Critical Review of Key Studies Cited in The Risk Assessment, Public Policymaking, and Judicial Decisions on the Toxicity and Carcinogenicity of Per-and Polyfluoroalkyl Substances (PFAS) on Humans

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Truth in Science

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1. Introduction and Background

Per-and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that includes perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), replacement chemicals such as GenX, and many others. PFAS have been manufactured and used in a variety of industries around the globe, including in the United States since the 1940s, and are reportedly found in more than 4,700 commercial products including food packaging; non-stick, waterproof, and stain-resistant coatings; household cleaners, as well as workplace operations (e.g., chrome plating, electronics manufacturing, oil recovery) (Buck et al. 2011; Duffek et al. 2020; Kissa 2001).

Some PFAS are commonly found in the blood (serum) of people and animals all over the world. Concerns have been raised regarding a potential link between PFAS exposure and cancers in humans. For example, in 2017, IARC listed PFOA as a potential (Group B2) carcinogen. That same year, the U.S. Centers for Disease Control (CDC) expressed less certainty regarding causality, stating that "more research is needed to assess the human health effects of exposure to PFAS." In 2017, California EPA added PFOA and PFOS to the Proposition 65 list as reproductive toxicants; however, more recently, the agency proposed modifying the listing for both chemicals to include carcinogenicity. The National Toxicology Program (NTP) concluded that both PFOA and PFOS should be "presumed to be an immune hazard to humans" based on evidence that the two compounds suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Other possible effects of PFAS include altered thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, and adverse reproductive and developmental outcomes (reviewed in Fenton et al., 2021).

To date, more than $2.45 billion has been paid worldwide through litigation settlements involving exposure to PFAS. During this time, the science linking PFAS exposure and adverse effects in humans has continued to evolve. Variations in study designs, the type, magnitude, and duration of PFAS exposure, and population characteristics may all contribute to inconsistent and conflicting conclusions regarding associations with key adverse effects. The reviewers identified several such examples while evaluating the studies that were the subject of this report.

Timeline

- 1940s-1950s: Scientists developed perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), two PFAS used in stain- and water-resistant products, protective coatings and firefighting foams.
- 1970s: Studies reported PFAS in the blood of occupationally exposed workers.
- 1990s: Some studies reported trace quantities in the blood of the general human population.
- 2001: Studies in the arctic report detectable levels of PFOS in marine mammals.

- 2003: 3M Corporation, the principal worldwide manufacturer and sole U.S. manufacturer of PFOS, announced a voluntary phase-out of PFOS, PFOA and related precursors.
- 2008: 3M completed phase-out of PFOS, PFOA, and related precursors.
- 2020: EPA published action plan on PFOS and PFOA.
- 2021 (April 27): EPA Administrator called for the creation of a new " EPA Council on PFAS" that is charged with building on the agency's ongoing work to better understand and ultimately reduce the potential risks caused by these chemicals.
- 2021 (June 10): EPA proposed steps to gather additional occurrence data, revisit rules that target certain long-chain PFAS in surface coatings applied to products, and expand the list of PFAS chemicals included in environmental monitoring programs.
- 2021: EPA initiated steps to propose designating PFOA and PFOS as " hazardous substances" through one of the available statutory mechanisms including potentially CERCLA Section 2 (Superfund Act).

How PFAS are Regulated in the U.S.

PFOS and PFOA were in production prior to the inception of the U.S. Environmental Protection Agency (EPA) and the relevant sections of legislation under which it derives authority: the Safe Drinking Water Act of 1974 (SDWA) and the Toxic Substances Control Act of 1976 (TSCA). TSCA, as enacted in 1976, dictated that all existing chemicals (it listed 62,000 of them) were considered to be safe for use and subsequently "grandfathered" in. TCSA also gave the EPA authority to evaluate the risks posed by a chemical before going into production. Additionally, the EPA can also regulate any chemicals that pose an "unreasonable" risk to human health or the environment and, under extreme circumstances, the EPA reserves the right to ban a chemical. This has only been done with five compounds: polychlorinated biphenyls (PCBs), chlorofluorocarbons (CFCs), dioxins, asbestos, and hexavalent chromium. The EPA's authority is restricted by the following caveat: it can only require safety testing and regulation after evidence is presented demonstrating a chemical poses a risk of harm. Consequently, the EPA has only required testing for about 200 of the more than 84,000 chemicals now registered for production or distribution within the U.S.

EPA has the authority under the Safe Drinking Water Act (SDWA) to set enforceable Maximum Contaminant Levels (MCLs) for specific chemicals and require monitoring public water supplies. There are currently no federal MCLs established for PFAS. In 2016, EPA issued health advisories for PFOA and PFOS, which are non-regulatory concentrations in drinking water implemented as thresholds at or below which exposures for a specified duration are unlikely to result in adverse effects.

In 2016, TSCA was amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act. The Act mandated the EPA to evaluate existing chemicals (those already grandfathered in) with clear and enforceable deadlines. All chemicals must now be assessed against a risk-based safety standard. In 2019, EPA published a 72-page plan outlining:

- Actions the agency has previously taken with respect to PFOS and PFOA
- Methods of reducing exposure

- Efforts to identify contaminated sites and water supplies
- The cost-effectiveness of different methods for removing PFAS from contaminated areas.

On February 26, 2020, the EPA press office released an update on their action plan, which included:

- A preliminary determination to regulate PFOA and PFOS in drinking water
- A supplemental proposal to ensure that persistent long-chain PFAS in surface coatings cannot be manufactured or imported into the United States for new uses without notification and review
- A new validated method to accurately test for 11 additional PFAS in drinking water
- Interim recommendations for addressing groundwater contaminated with PFOS and PFOA,
    - providing guidance for federal cleanup programs
    - $4.8 million in funding for research on managing PFAS in agriculture

Review Sponsor Objectives:

1. Determine whether it is possible to evaluate the toxicity and carcinogenicity of PFAS as a group, as a sub-group, or whether each compound should be evaluated separately.
2. Determine the extent to which scientific evidence demonstrates a clear causal link between exposure to PFAS and increased risk of developing cancer or other chronic illnesses.
3. As appropriate, apply accepted scientific methods that would help reviewers and meta-analysts determine the credibility of results of individual studies and use those methods in this research.
4. Using existing methods, assess the validity of each study's proposed hypothesis and protocols, and identify potential confounders that may have affected the study's findings.
5. Determine the reasons for conflicting or contradictory findings, if any, among existing studies.
6. Develop recommendations for methods and protocols for additional research that would yield more accurate, reliable, and trustworthy research results for both individual studies and meta-analysis.
7. Develop recommendations as to whether it is possible to evaluate the toxicity and carcinogenicity of PFAS compounds as a group, as a sub-group, or whether each compound should be evaluated separately.

This project solicited input from reviewers without disclosing the identify of the sponsor. Each reviewer was charged with reviewing a subset of the studies, but also invited to review and comment on any of the eleven studies selected by the sponsor.

2. Methods

2.1. Selection of Key Studies

Table 1 lists the eleven studies selected for this review, grouped into five topic areas. The selected studies include those conducted by a wide variety of organizations and institutions in both the U.S. and Europe, including government agencies, universities, and independent research laboratories; those that reported conflicting and contradictory results; or those that are among the most widely cited studies in the scientific, legal, and economic communities. The following additional selection criteria were considered:

- The study is a recent review of relevant information and discusses this information in a balanced way; or the study is a recent analysis of the data, or provides new data, either or both of which positively impacts an overall understanding of the chemical(s) of concern.
- The study forms the basis, in part, of a federal agency or health organization risk assessment position of the chemical(s), compound(s), or mineral(s) of concern that is subsequently used in rule-making.
- The study shows data and/or analysis that allow alternative explanations of established or draft risk assessment positions; data and/or analysis can include mode of action information, mathematical modeling, toxicological and/or epidemiological interpretation, and/or overall synthesis into a coherent picture of the toxicity of the chemical(s), compound(s), or mineral(s) in question.

Table 1. Key PFAS Studies Identified by Review Sponsor

Topic Area Group	Study	Brief Description
Toxicokinetics /Biomonitoring in Humans	1) Emmett et al., 2006	Investigation of community exposures to PFOA and serum concentrations.
	2) Olsen et al., 2007	Study on half-life of several PFAS compounds in the serum of retired production workers that is cited by all authorities in their assessment work.
Toxicokinetic Analyses	3) Russell et al., 2015	Compares apparent half-life to intrinsic (or actual) half-life of PFAS compounds through a mathematical approach; shows that many human observational studies have overestimated half life by failing to account for ongoing (background) exposures.
	4) Dourson et al., 2019	Comparison of human and experimental animal kinetics to develop an extrapolation factor and identify the appropriate dosimetric adjustment based on recommended guidelines of EPA.
Key Effects on Immune/Vaccine Response	5) Abraham et al., 2020	Internal exposures to PFAS matched with biological markers and vaccine responses; the principal study used by the European Food Safety Authority (EFSA) in its recent risk assessment.
	6) Grandjean et al., 2012	Serum vaccine antibody concentrations monitored in children exposed to PFAS chemicals highlighted by the EFSA in its recent risk assessment.
Key Effects on Reproduction / Development	7) Koskela et al., 2016	Short term gestational exposure in mice with implications for long term effects; this is the principal study used by the Agency for Toxic Substances and Disease Registry (ATSDR) in its determination of minimum risk level.
	8) Lau et al., 2006	Effects of PFOA on pregnancy in mice; this is the principal study used by EPA to estimate its health advisory of 70 ppt.
Key Effects in Rodents (other than reproduction /development)	9) Butenhoff et al., 2012	Chronic study in rats showing Leydig cell tumors; this is the principal study used by Health Canada in its 2018 assessment for PFOA.
	10) Perkins et al., 2004	Liver changes in rats associated with PFOA exposure; this is a principal study used by Health Canada in its 2018 assessment for PFOA.
	11) Luebker et al., 2005	Liver changes in rats associated with PFOS exposure; this is another principal study used by Health Canada in its 2018 assessment for PFOS.

2.2. Study Scoring

To support this review, SciPinion assembled a panel of experts as summarized in **Appendix A**:

(1) a panel of 15 topic experts to review and score the key PFAS studies; and
(2) a topic area lead scientist to support the review and preparation of this report.

Each of the 5 topic area groups was assigned 3 topic experts to serve as reviewers. Each reviewer was assigned to review and score the two papers in the topic area group. In addition, the topic lead scientist reviewed and scored all 10 studies. By this design, each of the 10 key papers received 4 reviews and scores (topic lead plus 3 panelists). Studies were scored on a scale of 1 to 10 (1=lowest, 10=highest) with respect to "degree of confidence", using the following charge questions:

- ***Methods:*** *What is your degree of confidence in the methods used in this study?*
- ***Results:*** *What is your degree of confidence in the results generated in this study?*
- ***Discussion/Conclusions:*** *What is your degree of confidence in the authors conclusions in this study?*
- ***Application to Risk Assessment Decision Making:*** *What is your degree of confidence in using this study to support decision-making for PFAS risk assessment?*

Reviewers were encouraged to briefly explain their rationale for each score. Study confidence scores were calculated by the following steps:

- Step 1: For each study, calculate the arithmetic mean of the four confidence ratings (methods, results, conclusions, application) for each reviewer, which yields an average rating from each reviewer.
- Step 2: Multiply the average rating from Step 1 by 10 to express the overall study score by reviewer on a scale of 0 to 100.
- Step 3: For each study, using the study scores by reviewers in Step 2, calculate the overall arithmetic mean, minimum, and maximum score across reviewers.

Factors to be considered by the reviewers when scoring were included in their instructional material (**Appendix B**).

The review was designed as follows:

- *Step 1: Reviewers and topic lead review and score their assigned studies;*
- *Step 2: Topic lead prepares this draft report, which incorporates the results from Step 1;*
- *Step 3: Reviewers review and provide input on the draft report from Step 2;*
- *Step 4: Topic lead finalizes this report based upon input received from the reviewers in Step 3.*

2.3. Identification of Additional Key Studies

Reviewers and topic lead were asked the following questions to help identify additional key studies for PFAS:

- *Considering the 11 key PFAS papers identified by the review sponsor in the introduction, are there other studies that you feel should be considered to be of equal or greater importance to PFAS risk assessment? If yes, please list the studies and briefly explain why they are important.*
- *Considering the Review Sponsor objectives are there other studies that you feel should be considered to meet these objectives? If yes, please list the studies and briefly explain why they are important.*

3. Results

3.1. Study Scoring

The results of the study scoring are summarized in **Figure 1** and discussed below below by topic area and study. Detailed input from the expert panel, including study ratings and scores and the explanatory text supporting them is provided in **Appendix C**.

3.1.1. Toxicokinetics / Biomonitoring in Humans

Two papers were selected on toxicokinetics and biomonitoring in humans. Both papers examined empirical data on serum measurements of PFAS among populations with elevated exposures in order to characterize toxicokinetic parameters, including serum elimination half-life values. The following table summarizes the range and arithmetic mean scores by charge question as well as the total score (multiplied by 10).

Table 2. Scoring results for two papers on Toxicokinetics / Biomonitoring in Humans. Arithmetic mean (Range based on 4 reviewers).

Category of Charge Question	Emmett et al. 2006	Olsen et al. 2007
Methods	8.25 (8 – 9)	7.25 (6 – 8)
Results	8.50 (8 – 9)	7.50 (6 – 9)
Discussion/Conclusions	8.50 (8 – 9)	7.25 (6 – 9)
Application to Risk Assessment Decision-Making	8.50 (7 – 10)	6.00 (4 – 8)
Total Score (Mean x 10)	*84.4 (80.0-87.5)*	*70.0 (55-85)*

Emmett et al. (2006)

Scores on the Emmett et al. (2006) study evaluation were very consistent between the four reviewers (inclusive of the topic area lead) for each charge question, differing by only 1 to 3 points. In addition, average scores between categories were also very consistent, ranging between 8.25 and 8.50, for a final score (multiplied by 10) of 84.4.

Each reviewer commented that Emmett et al. (2006) reports findings from a well-designed study that examines a wide range of plausible exposure pathways to demonstrate that drinking water and working at a fluorochemical plant can both contribute to elevated serum PFOA levels. The large sample size of both water and serum measurements provides high confidence that supports this key finding. In addition, the study includes several relevant demographic factors that may contribute to variability in the exposure/serum relationship, such as age of study participants and tenure of residence in the community. Potential shortcomings in the study methods include uncertainty in the exposure duration (given that the period of time that water was consumed was not addressed in the participant survey) and further, that different analytical methods were applied to serum samples without examining extraction efficiencies.

The reviewers found the presentation and interpretation of the results by Emmett et al. (2006), including tables and graphics, were clear and comprehensive. The relatively small sample size for private wells compared with public water supply wells precluded a rigorous statistical comparison by water source. In addition, two reviewers noted that the unequal number of repeat samples from the same well could have introduced bias in the summary statistics of data collected over the entire study period. One reviewer indicated that a more comprehensive exploratory data analysis was warranted to improve the confidence in the findings from the statistical analysis. Stated examples included an evaluation of potential outliers, sample variance, and probability distribution shapes.

The reviewers noted that the results from Emmett et al. (2006) are useful for risk assessment and risk communication. Several reviewers placed this study into the greater context of PFAS biomonitoring studies in humans. One reviewer noted that although this specific publication did not examine associations between serum PFOA levels and incidence rates of disease outcomes, subsequent epidemiological assessments were conducted that included the same cohort of the Little Hocking community to examine this question (Nolan et al., 2009, 2010). One reviewer cautioned against extrapolating such study findings to the general population, or other communities with lower total exposure and serum PFOA levels. In addition, one reviewer noted that while this study has been cited as supporting a serum/water PFOA ratio of approximately 100:1, the elevated serum concentrations among non-consumers of the community drinking water (Tables 4 and 5 of Emmett et al., 2006) introduces uncertainty in the ratio that is not discussed by the study authors. Worley et al. (2017a) used a physiologically based pharmacokinetic (PBPK) model for PFOA to illustrate that the serum levels reported by Emmett et al. (2006) for communities exposed to elevated PFOA in drinking water are more accurately predicted (i.e., correspondence between model predictions and empirical measurements is improved) serum levels from drinking water exposures when non-drinking water exposures are included.

Olsen et al. (2007)

Scores on the Olsen et al. (2007) study evaluation were more variable between reviewers, differing by 2 to 4 points per charge question. Average scores by category ranged from 6.00 to 7.50, for a final score of 70.0.

The reviewers noted this study is an important early contribution to the overall literature on variability in serum elimination half-lives of PFOA, PFOS, and PFHxS in humans. However, several limitations in the study methodology contributed to the lower confidence ratings. Each reviewer commented on the relatively small sample size (n=26), particularly for females (n=2), and the uncertainty in extrapolating estimates from an occupationally exposed cohort to the general population. The study authors noted the potential confounding influence of background sources to quantifying serum elimination kinetics, and this statement was underscored by one reviewer that noted the Emmett et al. (2006) publication demonstrated the importance of accounting for drinking water exposures among occupationally exposed community members. Reviewers commented on the sample preparation and analytical methods used, noting that the extraction method is different from methods applied in other studies and may underestimate concentrations if the mass fraction bound to albumin is not fully extracted. A second reviewer noted that the spiked sample recovery demonstrated a potential loss of 10%, which might be partially attributable to the use of plastic vials.

Because many studies have been reported by Olsen and co-authors since the publication from 2007 that was selected for review, care should be taken to consider the complete literature before using findings from one study to support a risk assessment. For example, regarding the applicability of the study findings to PFAS risk assessment, three reviewers compared the estimated half-lives reported by Olsen et al. (2007) to subsequent studies with this and other populations. Olsen et al. (2007) reported central tendency (i.e., geometric mean and arithmetic mean) elimination half-life estimates, which have been subsequently reevaluated to consider (subtract) background exposures (e.g., Li et al. 2018; Russell et al. 2015 [one of the 11 studies reviewed here]; Xu et al. 2020; Worley et al. 2017a,b). Russell et al. (2015) described the "apparent half-life", such as the values calculated by Olsen et al. (2007) that do not subtract background, as providing a biased overestimate of "intrinsic half-life", which is the more biologically relevant metric of kinetics. A side-by-side comparison of these various estimates using human serum data is provided in Table 3 below. The updated estimates include both recalculations of half-lives for the same study population evaluated by Olsen et al., as well as additional estimates of intrinsic half-lives based on serum measurements. Reviewers noted that the arithmetic mean (apparent) half-lives for PFOA and PFOS reported by Olsen et al. (2007) likely overestimate the intrinsic half-lives by a factor of 2-to 3, whereas the half-life reported by Olsen et al. for PFHxS is around the midpoint of a relatively wide range of half-lives calculated by others.

Table 3. Elimination Half-life Estimates of PFOA, PFOS, and PFHxS from Olsen et al. (2007) Compared with More Recent Updated Estimates based on Serum Data in Humans.

Chemical	Summary Statistic	Half-life (years) (Olsen et al. 2007)	Updated Half-life (years) (see footnotes)
PFOA	Geometric mean Arithmetic mean	3.5 3.8	-- 1.5[1], 2.1[2], 2.4[3], 2.7[4], 3.9[5]
PFOS	Geometric mean Arithmetic mean	4.8 5.4	2.9[1] 1.7[1], 3.3[5], 3.4[4]
PFHxS	Geometric mean Arithmetic mean	7.3 8.5	2.9[1] 2.8[1], 5.3[3], 15.5[5]

[1]Xu et al. 2020; [2]Worley et al. 2017a; [3]Russell et al. 2015; [4]Li et al. 2018; [5]Worley et al. 2017b

Olsen and Zobel (2007) reported no associations between serum PFOA concentrations in fluorochemical production workers and corresponding lipid, hepatic, and thyroid parameters. Similarly, there was no association between serum PFOA levels and an elevated risk of death from prostate cancer among the highest-exposed category of workers (Raleigh et al. 2014). This finding was a reassessment of a prior study by the same authors that was identified by one reviewer (Lundin et al. 2009); the reassessment corrected limitations in the earlier analysis, which had concluded exposure was associated (albeit inconsistently) with prostate cancer, cerebrovascular disease, and diabetes (Lundin et al. 2009).

3.1.2. Toxicokinetic Analyses

Two papers were selected on the toxicokinetics analysis of data in humans. Both papers reexamined estimates of serum elimination half-life values for selected PFAS and proposed guidelines for best practices for risk assessment and risk management decision making. The following table summarizes the range and arithmetic mean scores by charge question and the total score (mean multiplied by 10).

Table 4. Scoring results for two papers on Toxicokinetic Analyses. Arithmetic mean (Range based on 4 reviewers).

Category of Charge Question	**Russell et al. 2015**	**Dourson et al. 2019**
Methods	9.50 (8 – 10)	7.75 (6 – 10)
Results	9.75 (9 – 10)	7.75 (6 – 9)
Discussion/Conclusions	9.00 (7 – 10)	8.00 (7 – 9)
Application to Risk Assessment Decision-Making	9.75 (9 – 10)	8.25 (7 – 10)
Total Score (mean x 10)	*95.0 (90.0-100.0)*	*79.4 (65.0-92.5)*

Russell et al. (2015)

Scores on the Russell et al. (2015) study evaluation were very consistent between reviewers for each charge question, differing by only 1 point for two of the four questions, and 2-3 points for the other two questions. In addition, average scores between categories were also very consistent, ranging between 9.00 and 9.75, for a final score (multiplied by 10) of 95.0. This final score was at least 10 points higher than the final scores for other studies evaluated as part of this review.

Each reviewer considered the methods, results, discussion, and overall applicability of this study to be very strong. The presentation of the critical concept is clear and convincing in its logic and reproducibility. The simple numerical examples show how background exposures are relevant to kinetics analysis. Failure to properly account for such ongoing exposures, even at low levels, can yield biased estimates of intrinsic elimination rates in the liver and apparent elimination rates from plasma. While Russell et al. (2015) use PFOA as the case study, the concept is generally applicable for estimates of serum elimination half-lives across PFAS and other chemical classes that similarly exhibit a slow rate of oral absorption coupled with a rapid elimination in the liver.

Three of four reviewers assigned a perfect score of 10 to Russell et al. (2015) for the charge question that asked about applying the paper to risk assessment decision-making. As demonstrated and discussed in Russell et al. (2015), empirically derived serum elimination half-lives can introduce an upward bias in estimates of intrinsic half-life if ongoing (e.g., non-drinking water) sources are not accounted for in the calculation. Furthermore, as illustrated by the authors' reanalysis of the same data evaluated by Olsen et al. (2007; discussed in the prior section), the bias increases for communities with lower drinking water concentrations for whom non-drinking water sources comprise a more significant proportion of the total long-term average daily dose.

One reviewer noted that the key message from Russell et al. (2015) could be even more convincingly illustrated using a PBPK model rather than a classic first-order kinetics model. Such an analysis was published by Worley et al. (2017a), who applied the model to the same study populations evaluated by Emmett et al. (2006) and Olsen et al. (2007).

Dourson et al. (2019)

Scores on the Dourson et al. (2019) study evaluation were more variable between reviewers, differing by 2 to 4 points per charge question. Average scores by category ranged from 7.75 to 8.25, for a final score of 79.4.

The reviewers noted that the publication was innovative in its use of human clinical data (rather than community monitoring data). The reviewers stated that the paper provides an important contribution to risk assessment because it addresses two key uncertainties – 1) basis for establishing an internal dose as a point of departure: a) C_{max}; b) area under the curve (AUC); or c) average concentration during a sensitive developmental period; and 2) interspecies

variability assessed using robust datasets for both animals and humans. Both concepts may inform the set of uncertainty factors that are applied to a point of departure to calculate a reference dose.

Several reviewers noted that there were no serious flaws in the methodology, the sample sizes were appropriate for the study objectives, and potential confounders were appropriately acknowledged given the study populations were described by Dourson et al. (2019) as "*non-pregnant mice and humans, and in the case of humans, from individuals of both sexes with advanced disease*".

Dourson et al. (2019) introduced the work by summarizing the evolution of regulatory guidance on establishing the point of departure for developmental toxicants and that current default positions among regulatory agencies are not consistent. While this helps to establish how there can be a toxicologically meaningful difference between the use of C_{max} versus AUC, one reviewer cautioned that Dourson et al. (2019) ultimately outlines the opinions of the authors. In contrast, the references cited provide more objective regulatory guidance.

One reviewer independently reviewed the original data on the human subjects and recommended calculating kinetic parameters for only individuals with steady state concentrations rather than developing a multiplier based on the average of the C_{max} values across doses for all subjects. The same reviewer noted that Dourson et al. (2019) assumed the highest mice levels were representative of steady-state conditions, and that alternative choices could be explored, which would change the data derived extrapolation factor (DDEF) that is central to estimating a point of departure used to calculate a protective toxicity reference value for risk assessment. Dourson et al. (2019) would likely agree with this observation, presenting their example calculation as one interpretation that would be subject to change depending on the relationship between the time required to achieve a steady state and the "*relevant window of susceptibility for that endpoint*".

Similar to Russell et al. (2015), Dourson et al. (2019) observed that the published literature on the serum elimination half-life of PFOA is quite variable. Dourson et al. (2019) point out that the human data from Elcombe et al. (2013) would support a relatively short half-life estimate, and that this may be partly attributable to the biphasic elimination of PFOA that is observed in both humans and mice.

3.1.3. Key Effects on Immune/Vaccine Response

Two papers were selected on key effects associated with immune function and vaccine response in humans. Both papers evaluated correlations between serum PFAS levels and vaccine-induced antibody levels in infants and/or young children, though methods to control for potential confounders varied. The following table summarizes the range and arithmetic mean scores by charge question as well as the total score (mean multiplied by 10).

Table 5. Scoring results for two papers on Key Effects on Immune/Vaccine Response. Arithmetic mean (Range based on 4 reviewers).

Category of Charge Question	Abraham et al. 2020	Grandjean et al. 2012
Methods	8.25 (7 – 9)	7.75 (4 – 10)
Results	7.75 (7 – 9)	8.00 (4 – 10)
Discussion/Conclusions	8.00 (7 – 9)	7.00 (4 – 9)
Application to Risk Assessment Decision-Making	7.25 (6 – 8)	5.75 (3 – 9)
Total Score (Mean x 10)	*78.1 (70.0-85.0)*	*71.3 (37.5-92.5)*

Abraham et al. (2020)

Scores on the Abraham et al. (2020) study evaluation were very consistent between reviewers, differing by no more than 2 points per charge question. This resulted in very consistent average scores across reviewers, ranging between 7.25 to 8.25, for a final score (multiplied by 10) of 71.3.

Two reviewers described the study by Abraham et al. (2020) as well designed and executed, with appropriate consideration of potential confounders, and choices of biomarkers of immune system function. One reviewer emphasized that because the study examined exclusively vaccinated individuals, there is uncertainty in extrapolating findings to the general population. The results from this cross-sectional study demonstrate an inverse relationship between serum PFOS and PFOA levels in very young children, and their antibody responses to three different vaccines. The inclusion of an additional factor – breastfed versus formula-fed infants – is also relevant for purposes of informing public health advisories and risk assessment, though one author alludes to the potentially complex interactions between differences in PFAS concentrations (likely more elevated in breast milk) and differences in biomarkers of immunity in populations of breastfed and formula-fed infants generally (i.e., when PFAS exposures are not a factor). Inclusion of additional biomarkers would have increased the utility of the study. One reviewer suggested specific immunity markers that would have provided greater insight regarding changes in vaccine response.

Grandjean et al. (2012)

Scores on the Grandjean et al. (2012) study evaluation varied greatly between reviewers, differing by 5 to 6 points per charge question. Average scores by category ranged from 5.75 to 8.00, for a final score of 73.1. The final scores among reviewers ranged from 37.5 to 92.5.

Reviewers indicated that the study by Grandjean et al. (2012) is notable as being the first to examine potential immune system effects from exposure to PFAS in young children. Each reviewer expressed concerns over potential confounders that were not fully examined. As with the second study in this topic area, one reviewer suggested a potentially critical confounder is the role of vaccines themselves, which may alter the immune systems of young children,

implying the study design is deficient by excluding non-vaccinated individuals as a control group. One reviewer noted that the biochemical assays excluded a number of immunity markers that would have provided greater insight regarding changes in vaccine response. One reviewer expressed concern that the study population was exposed to elevated (i.e., greater than background) levels of multiple contaminants that are also linked to immune system effects, including PCBs, and that the statistical analysis did not adequately examine the potential for confounding effects. Grandjean et al. (2012) state, "*Results adjusted for PCBs in milk and 5-year serum as predictors of PCB immunotoxicity were not materially different*", however, since it is the 18-month serum PCB result that demonstrated the strongest association with antibody levels at 5 and 7 years (not the maternal or 5-year serum PCBs), based on work by these same authors reported by Heilmann et al. (2010), the rationale for selection of exposure periods that may contribute to statistical interactions was not well described.

3.1.4. Key Effects on Reproduction and Development

Two papers were selected on key effects on reproduction and development in mice. Both papers focus on effects of PFOA following exposure during pregnancy, including reproductive outcomes and developmental landmarks in pups, although the dosing protocols were different. The following table summarizes the range and arithmetic mean scores by charge question as well as the total score (mean multiplied by 10).

Table 6. Scoring results for two papers on Key Effects on Reproduction and Development. Arithmetic mean (Range based on 4 reviewers).

Category of Charge Question	Koskela et al. 2016	Lau et al. 2006
Methods	5.50 (1 – 9)	9.00 (8 – 10)
Results	5.75 (1 – 9)	9.00 (8 – 10)
Discussion/Conclusions	6.75 (5 – 10)	8.50 (6 – 10)
Application to Risk Assessment Decision-Making	3.75 (1 –7)	7.50 (5 – 10)
Total Score (mean x 10)	*54.4 (22.5-87.5)*	*85.0 (67.5-95.0)*

Koskela et al. (2016)

Scores on the Koskela et al. (2016) study evaluation varied greatly between reviewers, differing by 5 to 8 points per charge question. Average scores between categories varied from 3.75 to 6.75, for a final score (mean multiplied by 10) of 54.4, the lowest of the papers reviewed.

The relatively low scores given to the study by Koskela et al. (2016) reflect a wide range of observations noted by reviewers regarding the study methods, results, and interpretation, all of

which limit the applicability of the study in PFAS risk assessment. Examples of specific points raised by reviewers include:

- Use of a single dose group reported as 0.3 mg/kg/day, yet insufficient information is provided to determine the actual dose administered;
- Small sample size (n=6 pregnant mice in the treatment group, unspecified sample size of the control group), with only 2 animals involved in the examination of bone marrow stromal cells *in vitro*;
- Other than demonstrating that PFOA can accumulate in bone at 13 and 17 months, other conclusions regarding effects on bone morphology and density are speculative and potentially attributed to differences in the sizes of test animals (larger in treatment than control groups);
- No observed effect in biomechanical properties of the bone and no data are presented on the distribution of PFOA in bone to support the statement by Koskela et al. (2016) that the study extends findings by other researchers on the distribution of PFOA in bone; and
- No reporting of dietary PFOA levels or measured serum PFOA levels.

Lau et al. (2006)

Scores on the Lau et al. (2006) study evaluation were moderately consistent between reviewers, differing by 2 to 5 points per charge question. Average scores by category ranged from 7.50 to 9.00, for a final score of 85.0.

In contrast to the study by Koskela et al. (2016) which had methodological limitations and has not been replicated, the study by Lau et al. (2006) supports a dose-response relationship that can be applied to health risk assessment and the findings have been replicated by subsequent researchers. Reviewers noted that the study was well designed and executed, conducted by a EPA laboratory that is GLP compliant, used sufficient sample sizes to support criteria for statistical analysis, and was of sufficient study duration to examine the key reproduction and developmental toxicity endpoints. Reviewers also remarked that this was an important early study that supports the current understanding of species-specific differences in kinetics of PFAS in the mice and rats as models for establishing toxicity reference values for human health risk assessment. However, one reviewer felt Lau et al. (2006) overreached when stating that because there appears to be a similar lack of sex differences in PFOA elimination in humans, primates, and mice (compared with the rat), that mice are a preferred model. The reviewer suggested that a more rigorous examination of the literature using the Bradford Hill Criteria is needed to support broad claims regarding cause and effect stemming from differences in dose-response profiles across species. In addition, the study is not designed to evaluate the modes/mechanisms of action (e.g., there is no histopathology to accompany the finding of liver weight changes).

The Lau et al. (2006) study reports a BMD5 and BMDL5 of 0.20 mg/kg and 0.17 mg/kg, respectively, based on alterations (increase) in absolute maternal liver weight. One reviewer questioned the choice of this endpoint, stating that relative liver weight would be a preferred metric. In addition, one reviewer noted that the benchmark dose evaluation appears to have

relied on a single criterion (AIC), rather than a suite of criteria recommended by EPA today, including the factor difference between the calculated BMDL and the dose for the lowest treatment group. For this study, use of a benchmark response level (BMR) of 5 percent yields a BMDL that is approximately six times lower than the lowest dose of 1 mg/kg, which introduces uncertainty in the selection of this BMDL as a point of departure for risk assessment. One reviewer remarked on the significance of Lau et al. (2006) given that it is key study supporting EPA's 2016 drinking water health advisory for PFOA as well as similar benchmarks adopted by at least three States. The reviewer noted that although the study by Lau et al. (2006) is well designed and executed overall, some limitations have also been identified, which underscores the importance of applying a comprehensive literature search and systematic review process. It is critical to evaluate the entire toxicological and epidemiological data base to determine if the growing weight of evidence continues to support decisions that are based on the results reported in the earlier foundational studies.

3.1.5. Key Effects on Other Endpoints

Three papers were selected on key effects other than immune system function, reproduction, and development, in rats. The studies included a chronic (two-year) dietary and carcinogenicity study with PFOA in male and female rats, a 13-week dietary study with PFOA in male rats, and a two-generation reproduction and cross-foster study with PFOS in male and female rats. The following table summarizes the range and arithmetic mean scores by charge question as well as the total score (mean multiplied by 10).

Table 7. Scoring results for three papers on Key Effects on Other than Immune System Function, Reproduction and Development. Arithmetic mean (Range based on 4 reviewers).

Category of Charge Question	Butenhoff et al. 2012	Perkins et al. 2004	Luebker et al. 2005
Methods	8.50 (8 – 9)	7.75 (5 – 9)	8.00 (5 – 10)
Results	8.25 (5 – 10)	8.00 (7 – 10)	7.50 (4 – 9)
Discussion/Conclusions	8.50 (7 – 10)	7.00 (3 – 10)	7.00 (3 – 10)
Application to Risk Assessment Decision-Making	6.75 (3 – 9)	7.00 (4 – 9)	7.25 (4 – 9)
Total Score (x 10)	*80.0 (57.5-92.5)*	*74.4 (47.5-95.0)*	*74.4 (40.0-95.0)*

Butenhoff et al. (2012)

Scores on the Butenhoff et al. (2012) study evaluation varied greatly between reviewers, differing by 1 to 6 points per charge question. Average scores between categories varied from 6.75 to 8.50, for a final score (multiplied by 10) of 80.0.

Reviewers noted that the study by Butenhoff et al. (2012) was well conducted and well described, with appropriate levels of data validation and independent review. While it is an important early chronic rat study with PFOA, limitations in the study design include the use of only two dose levels and absence of measured blood or tissue levels. Nevertheless, extensive clinical chemistry and tissue histopathology accompanied the evaluation of systemic effects, which is particularly important for distinguishing between adaptive and adverse responses from liver hyperplasia. Butenhoff et al. (2012) specifically reported that the pathology review found that exposure for the high dose group (300 ppm) was associated with a slight increase in acinar cell hyperplasia, but not adenoma or carcinoma. Furthermore, male rats were observed to be more sensitive than female rats with respect to liver toxicity, which is an important finding that contributed to subsequent studies of mechanisms that can explain sex-specific and species-specific differences in urinary elimination of PFOA. One reviewer noted that although the authors identify neoplastic effects in the testes (i.e., Leydig cell tumors) as a critical effect endpoint for PFOA, and that the adenoma likely occurs through a non-genotoxic mode of action, they missed an opportunity to discuss uncertainty in extrapolating such effects from rats to humans.

Butenhoff et al. (2012) discuss the relevance of the differences in outcomes of the study compared with a similar study by Biegel et al. (2001) that was designed to further investigate the mode of action of PFOA. Reviewers found this discussion to be helpful, but stated that the discussion linking the study findings to a series of epidemiologic studies with PFOA detracted from the results of the animal study, in part because serum PFOA levels were not reported and the human equivalent dose was not evaluated.

Perkins et al. (2004)

Scores on the Perkins et al. (2004) study evaluation were also variable between reviewers, differing by 3 to 7 points per charge question. Average scores by category ranged from 7.00 to 8.00, for a final score of 74.4.

The study by Perkins et al. (2004) is described as well designed and executed, exceeding requirements of standard 90-day toxicity studies in rats, and supporting a dose-response analysis of liver effects across dose ranges that are relevant based on similar studies during the same period. The study included histopathology, with negative findings matching that of Butenhoff et al., (2012) described above. Doses were too low to replicate findings of Leydig cell tumors in the testes. Reviewers agreed with the proposed NOAEL of 1 ppm diet (0.06 mg/kg body weight) with doses of 10 ppm (0.64 mg/kg) and higher producing adaptive and reversible liver changes.

Although serum levels of PFOA were reported, one reviewer questions the accuracy of the calculated serum elimination half-life values, which appear to be much longer (29-54 days) than reported by the authors (7 days) based on steady-state concentrations at the end of treatment and the 8-week recovery period. In addition, there was no discussion of an apparent high-dose saturation based on dose-normalized serum concentrations which decrease from a maximum of 7.1 ng/mL at the low dose (1 ppm) to 1.4 ng/mL at the high dose (100 ppm).

Luebker et al. (2005)

Scores on the Luebker et al. (2005) study evaluation were also variable between reviewers, differing by 5 to 7 points per charge question. Average scores by category ranged from 7.00 to 8.00, for a final score of 74.4.

The study by Luebker et al. (2005) is the only study selected for review to focus on effects of PFOS in rats. The use of a cross-fostering design provides an opportunity to examine if effects in offspring are linked to effects in the dam or to direct exposure to the fetus. Additional study design elements that increase confidence in this study were the use of four dose groups spanning a dose range that did not induce pronounced toxicity that would interfere with observations in F1 and F2 generation offspring, and a sufficient sample size to support performance criteria of statistical tests. However, reviewers identified potential issues with the study design that may limit how the study findings can be compared with similar PFOS studies in rats. Specifically, dosing was performed via gavage rather than diet, which changes the kinetics of absorption, and may preclude observations of potential saturation effects (as was observed in data reported by Perkins et al., 2004). Furthermore, dosing was performed at GD-9 and LD-20, skipping other days during gestation and lactation.

Reviewers largely agreed with the results and discussion presented by Luebker et al. (2005). Relevant findings for risk assessment include support for a steep dose-response curve, evidence of *in utero* transfer of PFOS to the fetus and to pups by lactation, and relevance of reproduction as a critical effect endpoint in rodents. Some shortcomings of the analysis were also noted:

- milk appears to have been sampled from only two dams, which is insufficient for interpretation;
- cord blood and amniotic fluid, were not collected or analyzed, though one reviewer noted that these tissues cannot be obtained if pups are born naturally;
- blood from F1 pups was not collected or analyzed, which is a missed opportunity;
- no explanation is provided for reduction in feed consumption by control dams fostered by treated dams (CL/TD) and treated dams fostered by control dams (TL/CD)
- Inconsistency in the reported dose in F_0 females – stated as 0.1 mg/kg-day in Table 14, but 1.6 mg/kg in the text (p. 140)

Similar to the significance of Lau et al. (2006) study on PFOA, Luebker et al. (2005) is remarkable as it is key study supporting EPA's 2016 drinking water health advisory for PFOS as well as similar benchmarks adopted by at least three states on the basis of reduced pup body weight.

3.2. Identification of Additional Key Studies

The reviewers identified more than a dozen additional studies that provide additional insights regarding the exposure, toxicity including immunotoxicity and carcinogenicity, and kinetics of PFOA, PFOS, and other PFAS compounds (see Table 8).

Table 8. List of Additional Studies (Including Review Articles and Key Supporting Studies) Recommended by Expert Panel Members

Recommended Paper	Abstract
Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol. 2019 Mar;29(2):131-147. doi: 10.1038/s41370-018-0094-1. Epub 2018 Nov 23. PMID: 30470793; PMCID: PMC6380916.	Here, we review present understanding of sources and trends in human exposure to poly- and perfluoroalkyl substances (PFASs) and epidemiologic evidence for impacts on cancer, immune function, metabolic outcomes, and neurodevelopment. More than 4000 PFASs have been manufactured by humans and hundreds have been detected in environmental samples. Direct exposures due to use in products can be quickly phased out by shifts in chemical production but exposures driven by PFAS accumulation in the ocean and marine food chains and contamination of groundwater persist over long timescales. Serum concentrations of legacy PFASs in humans are declining globally but total exposures to newer PFASs and precursor compounds have not been well characterized. Human exposures to legacy PFASs from seafood and drinking water are stable or increasing in many regions, suggesting observed declines reflect phase-outs in legacy PFAS use in consumer products. Many regions globally are continuing to discover PFAS contaminated sites from aqueous film forming foam (AFFF) use, particularly next to airports and military bases. Exposures from food packaging and indoor environments are uncertain due to a rapidly changing chemical landscape where legacy PFASs have been replaced by diverse precursors and custom molecules that are difficult to detect. Multiple studies find significant associations between PFAS exposure and adverse immune outcomes in children. Dyslipidemia is the strongest metabolic outcome associated with PFAS exposure. Evidence for cancer is limited to manufacturing locations with extremely high exposures and insufficient data are available to characterize impacts of PFAS exposures on neurodevelopment. Preliminary evidence suggests significant health effects associated with exposures to emerging PFASs. Lessons learned from legacy PFASs indicate that limited data should not be used as a justification to delay risk mitigation actions for replacement PFASs.
Klaunig JE, Hocevar BA, Kamendulis LM. Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance. Reprod Toxicol. 2012 Jul;33(4):410-418. doi: 10.1016/j.reprotox.2011.10.014. Epub 2011 Nov 22. PMID: 22120428.	Perfluorooctanoic acid (PFOA) is an environmentally persistent chemical used in the manufacturing of a wide array of industrial and commercial products. PFOA has been shown to induce tumors of the liver, testis and pancreas (tumor triad) in rats following chronic dietary administration. PFOA belongs to a group of compounds that are known to activate the PPARα receptor. The PPARα activation Mode of Action was initially addressed in 2003 [9] and further refined in subsequent reviews [92-94]. In the intervening time, additional information on PFOA effects as well as a further refinement of the Mode of Action framework warrants a re-examination of this compound for its cancer induction Mode of Action. This review will address the rodent (rat) cancer data and cancer Mode of Action of PFOA for tumors of the liver, testes and pancreas.
Xu Y, Fletcher T, Pineda D, Lindh CH, Nilsson C, Glynn A, Vogs C, Norström K, Lilja K, Jakobsson K, Li Y. Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. Environ Health Perspect. 2020 Jul;128(7):77004.	Background: Firefighting foam-contaminated ground water, which contains high levels of perfluoroalkyl substances (PFAS), is frequently found around airports. In 2018 it was detected that employees at a municipal airport in northern Sweden had been exposed to high levels of short-chain PFAS along with legacy PFAS (i.e., PFOA, PFHxS, and PFOS) through drinking water. Objectives: In this study, we aimed to describe the PFAS profile in drinking water and biological samples (paired serum and urine) and to estimate serum half-lives of the short-chain PFAS together with legacy PFAS.

doi: 10.1289/EHP6785. Epub 2020 Jul 10. PMID: 32648786; PMCID: PMC7351026.	Methods: Within 2 weeks after provision of clean water, blood sampling was performed in all 26 airport employees. Seventeen of them were then followed up monthly for 5 months. PFHxA, PFHpA, PFBS, PFPeS, and PFHpS together with legacy PFAS in water and biological samples were quantified using LC/MS/MS. Half-lives were estimated by assuming one compartment, first-order elimination kinetics. Results: The proportions of PFHxA, PFHpA, and PFBS were higher in drinking water than in serum. The opposite was found for PFHxS and PFOS. The legacy PFAS accounted for about 50% of total PFAS in drinking water and 90% in serum. Urinary PFAS levels were very low compared with serum. PFBS showed the shortest half-life {average 44 d [95% confidence interval (CI): 37, 55 d], followed by PFHpA [62 d (95% CI: 51, 80 d)]. PFPeS and PFHpS showed average half-lives as 0.63 and 1.46 y, respectively. Branched PFOS isomers had average half-lives ranging from 1.05 to 1.26 y for different isomers. PFOA, PFHxS, and linear PFOS isomers showed average half-lives of 1.77, 2.87, and 2.93 y, respectively. Discussion: A general pattern of increasing half-lives with increasing chain length was observed. Branched PFOS isomers had shorter half-lives than linear PFOS isomers. https://doi.org/10.1289/EHP6785.
White SS, Stanko JP, Kato K, Calafat AM, Hines EP, Fenton SE. Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environ Health Perspect. 2011 Aug;119(8):1070-6. doi: 10.1289/ehp.1002741. Epub 2011 Apr 18. PMID: 21501981; PMCID: PMC3237341.	Background: Prenatal exposure to perfluorooctanoic acid (PFOA), a ubiquitous industrial surfactant, has been reported to delay mammary gland development in female mouse offspring (F1) and the treated lactating dam (P0) after gestational treatments at 3 and 5 mg PFOA/kg/day. Objective: We investigated the consequences of gestational and chronic PFOA exposure on F1 lactational function and subsequent development of F2 offspring. Methods: We treated P0 dams with 0, 1, or 5 mg PFOA/kg/day on gestation days 1-17. In addition, a second group of P0 dams treated with 0 or 1 mg/kg/day during gestation and their F1 and F2 offspring received continuous PFOA exposure (5 ppb) in drinking water. Resulting adult F1 females were bred to generate F2 offspring, whose development was monitored over postnatal days (PNDs) 1-63. F1 gland function was assessed on PND10 by timed-lactation experiments. Mammary tissue was isolated from P0, F1, and F2 females throughout the study and histologically assessed for age-appropriate development. Results: PFOA-exposed F1 dams exhibited diminished lactational morphology, although F1 maternal behavior and F2 offspring body weights were not significantly affected by P0 treatment. In addition to reduced gland development in F1 females under all exposures, F2 females with chronic low-dose drinking-water exposures exhibited visibly slowed mammary gland differentiation from weaning onward. F2 females derived from 5 mg/kg PFOA-treated P0 dams displayed gland morphology similar to F2 chronic water exposure groups on PNDs 22-63. Conclusions: Gestational PFOA exposure induced delays in mammary gland development and/or lactational differentiation across three generations. Chronic, low-dose PFOA exposure in drinking water was also sufficient to alter mammary morphological development in mice, at concentrations approximating those found in contaminated human water supplies.
Pizzurro DM, Seeley M, Kerper LE, Beck BD. Interspecies differences in perfluoroalkyl	Toxicokinetics are important for extrapolating health effects and effect levels observed in laboratory animals to humans for purposes of establishing health-based criteria. We conducted a comprehensive review of key

substances (PFAS) toxicokinetics and application to health-based criteria. Regul Toxicol Pharmacol. 2019 Aug;106:239-250. doi: 10.1016/j.yrtph.2019.05.008. Epub 2019 May 9. PMID: 31078680.	absorption, distribution, metabolism, and excretion (ADME) parameters across different mammalian species for five perfluoroalkyl substances (PFAS) and discussed how these data can be used to inform human health risk assessment of these substances. Our analysis revealed several notable differences among the different PFAS regarding species- and substance-specific tissue partitioning, half-life, and transfer to developing offspring via the placenta or lactation, as well as highlighted data gaps for certain substances. We incorporated these observations in an analysis of whether health-based values for specific PFAS can be applied to other PFAS of differing chain length or toxicological mode of action. Overall, our analysis provides one of the first syntheses of available empirical PFAS toxicokinetic data to facilitate interpreting human relevance of animal study findings and developing health-based criteria for PFAS from such studies.
Roberts M. 2016. A Critical Review of Pharmacokinetic Modelling of PFOS and PFOA to Assist in Establishing HGBVs for these Chemicals. Food Standards, New Zealand. Available at https://www1.health.gov.au/internet/main/publishing.nsf/content/2200FE086D480353CA2580C900817CDC/$File/7.Critical-Review-Pharmacokinetic-Modelling.pdf	This report presents a review of the US EPA reports on the perfluoroalkylated substances: perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), with a focus on the validity of the pharmacokinetic (PK) modelling applied by the US EPA, including a summary of assumptions, limitations and uncertainties. A hoped for outcome from this critical evaluation of the pharmacokinetic modelling used by the US EPA, and our development of what we believe are more realistic PK models, is that it may assist FSANZ to establish appropriate health based guidance values (HBGV).
Cordner A, De La Rosa VY, Schaider LA, Rudel RA, Richter L, Brown P. Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors. J Expo Sci Environ Epidemiol. 2019 Mar;29(2):157-171. doi: 10.1038/s41370-018-0099-9. Epub 2019 Jan 8. Erratum in: J Expo Sci Environ Epidemiol. 2019 Mar 29;: Erratum in: J Expo Sci Environ Epidemiol. 2020 May;30(3):585-586. PMID: 30622333; PMCID: PMC6455940.	Communities across the U.S. are discovering drinking water contaminated by perfluoroalkyl and polyfluoroalkyl substances (PFAS) and determining appropriate actions. There are currently no federal PFAS drinking water standards despite widespread drinking water contamination, ubiquitous population-level exposure, and toxicological and epidemiological evidence of adverse health effects. Absent federal PFAS standards, multiple U.S. states have developed their own health-based water guideline levels to guide decisions about contaminated site cleanup and drinking water surveillance and treatment. We examined perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) water guideline levels developed by the U.S. Environmental Protection Agency (EPA) and state agencies to protect people drinking the water, and summarized how and why these levels differ. We referenced documents and tables released in June 2018 by the Interstate Technology and Regulatory Council (ITRC) to identify states that have drinking water and groundwater guideline levels for PFOA and/or PFOS that differ from EPA's health advisories (HAs). We also gathered assessment documents from state websites and contacted state environmental and health agencies to identify and confirm current guidelines. Seven states have developed their own water guideline levels for PFOA and/or PFOS ranging from 13 to 1000 ng/L, compared to EPA's HA of 70 ng/L for both compounds individually or combined. We find that the development of PFAS guideline levels via exposure and hazard assessment decisions is influenced by multiple scientific, technical, and social factors, including managing scientific uncertainty, technical decisions and capacity, and social, political, and economic influences from involved stakeholders. Assessments by multiple states and academic scientists suggest that EPA's HA is not sufficiently protective. The ability of states to develop their own guideline levels and standards provides

	diverse risk assessment approaches as models for other state and federal regulators, while a sufficiently protective, scientifically sound, and enforceable federal standard would provide more consistent protection.
Worley, R.R., Yang, X., and Fisher, J. 2017. Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. Toxicol. Appl. Pharmacol., 330:9-21.	Background: Manufacturing of perfluorooctanoic acid (PFOA), a synthetic chemical with a long half-life in humans, peaked between 1970 and 2002, and has since diminished. In the United States, PFOA is detected in the blood of N99% of people tested, but serum concentrations have decreased since 1999. Much is known about exposure to PFOA in drinking water; however, the impact of non-drinking water PFOA exposure on serum PFOA concentrations is not well characterized. Objective: The objective of this research is to apply physiologically based pharmacokinetic (PBPK) modeling and Monte Carlo analysis to evaluate the impact of historic non-drinking water PFOA exposure on serum PFOA concentrations. Methods: In vitro to in vivo extrapolation was utilized to inform descriptions of PFOA transport in the kidney. Monte Carlo simulations were incorporated to evaluate factors that account for the large inter-individual variability of serum PFOA concentrations measured in individuals from North Alabama in 2010 and 2016, and the Mid-Ohio River Valley between 2005 and 2008. Results: Predicted serum PFOA concentrations were within two-fold of experimental data. With incorporation of Monte Carlo simulations, the model successfully tracked the large variability of serum PFOA concentrations measured in populations from the Mid-Ohio River Valley. Simulation of exposure in a population of 45 adults from North Alabama successfully predicted 98% of individual serum PFOA concentrations measured in 2010 and 2016, respectively, when non-drinking water ingestion of PFOA exposure was included. Conclusions: Variation in serum PFOA concentrations may be due to inter-individual variability in the disposition of PFOA and potentially elevated historical non-drinking water exposures.
East A, Anderson RH, Salice CJ. Per- and Polyfluoroalkyl Substances (PFAS) in Surface Water Near US Air Force Bases: Prioritizing Individual Chemicals and Mixtures for Toxicity Testing and Risk Assessment. Environ Toxicol Chem. 2021 Mar;40(3):859-870. doi: 10.1002/etc.4893. Epub 2020 Dec 15. PMID: 33026654.	Per- and polyfluoroalkyl substances (PFAS) are a large class of persistent chemicals used for decades in industrial and commercial applications. A key challenge with regard to estimating potential risk to ecological (and human) receptors associated with PFAS exposure lies in the fact that there are many different PFAS compounds and several to many can co-occur in any given environmental sample. We applied a data science approach to characterize and prioritize PFAS and PFAS mixtures from a large dataset of PFAS measurements in surface waters associated with US Air Force Installations with a history of the use of aqueous film-forming foams (AFFFs). Several iterations of stakeholder feedback culminated in a few main points that advanced our understanding of a complex dataset and the larger ecotoxicological problem. First, perfluorooctane sulfonate (PFOS) was often a dominant PFAS in a given surface water sample, frequently followed by perfluorohexane sulfonate (PFHxS). Second, a 4-chemical mixture generally accounted for >80% of the sum of all routinely reported PFAS in a sample, and the most representative 4-chemical mixture was composed of PFOS, PFHxS, perfluorohexanoic acid (PFHxA), and perfluorooctanoic acid (PFOA). We suggest that these results demonstrate the utility of formalized data science analysis and assessment frameworks to address complex ecotoxicological problems. Specifically, our example dataset results can be used to provide perspective on toxicity testing, ecological risk assessments, and field studies of PFAS in and around AFFF-impacted sites. Environ Toxicol Chem 2021;40:871-882. © 2020 SETAC.

Alexander BH, Olsen GW. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Ann Epidemiol. 2007 Jun;17(6):471-8. doi: 10.1016/j.annepidem.2007.01.036. Epub 2007 Apr 19. PMID: 17448680.	Purpose: To determine whether bladder cancer is associated with exposure to perfluorooctane sulfonate (PFOS) in an occupational cohort. Methods: Incidence of bladder cancer was ascertained by postal questionnaire to all living current and former employees of the facility (N = 1895) and death certificates for deceased workers (N = 188). Exposure to PFOS was estimated with work history records and weighted with biological monitoring data. Standardized incidence ratios (SIRs) were estimated using U.S. population-based rates as a reference. Bladder cancer risk within the cohort was evaluated using Poisson regression by cumulative PFOS exposure. Results: Questionnaires were returned by 1,400 of the 1895 cohort members presumed alive. Eleven cases of primary bladder cancer were identified from the surveys (n = 6) and death certificates (n = 5). The SIRs were 1.28 (95% confidence interval [CI] = 0.64-2.29) for the entire cohort and 1.74 (95% CI = 0.64-3.79) for those ever working in a high exposed job. Compared with employees in the lowest cumulative exposure category, the relative risk of bladder cancer was 0.83 (95% CI = 0.15-4.65), 1.92 (95% CI = 0.30-12.06), and 1.52 (95% CI = 0.21-10.99). Conclusions: The results offer little support for an association between bladder cancer and PFOS exposure, but the limited size of the population prohibits a conclusive exposure response analysis.
Raleigh KK, Alexander BH, Olsen GW, Ramachandran G, Morey SZ, Church TR, Logan PW, Scott LL, Allen EM. Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occup Environ Med. 2014 Jul;71(7):500-6. doi: 10.1136/oemed-2014-102109. Epub 2014 May 15. PMID: 24832944; PMCID: PMC4078701.	Objective: To evaluate mortality and cancer incidence in a cohort of ammonium perfluorooctanoate (APFO) exposed workers. Methods: We linked a combined cohort (n=9027) of employees from APFO and non-APFO production facilities in Minnesota to the National Death Index and to cancer registries of Minnesota and Wisconsin. Industrial hygiene data and expert evaluation were used to create a task-based job exposure matrix to estimate APFO exposure. Standardised mortality ratios were estimated using Minnesota population rates. HRs and 95% CIs for time-dependent cumulative APFO exposure were estimated with an extended Cox model. A priori outcomes of interest included cancers of the liver, pancreas, testes, kidney, prostate and breast, and mortality from cardiovascular, cerebrovascular and chronic renal diseases. Results: Mortality rates in the APFO-exposed cohort were at or below the expected, compared with Minnesota. The HR for dying from the cancer and non-cancer outcomes of interest did not show an association with APFO exposure. Similarly, there was little evidence that the incident cancers were associated with APFO exposure. Compared to the non-exposed population, modestly elevated, but quite imprecise HRs were observed in the higher-exposure quartiles for bladder cancer (HR=1.66, 95% CI 0.86 to 3.18) and pancreatic cancer (HR=1.36, 95% CI 0.59 to 3.11). No association was observed between APFO exposure and kidney, prostate or breast cancers. Conclusions: This analysis did not support an association between occupational APFO exposure and the evaluated health endpoints, however, the study had limited power to evaluate some conditions of interest.
Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, Jakobsson K. Half-lives of PFOS, PFHxS and PFOA after end of exposure to	Background: Municipal drinking water contaminated with perfluorinated alkyl acids had been distributed to one-third of households in Ronneby, Sweden. The source was firefighting foam used in a nearby airfield since the mid-1980s. Clean water was provided from 16 December 2013.

contaminated drinking water. Occup Environ Med. 2018 Jan;75(1):46-51. doi: 10.1136/oemed-2017-104651. Epub 2017 Nov 13. PMID: 29133598; PMCID: PMC5749314.	Objective: To determine the rates of decline in serum perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), and their corresponding half-lives. Methods: Up to seven blood samples were collected between June 2014 and September 2016 from 106 participants (age 4-84 years, 53% female). Results: Median initial serum concentrations were PFHxS, 277 ng/mL (range 12-1660); PFOS, 345 ng/mL (range 24-1500); and PFOA, 18 ng/mL (range 2.4-92). The covariate-adjusted average rates of decrease in serum were PFHxS, 13% per year (95% CI 12% to 15%); PFOS, 20% per year (95% CI 19% to 22%); and PFOA, 26% per year (95% CI 24% to 28%). The observed data are consistent with a first-order elimination model. The mean estimated half-life was 5.3 years (95% CI 4.6 to 6.0) for PFHxS, 3.4 years (95% CI 3.1 to 3.7) for PFOS and 2.7 years (95% CI 2.5 to 2.9) for PFOA. The interindividual variation of half-life was around threefold when comparing the 5th and 95th percentiles. There was a marked sex difference with more rapid elimination in women for PFHxS and PFOS, but only marginally for PFOA. Conclusions: The estimated half-life for PFHxS was considerably longer than for PFOS and PFOA. For PFHxS and PFOS, the average half-life is shorter than the previously published estimates. For PFOA the half-life is in line with the range of published estimates.
Ji J, Song L, Wang J, Yang Z, Yan H, Li T, Yu L, Jian L, Jiang F, Li J, Zheng J, Li K. Association between urinary per- and poly-fluoroalkyl substances and COVID-19 susceptibility. Environ Int. 2021 Aug;153:106524. doi: 10.1016/j.envint.2021.106524. Epub 2021 Mar 19. PMID: 33773143; PMCID: PMC7972714.	Background and objective: The growing impact of the COVID-19 pandemic has heightened the urgency of identifying individuals most at risk of infection. Per- and poly-fluoroalkyl substances (PFASs) are manufactured fluorinated chemicals widely used in many industrial and household products. The objective of this case-control study was to assess the association between PFASs exposure and COVID-19 susceptibility and to elucidate the metabolic dysregulation associated with PFASs exposure in COVID-19 patients. Methods: Total 160 subjects (80 COVID-19 patients and 80 symptom-free controls) were recruited from Shanxi and Shandong provinces, two regions heavily polluted by PFASs in China. Twelve common PFASs were quantified in both urine and serum. Urine metabolome profiling was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Results: In unadjusted models, the risk of COVID-19 infection was positively associated with urinary levels of perfluorooctanesulfonic acid (PFOS) (Odds ratio: 2.29 [95% CI: 1.52-3.22]), perfluorooctanoic acid (PFOA) (2.91, [1.95-4.83], and total PFASs (∑ (12) PFASs) (3.31, [2.05-4.65]). After controlling for age, sex, body mass index (BMI), comorbidities, and urine albumin-to-creatinine ratio (UACR), the associations remained statistically significant (Adjusted odds ratio of 1.94 [95% CI: 1.39-2.96] for PFOS, 2.73 [1.71-4.55] for PFOA, and 2.82 [1.97-3.51] for ∑ (12) PFASs). Urine metabolome-PFASs association analysis revealed that 59% of PFASs-associated urinary endogenous metabolites in COVID-19 patients were identified to be produced or largely regulated by mitochondrial function. In addition, the increase of PFASs exposure was associated with the accumulation of key metabolites in kynurenine metabolism, which are involved in immune responses (Combined β coefficient of 0.60 [95% CI: 0.25-0.95, P = 0.001]). Moreover, alternations in PFASs-associated metabolites in mitochondrial and kynurenine metabolism were also correlated with clinical lab biomarkers for

	mitochondrial function (serum growth/differentiation factor-15) and immune activity (lymphocyte percentage), respectively. Conclusion: Elevated exposure to PFASs was independently associated with an increased risk of COVID-19 infection. PFASs-associated metabolites were implicated in mitochondrial function and immune activity. Larger studies are needed to confirm our findings and further understand the underlying mechanisms of PFASs exposure in the pathogenesis of SARS-CoV2 infection.
Loccisano AE, Longnecker MP, Campbell JL Jr, Andersen ME, Clewell HJ 3rd. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. J Toxicol Environ Health A. 2013;76(1):25-57. doi: 10.1080/15287394.2012.722523. PMID: 23151209; PMCID: PMC3502013.	Perfluoroalkyl acid carboxylates and sulfonates (PFAA) have many consumer and industrial applications. Developmental toxicity studies in animals have raised concern about potential reproductive/developmental effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS); however, in humans conflicting results have been reported for associations between maternal PFAA levels and these outcomes. Risk assessments and interpretation of available human data during gestation and lactation are hindered due to lack of a framework for understanding and estimating maternal, fetal, and neonatal pharmacokinetics (PK). Physiologically based pharmacokinetic (PBPK) models were developed for PFOA and PFOS for the gestation and lactation life stages in humans to understand how the physiological changes associated with development affect pharmacokinetics of these compounds in the mother, fetus, and infant. These models were derived from PBPK models for PFOA/PFOS that were previously developed for adult humans and rats during gestation and lactation and from existing human pregnancy and lactation models developed for other chemicals. The models simulated PFOA and PFOS concentrations in fetal, infant, and maternal plasma and milk, were compared to available data in humans, and also were used to estimate maternal exposure. The models reported here identified several research needs, which include (1) the identification of transporters involved in renal resorption to explain the multiyear half-lives of these compounds in humans, (2) factors affecting clearance of PFOA/PFOS during gestation and lactation, and (3) data to estimate clearance of PFOA/PFOS in infants. These models may help address concerns regarding possible adverse health effects due to PFOA/PFOS exposure in the fetus and infant and may be useful in comparing pharmacokinetics across life stages.
Chou WC, Lin Z. Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats, monkeys, and humans: Development and performance verification. Environ Int. 2019 Aug;129:408-422. doi: 10.1016/j.envint.2019.03.058. Epub 2019 May 29. PMID: 31152982.	A challenge in the risk assessment of perfluorooctane sulfonate (PFOS) is the large interspecies differences in its toxicokinetics that results in substantial uncertainty in the dosimetry and toxicity extrapolation from animals to humans. To address this challenge, the objective of this study was to develop an open-source physiologically based pharmacokinetic (PBPK) model accounting for species-specific toxicokinetic parameters of PFOS. Considering available knowledge about the toxicokinetic properties of PFOS, a PBPK model for PFOS in mice, rats, monkeys, and humans after intravenous and oral administrations was created. Available species-specific toxicokinetic data were used for model calibration and optimization, and independent datasets were used for model evaluation. Bayesian statistical analysis using Markov chain Monte Carlo (MCMC) simulation was performed to optimize the model and to characterize the uncertainty and interspecies variability of chemical-specific parameters. The model predictions well correlated with the majority of datasets for all four species, and the model was validated with independent data in rats, monkeys, and humans. The model was applied to predict human equivalent doses (HEDs) based on reported points of departure in selected critical toxicity studies in rats and monkeys following U.S. EPA's guidelines. The lower bounds of the model-derived

	HEDs were overall lower than the HEDs estimated by U.S. EPA (e.g., 0.2 vs. 1.3 µg/kg/day based on the rat plasma data). This integrated and comparative analysis provides an important step towards improving interspecies extrapolation and quantitative risk assessment of PFOS, and this open-source model provides a foundation for developing models for other perfluoroalkyl substances.
Heilmann C, Budtz-Jørgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect. 2010 Oct;118(10):1434-8. doi: 10.1289/ehp.1001975. Epub 2010 Jun 1. PMID: 20562056; PMCID: PMC2957925.	Background: Polychlorinated biphenyls (PCBs) may cause immunotoxic effects, but the detailed dose-response relationship and possible vulnerable time windows of exposure are uncertain. In this study we applied serum concentrations of specific antibodies against childhood vaccines as sentinels of immunotoxicity. Objectives: The main objective was to assess the possible dependence of antibody concentrations against diphtheria and tetanus toxoids in children with regard to prenatal and postnatal PCB exposures. Methods: From a cohort of 656 singleton births formed in the Faroe Islands during 1999-2001, children were invited for examination with assessment of serum antibody concentrations at 5 years (before and after a booster vaccination) and at 7 years of age. Total PCB concentrations were determined in serum from ages 5 and 7 years, and data were also available on PCB concentrations in maternal pregnancy serum, maternal milk, and, for a subgroup, the child's serum at 18 months of age. Results: A total of 587 children participated in the examinations at ages 5 and/or 7 years. At age 5 years, before the booster vaccination, the antidiphtheria antibody concentration was inversely associated with PCB concentrations in milk and 18-month serum. Results obtained 2 years later showed an inverse association of concentrations of antibodies against both toxoids with PCB concentrations at 18 months of age. The strongest associations suggested a decrease in the antibody concentration by about 20% for each doubling in PCB exposure. At age 5 years, the odds of an antidiphtheria antibody concentration below a clinically protective level of 0.1 IU/L increased by about 30% for a doubling in PCB in milk and 18-month serum. Conclusions: Developmental PCB exposure is associated with immunotoxic effects on serum concentrations of specific antibodies against diphtheria and tetanus vaccinations. The immune system development during the first years of life appears to be particularly vulnerable to this exposure.

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4. Discussion and Conclusions

As would be expected, all key studies summarized by the reviewers were found to have strengths and weaknesses. However, differences in professional opinion regarding the usefulness of some of the studies to risk assessment decisions were apparent.

PFAS Toxicokinetics and Biomonitoring in Humans:

Biomonitoring data from humans can provide a key line of evidence to reduce uncertainty in estimates of toxicokinetics properties of chemicals. This information is particularly useful for developing a time course of predicted serum PFAS levels following changes in exposure conditions. Kinetics information has also been used to support the development of toxicity values and to justify grouping strategies to assess risks associated with exposure to mixtures. Four studies were reviewed on toxicokinetics – two specifically related to evaluations of human biomonitoring data on PFOA, PFOS, and PFHxS, in the context of large epidemiological studies of communities neighboring fluorochemical facilities, and two more recent studies that help to reexamine fundamental assumptions about the use of human data to improve estimates of toxicokinetics parameters for PFAS.

There was no indication of significant risk of bias for any of the studies. The reviewers consistently determined that the Emmett et al. (2006) study provided a higher confidence for use in risk assessment decision-making than Olsen et al. (2007) largely due to the improved study design. Reviewers described the study as having a strong methodology, scientifically sound derivation of results, the conclusions were well supported by data, and a strong (84 points out of 100) application to risk assessment decision making. However, reviewers noted several limitations with the Emmett et al. study that introduce uncertainty in extrapolating the results beyond the community in Little Hocking, WV. One reviewer cautioned that water and serum levels were higher than the general population, and one reviewer highlighted uncertainties in the serum/water PFOA ratio of approximately 100:1. Overall, reviewers supported the main conclusion from Emmett et al. (2006) that serum levels are strongly positively correlated with drinking concentrations of PFOA.

Some of the limitations and uncertainties noted have been addressed by subsequent publications that present a reanalysis of the same datasets to support the development of PBPK models (e.g., Worley et al., 2017a) and first-order kinetic models that account for non-drinking water sources of exposure (e.g., the Russell et al. (2015) study reviewed here). The study by Russell et al. (2015), which was ranked highest by reviewers, underscores the importance of factoring in background exposures when evaluating kinetics and the potential for biased results if not done so properly. Similarly, Dourson et al. (2019) observed that the published literature on serum elimination half-life of PFOA is quite variable and propose a biological mechanism (i.e., biphasic elimination due to potential saturation of resorption of PFOA in the kidney at high doses) that would support a relatively short half-life based on their analysis of the clinical human data from Elcombe et al. (2013). Both papers do not appear to be readily factored into current agency assessment of PFAS toxicokinetics and, taken together, suggest that current

estimates of elimination half-lives may be too high. Future research and regulatory evaluations can be improved by providing a more thorough assessment of on-going background exposures when evaluating elimination kinetics.

These concepts extend beyond PFOA and can be applied to support elimination half-lives for a broader range of PFAS in the general population. Until additional data become available, the PFOA half-life estimate based on Emmett et al. appears to be favored by reviewers to that presented by Olsen et al. (2007). This underscores the importance of using biomonitoring data from well-designed studies (including sample preparation and analytical methods) that includes (1) examination of a wide range of plausible exposure pathways, (2) large populations with exposures relevant to the general population, (3) paired water and serum measurements, (4) accounting for background exposures, and (5) careful evaluation of variability based on demographic factors.

Immune/Vaccine Response:

The potential impact of PFAS exposure on the childhood immune response receives a significant amount of attention. Recently, the EFSA used Abraham et al. (2020) human data in the derivation of their total weekly intake threshold. Importantly, both Abraham et al. (2020) and Grandjean et al. (2012) scored rather poorly across the reviewer categories, but with wide variability between the four reviewers. Limitations noted for both studies include potential confounders, the lack of an un-vaccinated control group, and lack of study of additional biomarkers. Neither study was identified as having potential risk of bias. Reviewers in particular noted concerns for use of the Grandjean et al. (2012) study. Although regulatory agencies have not incorporated this 2012 publication directly in the derivation of toxicity values and associated regulatory thresholds, the concern for potential immune system effects has been cited as one rationale for applying a database uncertainty factor of 3 or 10. The four reviewers had different views on the use of this study, given the inconsistency in human evidence for potential immune/vaccine responses. Risk assessment practitioners should, therefore, understand that this endpoint is still highly uncertain.

Toxicity Studies, including Reproductive and Development Effects, in Rodents:

Five rodent toxicity studies were reviewed that explored a range of effect endpoints of PFOA and/or PFOS. Spanning more than a decade of research, each has been selected by one or more federal and state environmental regulatory agencies (e.g., EPA, ATSDR, Health Canada) as one of the principal studies to support a current health benchmark for PFOA and PFOS. The reviewers stated there was no indication of significant risk of bias for any of the studies.

Two of the studies examined reproductive and development effects of PFOA in mice. Lau et al. (2006), which received the most consistent and highest scores (85 out of 100, ranging 67.5 to 95), established a dose-response relationship between administered dose of PFOA and increased maternal liver weight in mice. Not only did this study establish a link between maternal and development toxicity of PFOA in mice, but it also demonstrated that the renal elimination rate is similar between males and females. This provided strong evidence that the interspecies extrapolation uncertainty is likely greater for studies with rats, which exhibit a

much lower elimination rate in males compared with females. The reviewers scored Lau et al. (2006) more than 30 points higher than the study by Koskela et al. (2016) which examined the bioaccumulation potential of PFOA in bone of offspring following maternal exposure. Reviewers concluded that the effects on bone morphology and density are speculative due to multiple study design limitations.

Three studies in rats were selected that examined additional effect endpoints. The studies included a chronic (two-year) dietary and carcinogenicity study with PFOA in male and female rats (Butenhoff et al., 2012), a 13-week dietary study with PFOA in male rats (Perkins et al., 2004), and a two-generation reproduction and cross-foster study with PFOS in male and female rats (Luebker et al., 2005). Each study received a wide range of scores across reviewers. However, they received consistently high average scores (between 74 and 80), which is consistent with their utility as primary studies for PFOA and PFOS risk assessment. The studies by Butenhoff et al. (2012) and Perkins et al. (2004) with PFOA had notable differences in study design. Butenhoff et al. (2012) received a slightly lower score for application to risk assessment (67.5 compared with 70.0) because only two dose levels were administered and PFOA was not measured in blood or tissues. Nevertheless, both studies included extensive clinical chemistry and tissue histopathology, providing relatively strong and consistent evidence for adverse effect levels. Similarly, the reviewers generally agreed that the PFOS study by Luebker et al. (2005) had sufficient dose groups and key study design elements to demonstrate the potential for *in utero* transfer of PFOS to the fetus and to pups by lactation, and relevance of reproduction as a critical effect endpoint in rodents.

Collectively, while these studies were not designed to assess the mode(s) or mechanism(s) of action for toxicity, and each has notable limitations, they have provided the foundation for identifying some of the more sensitive effect endpoints and developmental stages for assessing risk. Because these studies were not specifically focused on carcinogenicity, they are insufficient to draw any firm conclusions as to whether specific PFAS are carcinogens. Health-protective toxicity values for cancer and non-cancer effects for PFAS will continue to depend on comprehensive, systematic reviews of peer-reviewed publications and data from toxicology and epidemiology studies.

5. Recommendations

The following recommendations are based on the comments from the reviewers:

1. The analysis of the additional studies suggested in Table 8 could increase the knowledge base for risk assessment, public policymaking, and judicial decisions on the toxicity/ carcinogenicity PFAS;
2. Additional longitudinal epidemiological studies are needed to address key data gaps regarding the causal link between PFAS exposure and disease, accounting for potential confounding effects of other chemical stressors; and

3. Toxicity studies that include sufficient data to evaluate mechanism of action are needed to further refine health risk assessment approaches for PFAS, particularly in the context of exposure to mixtures of PFAS.

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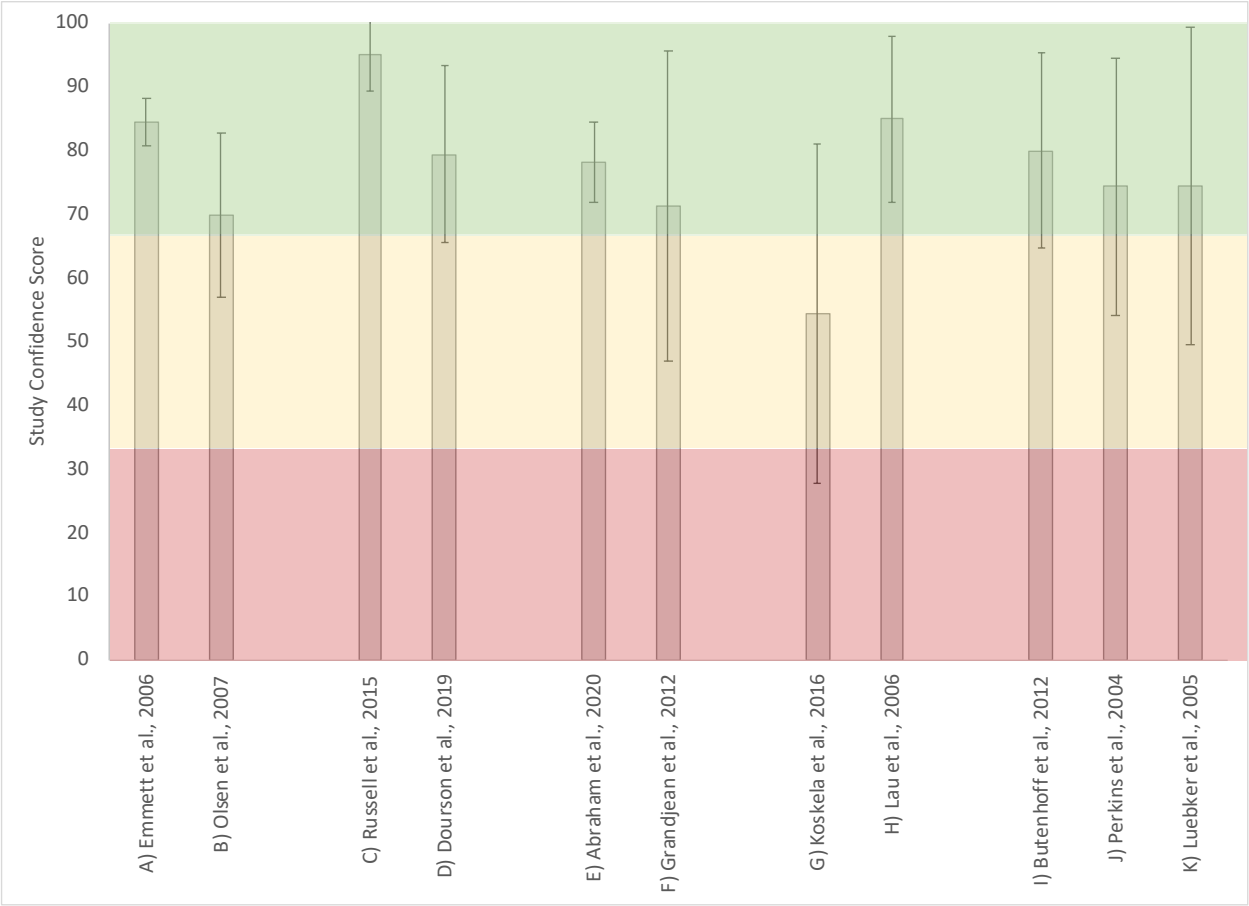
Figure 1. Summary of PFAS Study Confidence Scores



Appendix A: Panel Recruitment and Selection

Panel Recruitment

The goal of the panel recruitment is to cast as wide a net to reach out to as many potential candidates as is feasible. A total of 1708 potential candidates were identified as having relevant experience in toxicokinetics, cancer bioassays, reproductive/developmental effects, and exposure/biomonitoring using multiple sources, including: (1) SciPinion's internal database; (2) searches for authors of recent publications on the topic of interest in online databases (e.g., Pubmed; Google Scholar); (3) searches of profiles on social media databases (e.g., LinkedIn); (4) general internet searches; and (5) referrals. Email addresses were obtained for as many potential candidates as possible with the exception of those who were considered conflicted by either working in the industry of the sponsor or the regulatory agency that may receive the reviewed materials in a regulatory review. An email invitation was sent to all 1708 potential candidates, requesting interested candidates to volunteer on https://app.scipinion.com and upload a copy of their CV. A total of 83 applications were received.

Panel Expertise Verification

Based on the recruitment effort, 83 candidates volunteered for this opportunity. 4 applicants were excluded for failing to upload their CV or applying after the cutoff date. For the remaining 79 candidates, CVs were collected and reviewed. The source of panel candidate recruitment had no bearing on panel selection (i.e., candidates from all five sources listed above were treated equally). Expert panel members were selected from this pool.

Panel Selection

15 reviewers and 1 topic lead were selected from the available candidates based upon a consideration of their expertise based upon objective metrics for expertise (e.g., years of experience, number of publications, number of first/last author publications, key word counts), and topic specific expertise.

Panel Engagement

15 reviewers and 1 topic lead were placed under contract. Reviewers were compensated based upon level of effort. Email addresses corresponding to their SciPinion accounts were verified as belonging to the experts. During the review, panel members were blinded to the identities of their fellow panel members, the topic lead, and to the identity of the review Sponsor. The Sponsor remained blinded to the identities of the reviewers and topic lead until receipt of a draft of this report (i.e., post-review). Experts included in this panel are identified in **Table A-1**.

Table A-1. PFAS Panel Members

Topic Area	Name	Country	Affiliation	Degree	Years Experience (post degree)	Publications
All (Topic Lead)	Dr. Phil Goodrum	United States	GSI Environmental	PhD	22	16

Toxicokinetics /Biomonitoring in Humans	Dr. Kannan Kurunthachalam	United States	New York University School of Medicine	PhD	27	740
	Dr. Henriqueta Louro	Portugal	National Institute of Health, Portugal	PhD	7	37
	Dr. Sami Haddad	Canada	Université de Montréal	PhD	21	77
Toxicokinetic Analyses	Dr. Catherine Sherwin	United States	Wright State University Boonshoft School of Medicine	PhD	14	169
	Dr. Patrick Poulin	Canada	Consultant	PhD	23	61
	Dr. Ahmed Salem	United States	Abbvie, University of Minnesota	PhD	11	180
Key Effects on Immune/Vaccine Response	Dr. Peter Vogel	United States	St Jude Children's Research Hospital	PhD	30	225
	Dr. Rajeev Tyagi	India	CSIR- Institute of Microbial Technology	PhD	10	45
	Dr. Mahin Khatami	United States	Retired (National Cancer Institute)	PhD	41	60
Key Effects on Reproduction / Development	Dr. Babasaheb (Bob) Sonawane	United States	Retired (USEPA)	PhD	50	137
	Dr. Robert Kavlock	United States	Retired (USEPA)	PhD	44	200
	Dr. Alan Hoberman	United States	Argus International	PhD	39	115
Key Effects in Rodents (other than reproduction /development)	Dr. Wolfgang Dekant	Germany	University of Würzburg	PhD	37	450
	Dr. I Glenn Sipes	United States	Retired (University Arizona)	PhD	52	286
	Dr. Shakil Saghir	United States	Scotts Miracle-Gro	PhD	27	88

Appendix B. Reviewer Instructions

While reviewing and answering charge questions for your assigned key studies, please consider the factors listed in **Table B-1**.

Table B-1. Factors to be Considered in Key Studies

Category	Example Questions to be Considered
Causality	Did the results of the study establish a definitive causal link between exposure to the compound(s) and specific types of diseases/harmful effects on humans?
Replication	Were the results of each study replicated by either the original researchers or by other independent scientists? Are there other studies that resulted in identical or similar findings? How many? Did the original researchers provide enough data to facilitate efforts to replicate the experiment(s)? Has the study been cited by other scientists, advocates, and/or legal professionals? How often? How were these citations used?
Peer Review	Were the results of each study subject to a thorough and independent peer review process before it was conducted (i.e. NIH or EPA)? How were the results certified (i.e. an independent scientific panel such as a data safety and monitoring board, or another type of oversight committee)? How and when was it chose?
Data, Sample Size and Control Group	Did each study include appropriately sized sample and control groups? How might variations in sample size have affected the results? Was randomization used appropriately? Were there outliers that were excluded? What was the rationale for exclusion? Were the data appropriate to answer the study's research questions?
	Were/are the data transparent and available for review? Was there adequate controlling for all major confounders?
Duration	Were each of the studies conducted over an appropriate time period? How long was the follow-up period and what was the rationale? How might variations in durations of the studies have affected the results?
Human vs. Animal Subjects	Did the studies use human and/or animal subjects? If animals, how might this affect the applicability of the results to humans? Are findings from animal models supported by observational studies in humans? Were mechanisms identified?
Exposure / Dosage Levels	Were the subjects (human and/or animal) exposed to exaggerated physiological concentrations of the chemical or compound? Was the dose spacing appropriate for the compound? How might exaggerated dosage levels have affected the results?
Methodology and Protocols	What was the research question(s) being answered by the study? Were they hypotheses driven? Were these proposed *a priori* (i.e., before the study was designed? Was the methodology utilized in each study adequate to answer the research question(s)? How might alternative methods have affected the results? Did the study adhere to definitive designs, definitions, outcomes, and analytical modes or were they modified during the process? If so, were the changes made so great that results have been affected?
Financial Interest, Competition, and Conflicts of Interest	What, if any, conflicts of interest were declared by the study authors in their published results?
Refutation	Have the results of any of the studies been refuted by new or additional evidence presented by either the original researcher or other scientists. Did the original researcher acknowledge the new/additional findings in subsequent research and/or public statements? Were there reactions or responses by other scientists to the study? Did the journals that published the final results run one or more editorials with either supporting or non-supporting views?

Bias	What were any study design, data analysis, or other factors that may have influenced results? Were these discussed by the authors in their publication or presentation of the findings? How adequately were they presented? Were funders involved in the study resign or any other parts of the process, including conducting the study and presenting results? Did this involvement influence the process or outcome? How could this be substantiated?
Publication	Were findings published in a peer-reviewed journal? Which one? What is the impact factor of the journal in which the study was published? How many publications resulted from the work?
Pre-Registration and Data Transparency	Was the study protocol model pre-registered? Was it published before study results? Were the data made available to other researchers when the final results were published? Are all of the data and other materials currently available to other researchers?
Perspective	Did the study appropriately place the results in perspective with previous studies? What was the conclusion of the study authors? Was it appropriate based on their results?
Effect Size	Were the effects appropriately reported? What levels of statistical significance were chosen? Are these substantiated? What was the effect size and how was it derived?

Please note that these are considered to be general guidelines for you to consider rather than a check list. Not all factors apply to all studies, and you are welcome to include additional factors as you see fit. Some examples of additional factors include, but are not limited to, statistical power, confounders, inadequate literature citations (i.e., not citing null or negative studies), the likelihood of missing, null, or negative studies, frequency of exposure data, data transformation, partial data, probability hacking (i.e., trying different models until a statistical significant finding occurs), multiple outcomes measured without adjustment for probability assessment, and reviewer and publication bias. Any insight on how factors might have skewed the results of the original research would be appreciated. Please be sure to include relevant details in your explanations to your charge question responses.