248
8	1.912	12	1.615

8	2.084	12	1.619
8	2.162	12	2.447
8	2.360	12	2.704
9	2.236	12	3.351
10	1.599	14	2.896
10	1.904	15	2.229
10	2.040	15	3.038
10	2.056	20	3.056
11	2.485	31	2.713
12	1.817	32	1.658
12	2.284	32	2.724
13	2.893	33	2.838
15	2.009	35	3.094
16	2.446	38	2.419
18	3.387	39	1.553
21	3.007	39	3.037
32	5.394	40	4.788
35	2.313	41	2.552
39	1.971	41	2.809
39	3.519	41	3.009
40	1.931	42	1.877
40	3.273	42	1.905
41	4.196	42	2.781
41	4.215	42	3.037
42	2.914	42	3.818
43	2.843	43	3.142
44	2.757	43	3.675

44	2.835	44	1.839
44	3.314	44	3.303
45	3.339	48	2.723
47	3.064	49	3.484
47	3.681	49	3.713
49	3.845	50	2.115
49	3.930	50	2.486
58	2.844	50	3.512
64	3.967	55	2.942
64	4.614	55	4.084
66	3.169	56	3.722
67	4.148	62	3.408
67	4.272	62	4.360
68	2.721	63	2.713
69	4.006	63	3.284
74	2.788	63	4.749
76	7.402	65	4.151
77	4.739	66	1.582
78	2.996	66	2.990
81	3.311	66	4.874
		67	3.818
		67	4.360
		69	3.827
		70	2.704
		71	3.997
		71	4.521
		79	4.750

Table S2 provides an estimate of the effect of a mix of isomers on the relative serum concentration to input dose ratio compared with that for L-PFOS alone, assuming the initial concentrations corresponded to input-output equilibrium. The initial serum concentrations are the geometric means, and the half-life estimates are medians from Tables 2 and 4 of Li et al. (2022) respectively. The ratio (1.11 in Table S2) will vary with isomer distribution; the linear/branched ratio observed here (56:44) corresponds to the contamination with AFFF at the nearby airbase modified by environmental transport, so probably reflects an original electrochemical fluorination (ECF) production process.

Table S2 Estimated relative input of an isomer mix to produce the observed initial serum concentrations.

Isomer	Initial serum conc.	Relative fractions	Half-life (yrs)	Estimated relative input
L-PFOS	150	0.5085	2.89	150.00
1 m-PFOS	23	0.0780	5.57	11.93
3/4/5m-PFOS	73	0.2475	3.83	55.08
2/6m-PFOS	49	0.1661	2.87	49.34
Total	295			266.36

To account for the potential increased average half-life due to an isomer mix, we multiply the 97.5th percentile of 1.869 times the median by this factor of 1.11 to obtain an estimate of 2.1 for the human toxicokinetic uncertainty factor.

Monkey 6-month experiment

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Assumptions:

- Liver weight/body weight ratio is determined by the average serum concentration of PFOS over the 26 weeks (182 days) of the study.
- Serum concentration increased during dosing according to the 1-compartment model
$$C(t) = C_m\left(1 - exp(-\gamma t)\right)$$
with the error model
$$\ln(C_t/C(t)) \sim N(0, \omega^2)$$
where C_t is the measured concentration at time t (days).
- Serum measurements were taken on weeks 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, and 26; and these are assumed to correspond to dosing days 0, 7, 14, 27, 37, 51, 79, 107, 135, 163, and 182 (except for week 1, actual days were not documented). Day 1 was the first day of dosing, Wednesday, 8/26/98. The assumed days correspond to Tuesdays for week 1, 2, and 26, Monday for week 4, and Thursdays for the remainder. Some of these days correspond with the documented days for other blood measurements, but others cannot be so matched.

Methodology:

- The average serum concentration over the 182 days of the study was obtained from the maximum likelihood estimate of the 1-compartment model parameters.
- Three of the measured concentrations were discarded as being measurement errors, since they were clearly outliers and probably analytical errors. A similar measurement error was noted in liver concentration measurements, where re-measurement was possible. Serum concentrations presumably could not be re-measured through lack of sufficient sample; and many of the measurements are flagged as having less than method-specified sample sizes.

- The control group had measurable PFOS serum concentrations starting at week 8 in some cases, but the maximum ever measured was 0.074 mg/L (at week 26), compared with the minimum measurement of 0.79 mg/L in a 0.03 mg/kg/d animal at week 6. Estimates of lifetime average in the control group (using the modeling described above and also trapezoidal rule estimates) are less than 0.05 mg/L. In what follows, control group animals are assigned serum concentrations of zero.

Results:

- Half-life estimates (from the 1-compartment model above) in the 0.03 and 0.15 mg/kg/day groups combined had mean 379 days, SD 608 days, median 149 days, min 76 days, max 2836 days and were not significantly different in distribution between these two dose groups; although some, especially the longer, of these half-life estimates were sensitive to small changes in selection of the days of dosing within specified weeks. The high dose group (0.75 mg/kg/d) had significantly lower half-lives – mean 48 days, SD 6.3 days, median 46 days, min 42 days, max 63 days, with little sensitivity to selection of dosing days within specified weeks.
- The 1-compartment model error estimates ranged from w = 0.11 to 0.29.
- Individual liver weight/body weight ratios are available for 31 animals at 182 days of dosing, and plotted vs. average serum concentrations suggest (visually) a linear increase.
- The dose-response relationship was modeled using BMDS type modeling. BMDS online (https://bmdsonline.epa.gov/) was used to confirm that a linear model is as acceptable as any (all were considered questionable – but probably because of errors in the software, see note below) and provides the lowest estimate of BMDL and lowest AIC (no model was recommended). However, actual computations for the linear model were performed in Excel.
- [Note: for every dataset tried of individual animal data with continuous response the downloadable version of BMDS 3.3.2 crashes. The online version provided close to accurate BMDL values (not all significant figures provided are correct) but failed to correctly count degrees of freedom and mis-states the significance of some tests. It suggests that all the available models are questionable because of "Zero degrees of freedom; saturated model; Control stdev. fit greater than 1.5; Constant variance test failed (Test 2 [*or Test 3*] p-value < 0.05)" for an assumption of constant variance [or non-constant variance]. The online BMDS statement of saturation of degrees of freedom was incorrect, and (possibly as a result of that error) the "Test 2" and "Test 3" results were incorrect. The "stdev. fit greater than 1.5" was also incorrect (it should have states this is "1.5x actual response stdev at control" according to the BMDS 3.3 manual.)]
- The dose-response data are shown in Figure S1 (the fits are explained below). Males and females are significantly different.
- Treating males and females separately, BMDS-type analyses show that the linear model is better than any others of those available in BMDS online (see note above).
- For males, a constant variance is not rejected. For females, a constant variance is rejected. However, this is entirely due to the four animals in the 0.03 mg/kg/day group, which are

barely distinguishable on Figure S1. BMDLs for males assuming constant variance are 21.6 mg/L for 1 SD of modeled control value and 31.4 mg/L for 20% increment from modeled control value. For females, assuming non-constant variance (a power law as in BMDS), the BMDLs are 7.9 mg/L for 1 SD of modeled control value, and 33.4 mg/L for 20% increment over modeled control value.

- Treating males and females independently, except having the same linear slope (but allowing non-constant variance) is not rejected.
- The rejection of a constant variance for females is considered to be a fluke, due to the happenstance of the four low dose females having very similar liver wt./body wt. ratios; all other dose groups have substantially larger variation. The non-constant variance assumption is therefore rejected.
- With constant variance for both males and females the slope and variance can be common to both males and females, but the intercepts are different. The MLE fits for these conditions are shown on Figure S1.
- With these conditions, the BMDL is 21.1 mg/L for a 1 SD increment over the modeled control value, and 32.8 mg/L for 20% increment over the male modeled control value (which is the smaller of male and female).
- If males and females are considered as entirely equivalent, the variance is not significantly non-constant; and using constant variance the BMDL is 43.1 mg/L for 1 SD, and 55.0 mg/L for 20% increment above modeled control value.
- The selection of 20% increment over the control group is suggested by the agreement of the ESTP liver hypertrophy expert group that an increase in liver weight of at least 20% is required to histologically detect a change in hepatocyte cell size, combined with the apparent non-adversity of the relative liver weight changes (Hall et al, 2012).

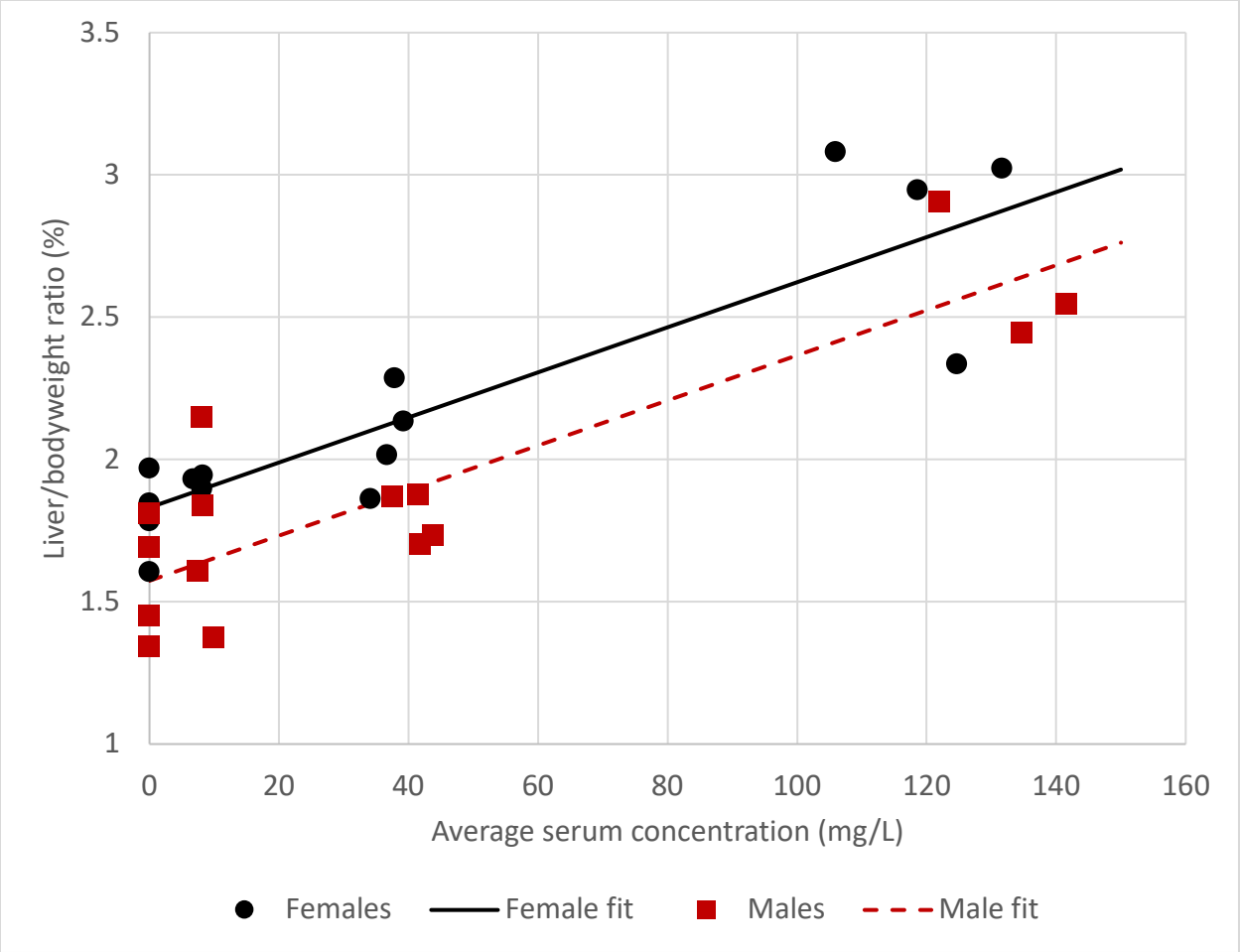

Figure S1. Liver/bodyweight ratio vs. average serum concentration for individual monkeys in the 6-month experiment.

Rat 2-year experiment

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Assumptions

- Non-neoplastic liver effects at sacrifice are determined by the serum concentration of PFOS at sacrifice. The rat half-life is sufficiently short that the animals would be at input-output equilibrium at sacrifice (all other factors being constant), and the liver response to variation in serum concentration is assumed to be relatively fast.
- Based on this assumption, all animals were included in the analysis, no matter what study date they were sacrificed (from 4 weeks to 106 weeks) and including the recovery group.

Methodology

- The most sensitive endpoint is hepatocellular centrilobular hypertrophy in the male rats (Figure S2) —this endpoint was always noted with occasional polykaryocytosis in both males and females.
- This endpoint was graded as absent, minimal, slight, moderate, or moderately severe (coded in Figure S2 as 0, 1, 2, 3, and 4 for display purposes).
- BMDS analysis was performed on the results for males, using presence or absence only and ignoring the grade.
- The default extra risk of 0.1 is used as the criterion.

Results

- BMDS online cannot (at 12/20/24) handle more than 30 dose points, so cannot handle the individual dichotomous results for the 154 male rats. However, BMDS 3.3.2 (download Excel version) can handle the 154 results. All models except Weibull are assessed as viable, although the “questionable” for Weibull is apparently based on inappropriate statistics for individual animal results.
- BMDS analysis provided a lowest estimate of 2.09 mg/L for BMDL using a dichotomous Hill model with parameter estimates making this model equivalent to the log-logistic.
- The BMD for the dichotomous Hill is 8.45 mg/L. At the BMDL the dichotomous Hill model becomes linear with intercept zero – so there is just one non-bounded parameter. However, the dichotomous Hill (and log-logistic) had highest AIC and BMD/BMDL>3, while the log-probit also produced BMDL/BMD > 3.
- The quantal linear model (and gamma and multistage 1, which both reduced to the quantal linear), with BMDL of 2.76 mg/L, gave lowest AIC and acceptable BMD/BMDL ratio. In addition, the loglikelihood was higher for the quantal linear than the dichotomous Hill model, despite using fewer parameters. These results for the dichotomous Hill and quantal linear model were checked independently in Excel. Other viable models gave BMDLs ranging up to 8.6 mg/L in BMDS 3.3.2.

- Arbitrarily dividing the range of serum concentrations into approximate deciles – the 18 non-detects set at zero, with 15 each in 8 deciles, and 16 in the top decile – gives Figure S3 showing empirical average response against the average of upper and lower concentrations required to perform this decimation. Included are the 80% confidence intervals, individual observations (0 or 1), and MLE curves from the individual animal analysis. Two points coalesce visually at the lower end, one at the origin, one at 0.1 mg/L, both with zero positives.
- For comparison, the liver weight/body weight ratio in these mice increased relatively slowly and linearly with serum concentration, so that a 5% increment in liver weight/body weight ratio corresponded to a serum concentration of ~32 mg/L.

Unselected values

The estimated serum concentrations for the NOAELs listed in the paper (Table 3) were obtained from Table 7 of Butenhoff et al. (2012) using trapezoidal integration, with the values given at the 0.5, 2 and 5 ppm dietary concentrations augmented by setting the values at 53 weeks equal to those at 14 weeks, matching the pattern measured in the 20 ppm dietary concentration group. Omitting these would give slightly lower estimates using trapezoidal integration

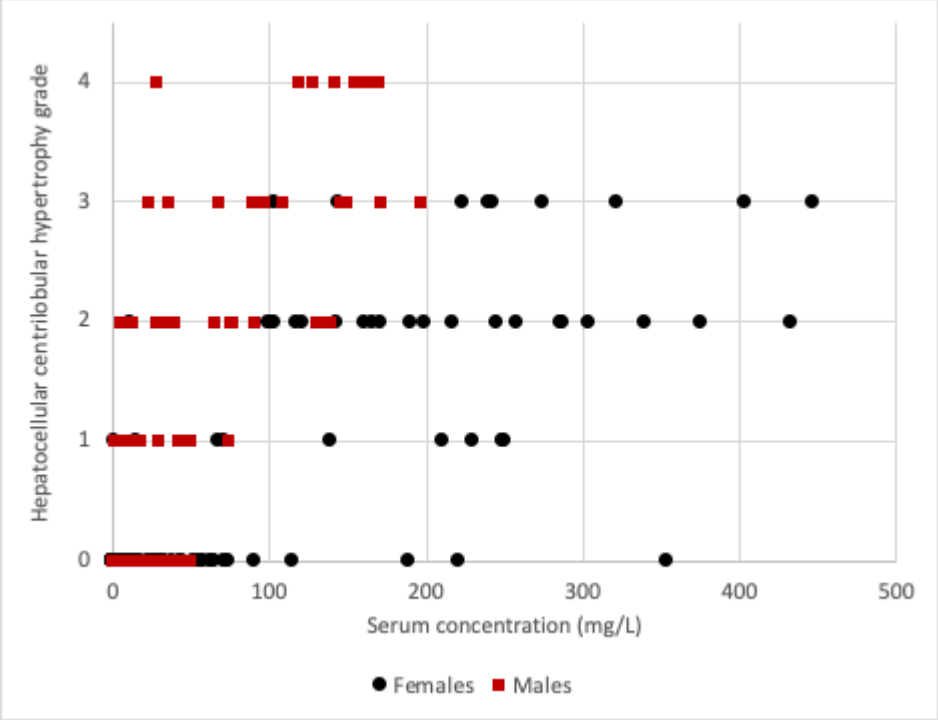

Figure S2. Grade of hepatocellular centrilobular hypertrophy in individual male and female rats, showing the higher sensitivity of males.

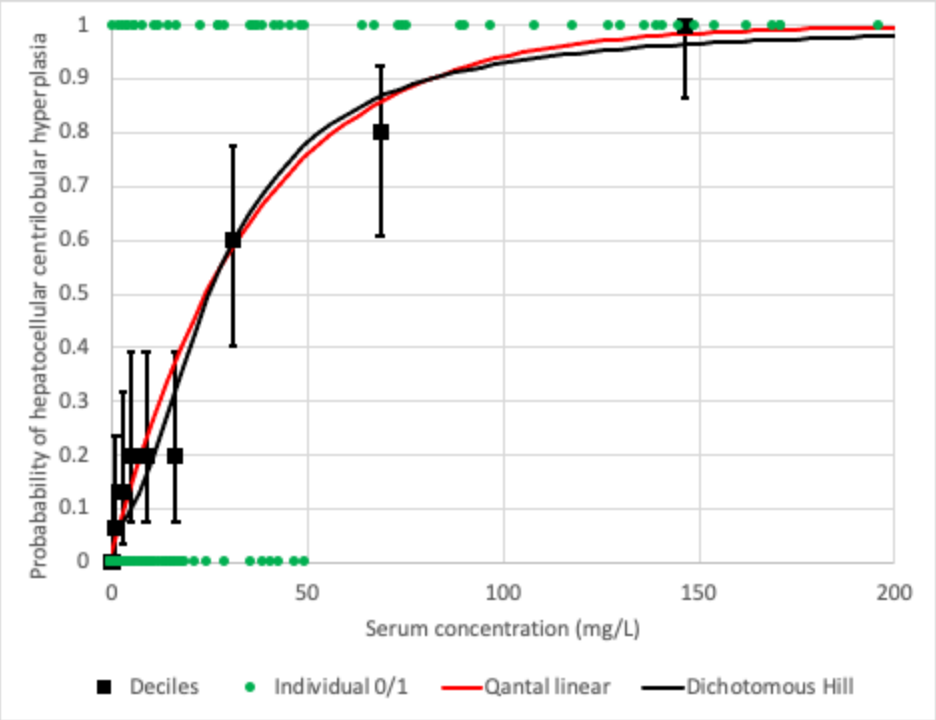

Figure S3. Individual and grouped concentration-response for hepatocellular centrilobular hypertrophy in male rates. Lines are maximum likelihood estimates using the individual results. Error bars are 80% confidence intervals.

Thibodeaux et al. (2003) and Lau et al. (2003)

References

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Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicological Sciences. 2003 Aug 1;74(2):382–392. https://doi.org/10.1093/toxsci/kfg122.

Grasty RC, Grey BE, Lau CS, Rogers JM. Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat. Birth Defects Research Part B: Developmental and Reproductive Toxicology. 2003 Dec;68(6):465–471. https://doi.org/10.1002/bdrb.10046.

Assumption

Based on the results of Grasty et al (2003) showing that neonatal mortality was controlled by maternal serum concentration within the last few days of gestation, it was assumed that effects on the most sensitive endpoints documented in Lau et al. (2003) were also controlled by maternal serum concentrations in this time period, and that this serum concentration could be approximated by the measurement at 21 days.

Methodology and Result

Correspondence with Dr. Lau indicated that it would be impossible to track individual animal data due to their experimental design -- although perhaps it would be available for the dams. However, it would require extreme effort and time to decipher the material (including handwritten reports) as well as an unknown but probably long time to retrieve the records from Federal Archives. The NOAEL reported in Table 3 of the paper is the best estimate obtained by digitization of Figure 3 of Thibideaux et al. (2003) for average maternal serum concentration at 21 days.

Luebker et al. (2005)

References

Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague–Dawley rats: dose–response, and biochemical and pharmacokinetic parameters. Toxicology. 2005 Nov 5;215(1-2):149–169. https://doi.org/10.1016/j.tox.2005.07.019.

Dose-response (NOEL) and mevalonic acid/cholesterol supplement studies

Argus, 2007. Final Report. One generation reproduction study of PFOS - mevalonic acid/cholesterol challenge and NOEL investigation in rats. Sponser study number T-6295.25. Argus Research Protocol Number 418-018. Available in EPA Administrative Record AR226-1357. [Also available in other AR226-1206. Includes analysis of sera.]

Pharmacokinetic study

Argus, 1999. Final Report. Protocol 418-013. Oral (gavage) pharmacokinetic study of PFOS in rats. Sponsor's study number T-6295.12. Available in EPA Administrative Record AR226-565.

Nold JB. 1999. Pathology Report (Ancillary Study). Electron microscopic evaluation of liver and lung and light microscopic evaluation of liver in CRL:CD®BR VAF/PLUS® rats. Oral (gavage) pharmacokinetic study of PFOS in rats. Client Study Number 418-013, PAI Study Number 99-11

(EM-99_39) Pathology Associates International. Prepared for 3M Corporate Toxicology. Available in EPA Administrative Record AR226-572.

3M. 2001. Oral (gavage) pharmacokinetic study of PFOS in rats. Determination of the presence and concentration of perfluorooctanesulfonate (PFOS) in serum, liver, urine, and feces samples. 3M Medical Department Study: T6295.12. Analytical Report FACT TOX-110. LRN-U2849. Available in EPA Administrative Record AR226-1030A004.

Assumption

The relevant period of dosing for the endpoints demonstrated is unclear. It was assumed that, as above, maternal serum concentration at the end of gestation is the controlling factor.

Methodology and result

This paper discusses a dose-response study, a pharmacokinetic investigation, and in passing an attempt to prevent neonatal mortality by co-administering mevalonic acid lactone or cholesterol supplements. The dose-response/co-administering study obtained sera from gravid dams on GD21, but only at doses of 1.6 and 2 mg/kg/day, while the pharmacokinetic study obtained sera from gravid dams on GD0, 7, 15, and 21 at doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day, but all dams were sacrificed at GD21. Thus neither of these studies is suitable for development of an individual-animal-serum concentration-based reproductive/developmental BMD in female rats – the former because only sera at two high doses were available, the latter because the offspring were not available for study.

Both the dose-response and pharmacokinetic studies showed significant effects at 0.4 mg/kg/day, but no effect was demonstrated at 0.1 mg/kg/day in the pharmacokinetic study. Average maternal serum concentration at GD21 (Table 9 of Luebker et al., 2005) was selected as a serum-based NOAEL.